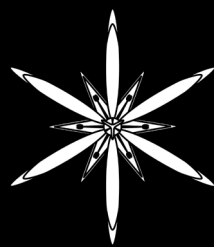
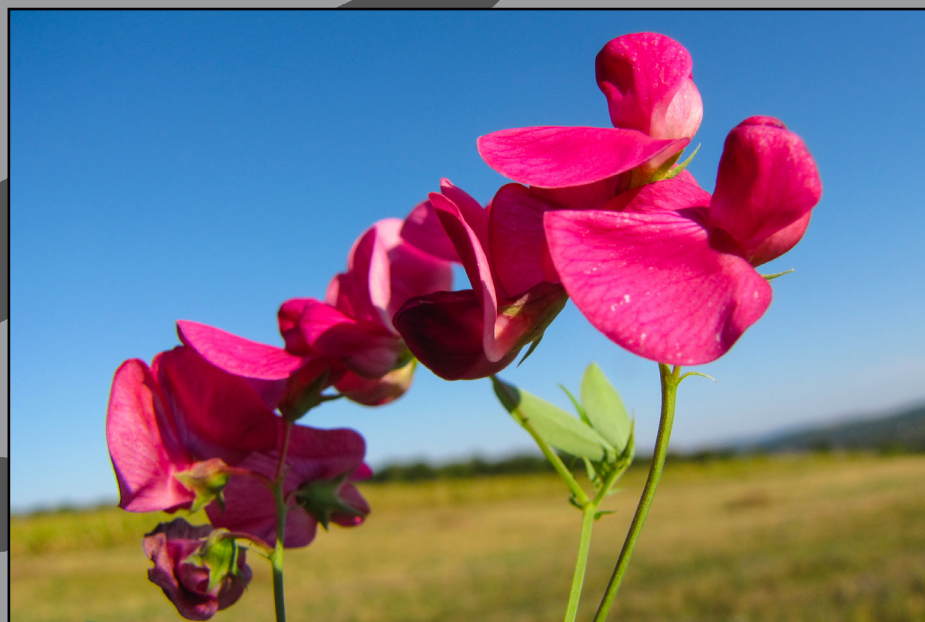


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CORRESPONDENCE

On the recent record of “*Chenopodium badachschanicum*” (Chenopodiaceae) from Iran

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Abstract

Chenopodiastrum badachschanicum (Tzvelev) S. Fuentes, Uotila et Borsch has been recently reported (as *Chenopodium badachschanicum* Tzvelev) from Iran in the article published in *Modern Phytomorphology*. Unfortunately, the article contains some errors and outdated information on taxonomy of this species. Most importantly, the image of herbarium specimens reproduced in the article definitely represents not *C. badachschanicum* but, undoubtedly, a species of *Spinacia* L., most probably *S. turkestanica* Iljin. Judging from its distribution pattern, *C. badachschanicum* may be expected in Iran, especially in eastern mountain areas. However, there is no positive evidence of its occurrence in Iran yet and its recent record is based on a misidentification.

Keywords: Chenopodiaceae, *Chenopodium*, *Chenopodiastrum*, taxonomy, distribution, Iran

Introduction

In one of the latest issues of *Modern Phytomorphology*, *Chenopodium badachschanicum* Tzvelev (Chenopodiaceae) was reported from Iran, supposedly for the first time for the flora of that country (Keshavarzi *et al.* 2016). However, there are some evident problems with that record and the article itself, which stimulated the present note, where I provide corrected and updated information about

the species, as well as some comments on the record itself.

Taxonomy of *Chenopodiastrum badachschanicum* and related taxa: a brief overview

Chenopodium badachschanicum was described by Tzvelev (1960) from the Pamir Mountains in Tajikistan (holotype, LE: “Tadzhikistania,

Pamir occidentalis, in declivitate lapidosa paulo ruderata in valle fl. Murgab 3–4 km intra ostium fl. Pschart occidentalis, alt. circa 3300 m s. m., 19 VI 1958, № 220, N. Tzvelev”; “Таджикская ССР, Западный Памир, несколько засоренная осыпь мелких камней по склону правого берега р. Мургаб, в 3–4 км ниже устья р. Западный Пшарт, около 3300 м над ур. м., 19 VI 1958, № 220, Н. Цвелев”). When discussing his new taxon, Tzvelev did not mention its affinity to *Chenopodium hybridum* L. (now *Chenopodiastrum hybridum* (L.) S. Fuentes, Uotila & Borsch: see Fuentes-Bazan *et al.* 2012b) but instead considered some other supposedly related taxa of *Chenopodium* L., viz. *C. bryoniifolium* Bunge, *C. atripliciforme* Murr, and *C. murale* L. For some time, this species was indeed considered a relative of *C. bryoniifolium* and/or *C. atripliciforme*.

Uotila (1993, 1997, 2001) clarified the taxonomic position and patterns of geographical distribution of *Chenopodium atripliciforme* and *C. badachschanicum* and demonstrated the close relationships of *C. badachschanicum* and *C. hybridum*. Later the *Chenopodium hybridum* group (including the widespread but originally probably European – western Asian *C. hybridum* s. str., mountain Asian *C. badachschanicum*, and North American *C. simplex* (Torrey) Raf.) was transferred to a new genus *Chenopodiastrum* S. Fuentes, Uotila & Borsch, and the relevant nomenclatural combinations have been made for these three species (Fuentes-Bazan *et al.* 2012b). These taxa belong to the Eurasian–North American section *Chenopodiastrum* sect. *Grossefoveata* (Mosyakin) Mosyakin (see nomenclatural citations and synonymy in Mosyakin (1993, 2013)). As I noted earlier (Mosyakin 2013), “This section includes three currently recognized species, *Chenopodiastrum hybridum*, *C. badachschanicum* (Tzvelev) S. Fuentes, Uotila, & Borsch (*Chenopodium badachschanicum* Tzvelev), *C. simplex* (Torr.) S. Fuentes, Uotila, & Borsch (*Chenopodium simplex* (Torr.) Raf.; *C. gigantospermum* Aellen), and probably one yet undescribed East Asian entity (species or subspecies) discussed by Baranov (1964) and mentioned by Zhu *et al.* (2003)”. Additional

comments on still problematic and probably undiscovered taxa of that group are available from Sukhorukov (2014) and Sukhorukov & Kushunina (2014). Carpology of taxa of *Chenopodium* and related genera (including the *Chenopodiastrum hybridum* aggregate) was recently studied (Sukhorukov & Zhang 2013; Sukhorukov 2014) in light of new phylogenetic evidence.

The Iranian record as reported by Keshavarzi *et al.* (2016)

Keshavarzi *et al.* (2016) cited in their article several rather irrelevant references but failed to mention and cite the publications directly relevant to the species under discussion. For example, they mentioned the molecular phylogenetic study by Fuentes-Bazan *et al.* (2012a), in which the *Chenopodium hybridum* – *C. murale* group was already reported as a clade separate from *Chenopodium* s. str., but did not cite the following article by Fuentes-Bazan *et al.* (2012b) with phylogenetic information updated and a new taxonomic treatment proposed. Some other important references that were glaringly missing in Keshavarzi *et al.* (2016) are cited here. A simple Google search with the keywords “*Chenopodium badachschanicum*” easily brings most of those publications; many of them are open access resources.

The text of the article was evidently in need of considerable editing, both linguistic and scientific. Some references were also cited incorrectly. For example, Uotila is cited as the author of the treatment of Chenopodiaceae in Flora Iranica. In fact, he prepared the treatment of *Chenopodium* (Uotila 1997), while the whole treatment of Chenopodiaceae in Flora Iranica was authored by seven contributors (see Hedge *et al.* 1997). There are some other problems, which are not discussed here for brevity’s sake.

Most importantly, the image of the herbarium specimen(s) reproduced in the article definitely represents not *Chenopodiastrum badachschanicum* but, without any doubt, a species belonging to a different genus (not *Chenopodiastrum* and

not *Chenopodium*), and even to a different tribe. In my opinion, the plants illustrated on Fig. 2 (Keshavarzi *et al.* 2016: 33) belong to *Spinacia turkestanica* Iljin, a species previously known for Iran (Hedge *et al.* 1997). Other illustrations (close-ups; Keshavarzi *et al.* 2016: 34, Fig. 3) most probably also represent parts of inflorescences of staminate individuals of that species. My identification of the plants illustrated in Keshavarzi *et al.* (2016: 33, Fig. 2) has been confirmed also by other experts in Chenopodiaceae, Alexander Sukhorukov and Pertti Uotila (personal communications, email messages to Sergei Mosyakin). Of course, our identification of the specimen from the ALH herbarium is based on images only. However, the characters visible on the images are sufficient. In particular, the characteristic features are: general branching habit, remnants of the basal leaf rosette, peculiar hastate leaves with rather long horizontal basal lobes, and the inflorescence shape. It is also worth noticing that only staminate flowers seem to be visible on close-up images, which is what to be expected if only staminate plants of a dioecious *Spinacia* were studied. Keshavarzi *et al.* (2016) also did not report any original information on morphology of fruits – the most important characters for distinguishing *C. badachschanicum* from *C. hybridum* and other similar taxa. Instead, they for some reason provided non-diagnostic images of the leaf surface and pollen grains.

Following the request by the editor of *Modern Phytomorphology*, Andriy Novikov (Andrew Novikoff), made already after the article has been published, the authors provided some field photographs showing plants probably indeed belonging to a species of *Chenopodiastrum* (personal communication by the editor). Positive species-level identification of plants on those images is impossible or at least highly problematic because no reliable diagnostic characters are visible (in particular, fruits: see Uotila (1997, 2001), Sukhorukov (2014) and references therein), while the general appearance of plants can be misleading. By the date of the completion of the present note the authors did not provide any additional herbarium specimens or their scanned images,

except for the one illustrated in the article (that of *Spinacia turkestanica*). Thus, at present there is no reliable evidence supporting their claim of a new country record of *C. badachschanicum* and its recent reporting for Iran is based on a misidentification.

Conclusions

The article reporting “*Chenopodium badachschanicum*” from Iran is evidently not an achievement of the authors, reviewers and editors. Forthcoming articles submitted to *Modern Phytomorphology*, a journal that is gaining popularity and outreach, should be prepared for publication with better scientific and editorial scrutiny. I do hope that this brief critical note will result in improving future articles of this journal.

Judging from its distribution pattern, *Chenopodiastrum badachschanicum* may be indeed expected to be found somewhere in Iran, especially in the mountain areas of the eastern part of the country. If a species of *Chenopodiastrum* has been indeed found in Iran by the authors, as they claim, solid evidence on its identity has to be presented. Probably new collections and morphological studies will be needed. However, now we should conclude that at present there is no positive evidence of *C. badachschanicum* occurring in Iran.

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I am grateful to Pertti Uotila (Botanical Museum, Helsinki University, Helsinki, Finland) and Alexander Sukhorukov (Lomonosov Moscow State University, Moscow, Russia) for discussing the identity of plants illustrated in Keshavarzi *et al.* (2016). Kind cooperation of Andriy Novikoff (State Natural History Museum NAS of Ukraine, Lviv, Ukraine) is also appreciated.

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RESEARCH ARTICLE

Seed morphology of *Silene commelinifolia* Boiss. complex (Caryophyllaceae Juss.)

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Abstract

Silene (Caryophyllaceae) is a large genus with about 700 species distributed worldwide. *Auriculatae* is the largest section of this genus with 21 endemic species represented in Iran. Seed micromorphology of *S. commelinifolia*, *S. lucida*, *S. nurensis* and *S. eremicana* from 18 populations was studied by the light microscopy. As a result, 6 populations were adopted for further SEM studies. A great variation was observed in seed shape and size, lateral and peripheral surfaces of seeds, and shape of testa cells edge. Totally, 16 quantitative and qualitative seed features were measured and evaluated. Seed size varied from 1.28 × 1.43 mm in Alvand population of *S. eremicana*, to 1.97 × 1.50 mm in Bozghosh population of *S. lucida*. PAST and SPSS software was applied to demonstrate the species relationships. *S. eremicana* and *S. lucida* were closely grouped, which is in accordance with their morphological similarities. Seed micromorphology revealed that the species, which are similar to *S. commelinifolia* are clearly separated from each other. Therefore seed morphology is of taxonomic importance in the studied group.

Keywords: *Silene*, seed, micromorphology, SEM, Iran

Introduction

Silene L. (Caryophyllaceae Juss.) is a large genus with worldwide distribution, containing about 700 species. These species are mainly

hermaphrodite, although a few species are dioecious or gynodioecious (Bari 1973; Greuter 1995). These are annual, biennial or perennial herbs. The section *Auriculatae* (Boiss.) Schischkin is the largest within this

Table 1. Voucher details of studied *Silene* taxa.

Nr	Taxon	Locality	Code	SEM
1	<i>S. commelinifolia</i> var. <i>ovatifolia</i>	West Azerbaijan, Piranshahr to Naghadeh, Gerd Kashaneh, Lik Bin Village, Landi Sheykh Mountain, 36 41 7.5 N, 45 26 27.1 E, 2400 m, 02.07.2010, A. Gholipour, 890277		
2	<i>S. commelinifolia</i> var. <i>ovatifolia</i>	Tehran, Darakeh mountain, 35 49 37.3 N, 51 22 47.3 E, 1925 m, 20.06.2008, A. Gholipour, 8768	Com12	*
3	<i>S. commelinifolia</i> var. <i>ovatifolia</i>	West Azerbaijan, Urmia, Anhar, Marmisho, Solok, 37 29 0.33 N, 44 45 0.22 E, 2327 m, 20.07.2011, A. Gholipour, 900832		
4	<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Mazandaran, Baladeh, Kamarbon, Gosfandsarai-e chai khaksar, 36 14 16.1 N, 51 22 17.1E, 2852 m, 07.07.2011, A. Gholipour, 900624		
5	<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Tehran, Haraz Road, Polur, 35 48 899 N, 52 01 643 E, 2405 m, 10.06.2007, A. Gholipour, 8637		
6	<i>S. commelinifolia</i> var. <i>commelinifolia</i>	West Azerbaijan, Urmia, Anhar, Marmisho, 37 29 03.2 N, 44 36 24.7 E, 3007 m, 02.07.2012, A. Gholipour, 91312		
7	<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Hamadan, Alisadr cave, 29.06.2010, A. Gholipour		
8	<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Tehran, Touchal, 35 52 572 N, 51 24 131 E, 2700 m, 23.06.2008, A. Gholipour, 8771	Com9	*
9	<i>S. commelinifolia</i> var. <i>commelinifolia</i>	Tehran, Dizin, Gajerah, Velayatroud village, 36 03 N, 51 23 E, 2500 m, 15.07.2008, A. Gholipour		
10	<i>S. cf. commelinifolia</i>	Ardabil, km 30 Ardabil to Kivi, before Neor lake, 38 00 549 N, 48 55 225 E, 2590 m, 16.07.2011, A. Gholipour, 900701		
11	<i>S. cf. commelinifolia</i>	Ardabil, Neor lake, 09.08.2008, A. Gholipour		
12	<i>S. cf. commelinifolia</i>	East Azerbaijan, Sarab, Shalagoon Village, Bozqush Mountain, 37 45 54 N, 47 35 31 E, 2650–3000 m, 08.07.2012, A. Gholipour, 91387	Com3	*
13	<i>S. eremicana</i>	Hamadan, Alvand Mountain, Ganjnameh, 34 43 475 N, 48 25 039 E, 2800 m, 26.06.2007, A. Gholipour, 86105	ere	*
14	<i>S. lucida</i>	East Azerbaijan, Sarab, Shalagoon village, Bozqush Mountain, 37 45 54 N, 47 35 31 E, 2650–3000 m, 08.07.2012, A. Gholipour	Luc2	*
15	<i>S. lucida</i>	Gilan, Kelachay, Rahim Abad, Eshkevarat, Chakol, Boza kuh, 2800–3100 m, 29.06.2007, A. Gholipour, 86139		
16	<i>S. lucida</i>	Piranshahr to Naghadeh, km 5, Zarkanan and Silveh villages, Kuh-e Sepiarez, 36 50 59.9 N, 44 58 24.8 E, 2820 m, 01.07.2010, A. Gholipour, 890257		
17	<i>S. nurensis</i>	Chaharmahal and Bakhtiari, Farsan, Kuhrang, Zardkuh, 32 18 704 N, 50 08 574 E, 3300–3400 m, 29.07.2008, A. Gholipour, 8782	Nur1	*
18	<i>S. cf. commelinifolia</i>	Lorestan, Azna, Daretakht, Oshtorankuh, 33 20 522 N, 49 20 427 E, 2535 m, 09.08.2008, A. Gholipour, 8787		

genus, representing 21 endemic species in Iran (Melzheimer 1980).

According to Bittrich (1993), seeds of Caryophyllaceae are small or very small (0.4–3 mm long), black, brown or nearly white, reniform, pyriform, or orbicular, and

mostly laterally compressed. Different seed morphological studies had revealed the taxonomic importance of seed characters in *Silene* (Yildiz & Cırpıcı 1998; Hong et al. 1999; Fawzi et al. 2010). Chowdhuri (1957) found that seeds morphology in *Silene* is of

Table 2. Qualitative and quantitative characteristics of seed of taxa studied of *Silene*.

Nr	Character	States / Units
1	seed shape	round-reniform (0), symmetrical-reniform (1), cordate-reniform (2), asymmetrical-reniform (3), semi-circle reniform (4)
2	peripheral surface of seed	flat (0), concave (1), convex (2)
3	lateral surface of seed	flat (0), concave (1), convex (2)
4	testa cells edge	v-shaped (0), undulate (1), smooth (2), sinuate (3)
5	shape of peripheral cells	elongated polygonal (0), polygonal (1)
6	trichomes at hillum	determinate (0), indeterminate (1)
7	seed Length	mm
8	seed Width	mm
9	seed length to width ratio	
10	testa cell length	µm
11	testa cell width	µm
12	testa cell length to width ratio	
13	width of peripheral surface	mm
14	hilum region length	µm
15	hilum region width	µm
16	hilum length to with ratio	

taxonomic importance on the section level. [Melzheimer \(1987\)](#) considered testa cells shape studied by SEM for *Silene* species separation. Seed morphology of some *Silene* species in Pakistan was studied by [Ghazanfar \(1983\)](#). Numerical analysis of seed features by [Zareh \(2005\)](#) showed that these features are more diagnostic whenever used with a set of macromorphological data. [Camelia \(2011\)](#) studied some *Silene* and recorded that most seeds are uniform but testa cells shape is informative.

Seed morphological studies in this genus can provide additional taxonomic information to help taxa distinguishing. This is the first seed morphological studies for *S. commelinifolia* Boiss., *S. lucida* Chowdh., *S. nurensis* Boiss. et Hausskn., and *S. eremicana* Stapf.

Material and methods

Eighteen populations of 4 *Silene* (namely *S. commelinifolia*, *S. lucida*, *S. nurensis*, and *S. eremicana*) growing in Iran were studied at the beginning, and then seeds from 6

populations were used for further SEM and statistic analyses ([Tab. 1](#)). The specimens were collected from nature, and later the vouchers were deposited at the Herbarium of Shahid Beheshti University (HSBU) and Payame Nour University (PNUSH).

Beforehand, the samples were studied by a Dino-Lite Pro hand stereomicroscope. For SEM studies, selected samples were directly transferred by fine pipette to a metallic stub using double-sided adhesive tape, and then coated with gold in a sputtering chamber (Sputter Coater BALTEC, SCDOOS). The coating was restricted to 100 Å. The SEM examination was carried out on EM32000 KV25 microscope. The measurements were calculated on the base of 10–20 readings from each specimen by use of ImageTool ver. 3.

In general, 16 qualitative and quantitative seed characteristics were analyzed ([Tab. 2](#)). To reveal species relationships we applied cluster analysis, principal components analysis (PCA) and principal coordinate analysis (PCO) plotting. For multivariate analysis, the mean

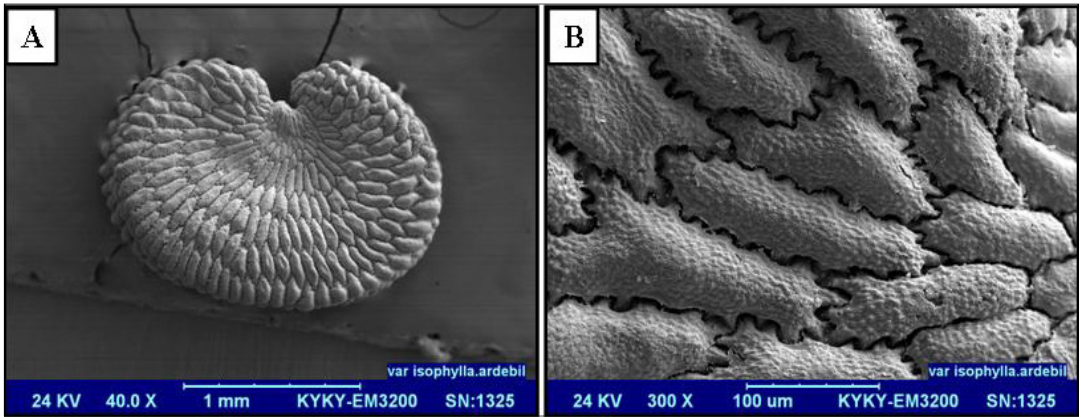


Fig. 1. SEM micrographs of seed surface in *Silene cf. commelinifolia* (Bozqush population): **A** – lateral surface; **B** – testa cells shape.

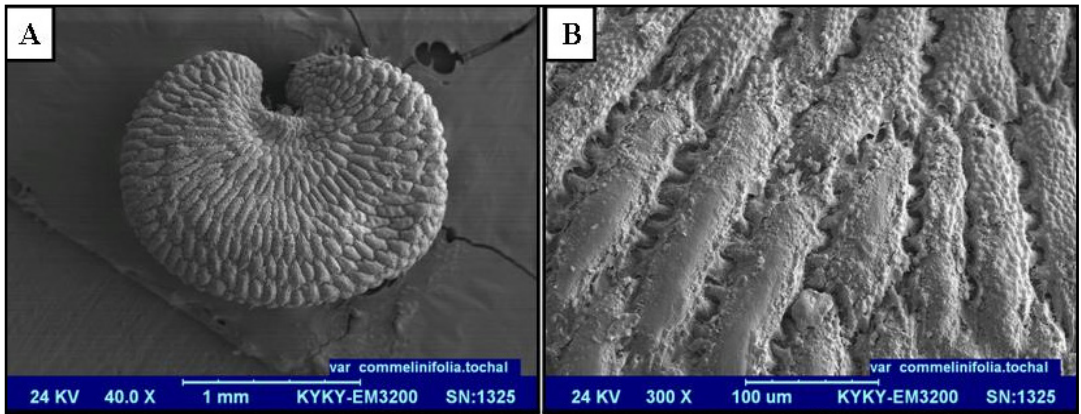


Fig. 2. SEM micrographs of seed surface in *Silene commelinifolia* var. *comelinifolia* (Touchal population): **A** – lateral surface; **B** – testa cells shape.

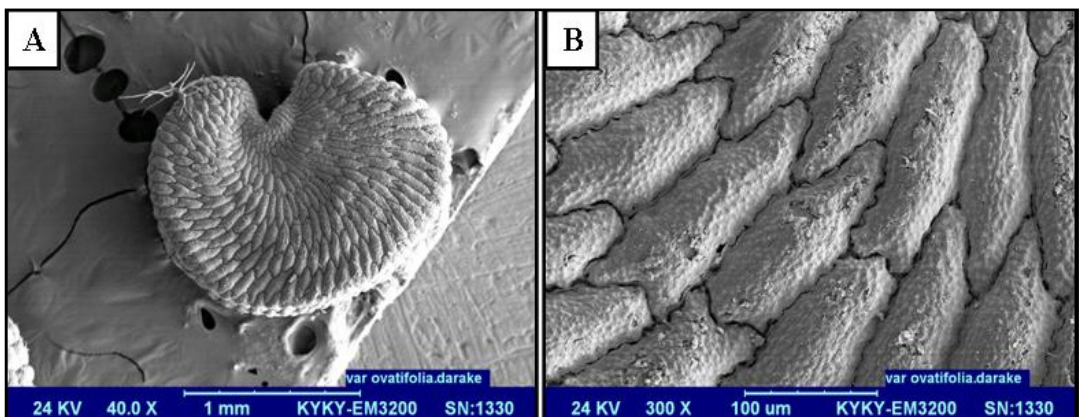


Fig. 3. SEM micrographs of seed surface in *Silene commelinifolia* var. *ovatifolia* (Darake population): **A** – lateral surface; **B** – testa cells shape.

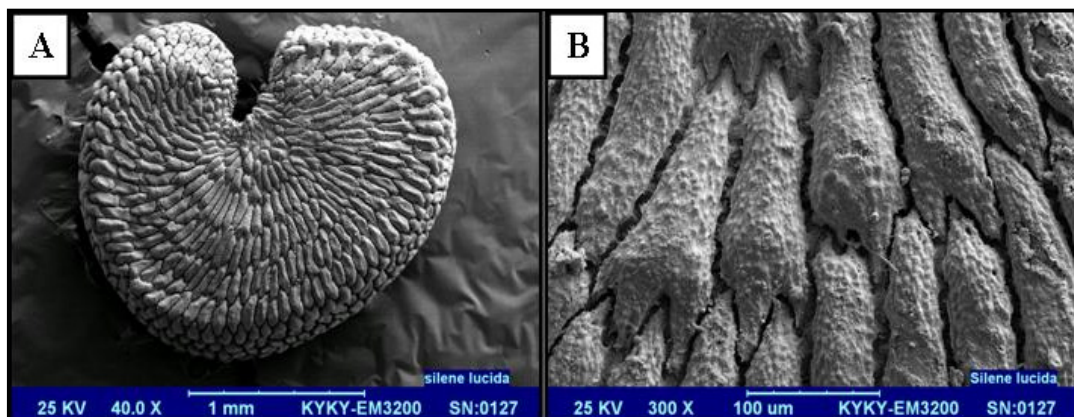


Fig. 4. SEM micrographs of seed surface in *Silene lucida* (Bozqush population): **A** – lateral surface; **B** – testa cells shape.

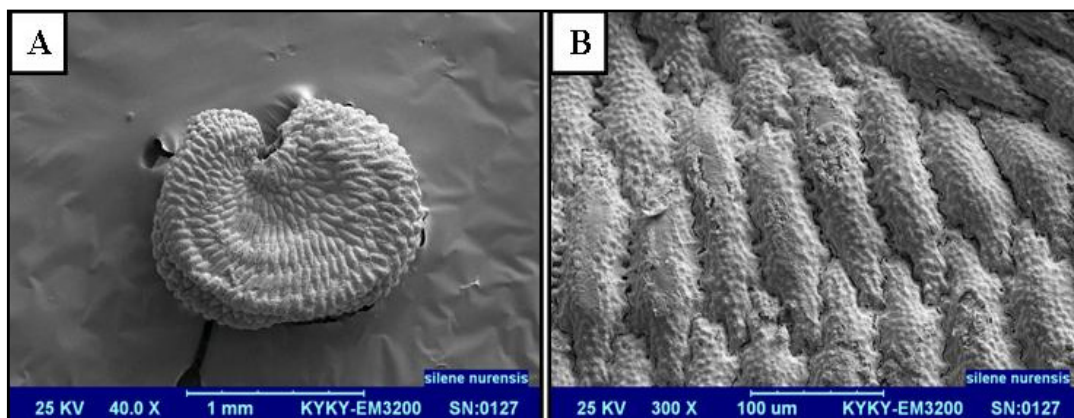


Fig. 5. SEM micrographs of seed surface in *Silene nurensis* (Zardkuh population): **A** – lateral surface; **B** – testa cells shape.

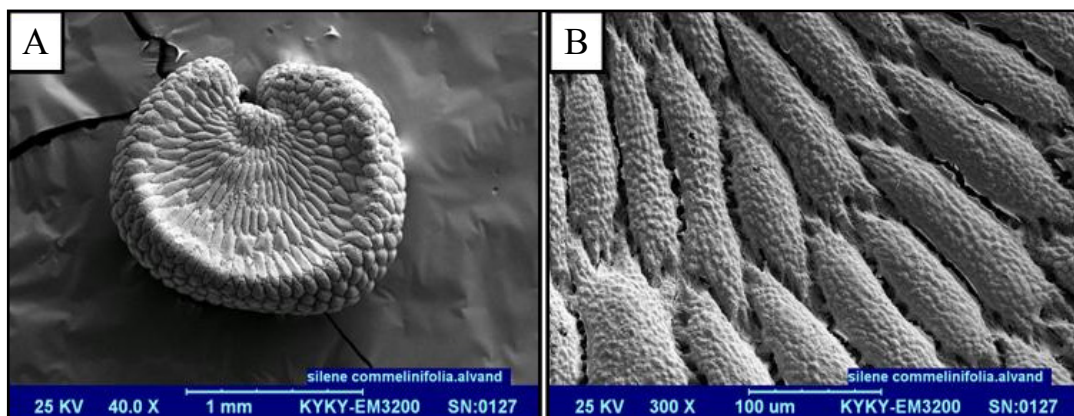


Fig. 6. SEM micrographs of seed surface in *Silene eremicana* (Alvand population): **A** – lateral surface; **B** – testa cells shape.

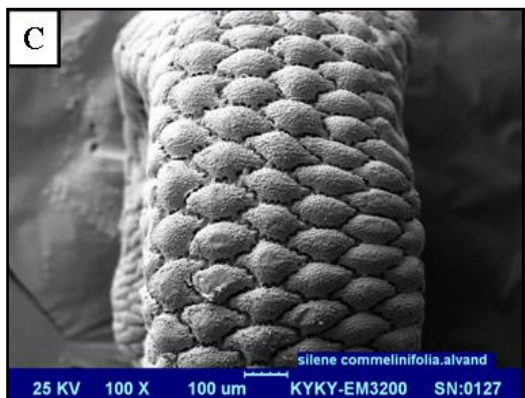
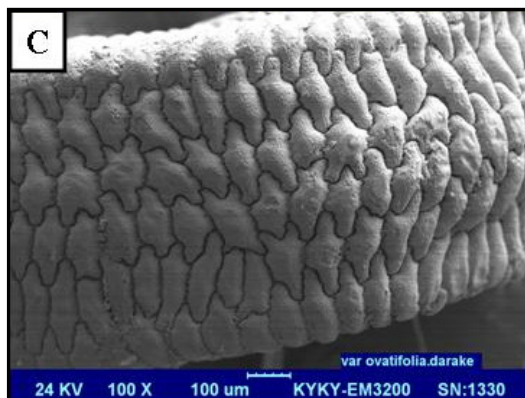
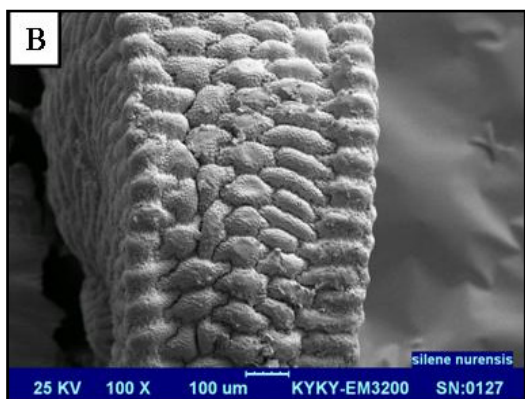
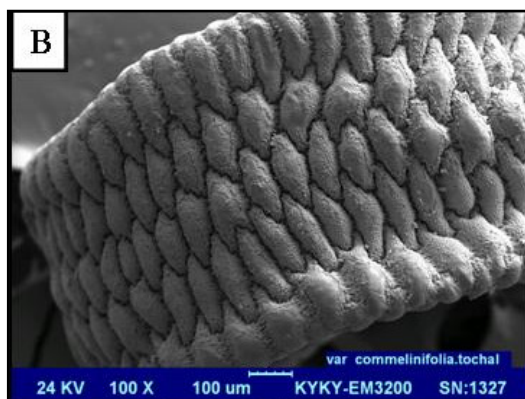
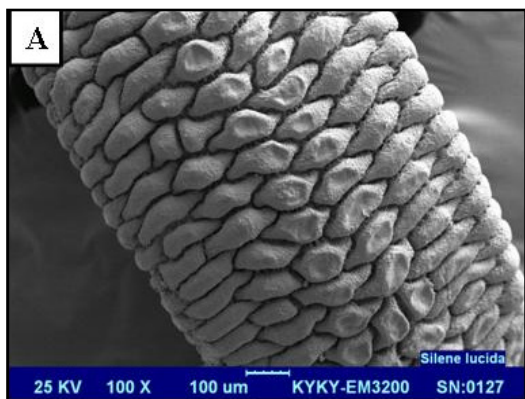
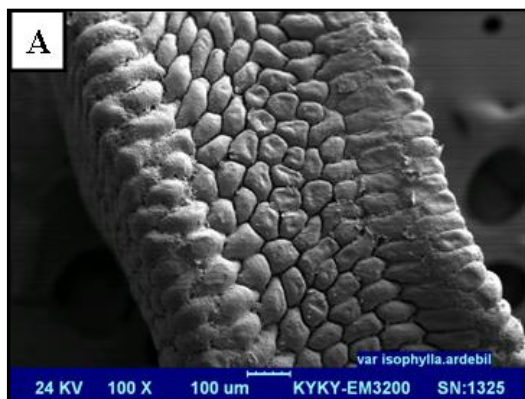


Fig. 7. SEM micrographs of seed surface in peripheral view: **A** – Bozqush population of *Silene* cf. *commelinifolia*; **B** – Touchal population of *S. commelinifolia* var. *commelinifolia*; **C** – Darake population of *S. commelinifolia* var. *ovatifolia*.

Fig. 8. SEM micrographs of seed surface in peripheral view: **A** – Bozqush population of *Silene lucida*; **B** – Zardkuh population of *S. nurensis*; **C** – Alvand population of *S. eremicana*.

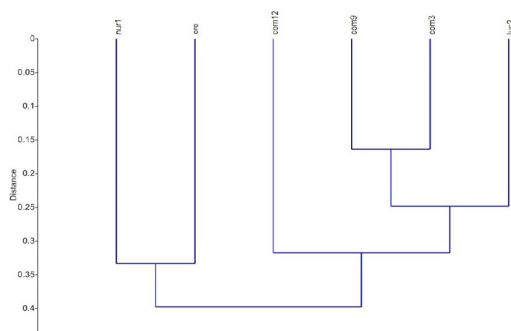


Fig. 9. Cluster analysis by UPGMA based on seed features: **com9** – *Silene commelinifolia* var. *commelinifolia*; **com12** – *S. commelinifolia* var. *ovatifolia*; **com3** – *S. cf. commelinifolia*; **nur1** – *S. nurensis*; **luc2** – *S. lucida*; **ere** – *S. eremicana*.

of the quantitative characters was used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean = 0, variance = 1) were applied in the statistical analysis. The average taxonomic distances and squared Euclidean distances were used as dissimilarity coefficients in the cluster analysis of seed morphological data. SPSS ver. 20 and PAST (Hammer *et al.* 2001) were used for statistical analysis. Cophenetic correlations were determined to fit the obtained dendrogram.

Results

Studied populations showed some differences in seed shape, size, and in lateral and peripheral surface characteristics. Main differences were related to such seed features as testa cells size and shape of their edges. General shape of seeds was round-reniform, symmetrical-reniform, cordate-reniform, asymmetrical-reniform and semi-circle reniform (Figs. 1–6).

Seed size varied from 1.28 × 1.43 mm in Alvand population of *S. eremicana* to 1.97 × 1.50 mm in Bozghosh population of *S. lucida*. Lateral seed surface is convex in Alvand population of *S. eremicana*, and flat in other studied populations (*S. lucida*, *S. nurensis*, *S. cf. commelinifolia* and *S. commelinifolia*).

Peripheral surface of seeds is concave in Zardkuh population of *S. nurensis* and Bozghosh population of *S. cf. commelinifolia* (Fig. 7) and convex in Alvand population of *S. eremicana* (Fig. 8).

Testa cells shape in studied populations was fusiform. The edges of testa cells were v-shaped in Bozqush population of *S. cf. commelinifolia* (Fig. 1 B) and Touchal population of *S. commelinifolia* var. *commelinifolia* (Fig. 2 B), while it was smooth and undulate in Darakeh population of *S. commelinifolia* var. *ovatifolia* (Fig. 3 B). Bozqush population of *S. lucida* (Fig. 4 B) and Alvand population of *S. eremicana* (Fig. 6 B) showed semi smooth testa cells edges. In Zardkuh population of *S. nurensis* testa cells edges were irregular and sinuate (Fig. 5 B).

UPGMA dendrogram (Fig. 9), PCA (Fig. 10) and PCO (Fig. 11) plots of micromorphological characters clearly separated studied taxa. UPGMA dendrogram showed two main clusters (Fig. 9). First cluster composed of two subsets composed of Zardkuh population of *S. nurensis* (*nur1*) and Alvand population of *S. eremicana* (*ere*). Second cluster composed of two subsets and contained Darakeh population of *S. commelinifolia* var. *ovatifolia* Melzh. (*com12*), Touchal population of *S. commelinifolia* var. *commelinifolia* (*com9*), Bozqush populations of *S. lucida* (*luc2*) and *S. cf. commelinifolia* (*com3*). Cluster analysis by Ward's method showed the same division.

Principal components analysis of seed micromorphological data revealed that two first components comprised about 70% of the total variance. In the first component with about 46% of total variance, seed morphological characters, including seed shape, testa cells edge, seed length, seed width, length/width ratio, width of peripheral surface, hilum region length, testa cell length, testa cell width and length/width ratio showed the highest correlation (>0.7). In the second component with about 24% of total variance, lateral and peripheral surface had the highest correlation (>0.7). PCA indicated that seed characters (such as seed shape and size, lateral and peripheral surface, the size and edge of testa cells) are useful in identification and

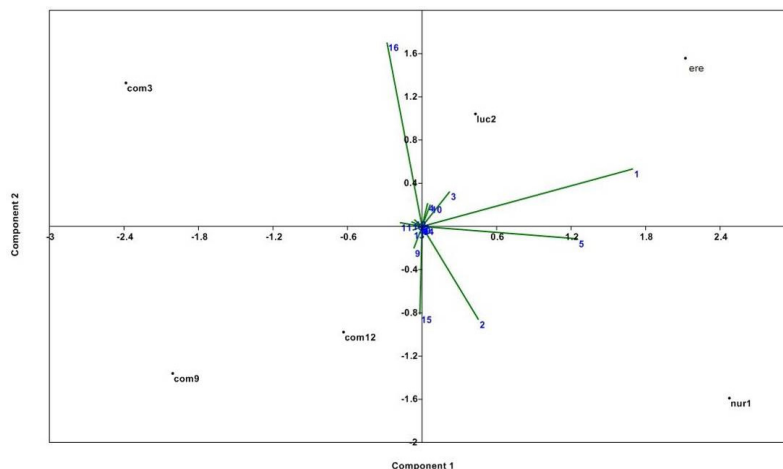


Fig. 10. PCA ordination scatter diagram of studied accessions based on seed features: **com9** – *Silene commelinifolia* var. *comelinifolia*; **com12** – *S. commelinifolia* var. *ovatifolia*; **com3** – *S. cf. commelinifolia*; **nur1** – *S. nurensis*; **luc2** – *S. lucida*; **ere** – *S. eremicana*.

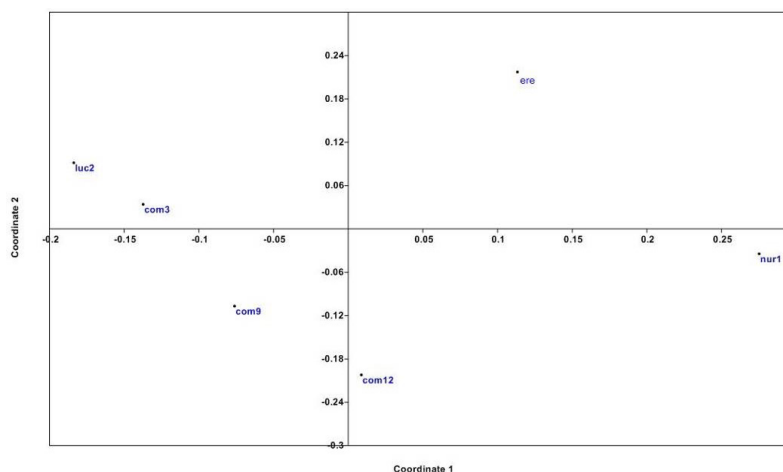


Fig. 11. PCO scatter diagram based on seed features in studied taxa. **com9** – *Silene commelinifolia* var. *comelinifolia*; **com12** – *S. commelinifolia* var. *ovatifolia*; **com3** – *S. cf. commelinifolia*; **nur1** – *S. nurensis*; **luc2** – *S. lucida*; **ere** – *S. eremicana*.

classification of the studied species and varieties (Fig. 10). Principal coordinate analysis plot showed taxa relationships and indicated the clear separation of taxa (Fig. 11). Cluster analysis, as well as PCA and PCO based on *Silene* seed morphology showed more or less similar outcomes.

Discussion

The results of present study (Figs. 1–6) are in accordance with several previous works, reported that the most *Silene* species have generally reniform and small seeds (Yildiz & Cirpici 1998; Zareh 2005; Fawzi et al. 2010).

We found that such micromorphological features as shape, size, lateral and peripheral

surface of seeds, the size and edge of testa cells can be effectively applied for separation of studied species and varieties. For example, rounded reniform seed shape with v-shaped testa cells edge were the seed features for *S. commelinifolia*.

Studied taxa are clearly separated, what confirms previous morphological findings of Atazadeh *et al.* (2014). Although species are separated from each other, there is evident relationship between *S. eremicana* and *S. lucida*. Both varieties of *S. commelinifolia* (var. *ovatifolia* and var. *commelinifolia*) showed a high similarity in their seed features.

Atazadeh *et al.* (2014) pointed the unique position of *S. cf. commelinifolia* populations (such as Bozqush population) defined on the base of morphological and karyotype studies, and suggested existence there of new subspecies. The results of present study are in accordance with that conclusion. Populations of *S. cf. commelinifolia* in present study showed similarity in their seed micromorphology (*i.e.* shape, size, lateral and peripheral surface of seeds, the size and edge of testa cells) and probably represent new subspecies of *S. commelinifolia*.

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CORRESPONDENCE

Glandular trichomes on vegetative and reproductive organs of *Lamium orientale* (Lamiaceae)

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Abstract

The types of glandular trichomes and their distribution on the vegetative and reproductive organs of *Lamium orientale* (syn. *Wiedemannia orientalis*) are studied for systematic purposes in this paper. Two morphologically different types of glandular trichomes (peltate and capitate) are described. Peltate trichomes are characterized by a short stalk, which is connected with a large spherical head composed of four cells in a single layer. Capitate glandular trichomes are subdivided into capitate type A and capitate type B. On the vegetative organs peltate trichomes are abundant, while on reproductive organs capitate trichomes are numerous and the peltate trichomes are rare or absent.

Keywords: *Lamium orientale*, Lamiaceae, trichomes

Introduction

Lamiaceae Martinov is one of the largest plant families represented by about 258 genera and 3500 species in the world (Duarte & Lopes 2007). According to Davis (1982), Lamiaceae family has 45 genera and about 546 species distributed in Turkey. Turkey is a gene center of this family to which genus *Lamium* L. belongs (Başer 1993).

The most important characteristic of the Lamiaceae are glandular trichomes distributed

on vegetative and reproductive organs (Werker 2006). These trichomes produce essential oils and their structure has been studied for systematic purpose (Hanlidou *et al.* 1991; Vrachnakis 2003). The commercial value of these essential oils is recorded in lots of special studies, as well as in relation with the morphology, structure and secretion of trichomes (Amelunxen *et al.* 1969; Heinrich *et al.* 1983; Dudai *et al.* 1988; Antunes & Sevinate-Pinto 1991).

Plant organ	Capitate glandular trichomes						Peltate trichomes	
	Type A			Type B			Center cell	Peripheral cells
	Head cells	Stalk cells	Base cells	Head cell	Stalk cells	Base cells		
Stem	1	2	2	-	-	-	1	4
	2	1	1	-	-	-	-	-
Leaf	-	-	-	-	-	-	1	4
Petiole	-	-	-	-	-	-	1	4
Calyx	1	2	1	1	1	1	1	4
	2	1	1	-	-	-	-	-
	2	2	1	-	-	-	-	-
Corolla	1	2	1	1	2	1	-	-
	1	1	1	1	1	1	-	-
	2	1	1	-	-	-	-	-
	4	1	1	-	-	-	-	-

Table 1. Glandular trichomes variation in *Lamium orientale*.

This paper provides first comparative investigation on glandular trichomes of *L. orientale* (Fisch. et C.A. Mey.) E.H.L. Krause formerly known as *Wiedemannia orientalis* Fisch. et C.A. Mey. (Mill 1982) and nested in *Lamium* genus on the base of recent phylogenetic studies (Bendiksby *et al.* 2011; Atasagun *et al.* 2015). It is aimed to evaluate the usefulness of characters of glandular trichomes for systematic purpose.

Material and methods

Plant material of *L. orientale* was collected in Amasya (on roadside between Yedikuğular Bird Paradise protected area and Ortaova village, 500 m, April 2008, İ. Öztürk Çalı 376), which is a city in the Black Sea region of Turkey. The specimens were kept as a herbarium material deposited at Gazi University Herbarium (GAZI). Flora of Turkey (Davis 1982) was used for taxonomical description.

The plant material was fixed in 70% alcohol for trichome evaluation properties. Glandular trichomes were obtained from transverse and surface sections of vegetative (stem, leaf blade, petiole) and reproductive organs (calyx, corolla) of *L. orientale*. Glandular trichomes preparations were made by hand using commercial razor

blades under a Leica ICC50 HD binocular light microscope. Sartur reagent was applied to the sections for investigation of anatomical tissues (Çelebioğlu & Baytop 1949). The types of glandular trichomes and their distribution were described and classified according to Metcalfe & Chalk (1972), Payne (1978) and Navarro & El Oualidi (2000).

Results and discussion

The great diversity of plant trichomes has interested botanists by their adaptive and taxonomic values. The morphology and distribution of glandular trichomes are often applied as taxonomic characters at subfamilial level in Lamiaceae family (El-Gazzar & Watson 1970; Abu-Asab & Cantino 1987; Cantino 1990).

There are two different types of glandular trichomes on vegetative and reproductive organs of *L. orientale* – peltate and capitate (Tab. 1).

Peltate glandular trichomes of *L. orientale* have a basal epidermal cell, a very short monocellular stalk and a broad round multicellular secretory head consisting of four cells in the single shield (Tab. 1; Fig. 1 A, B). The anticlinal wall of the stalk cell is cutinized.

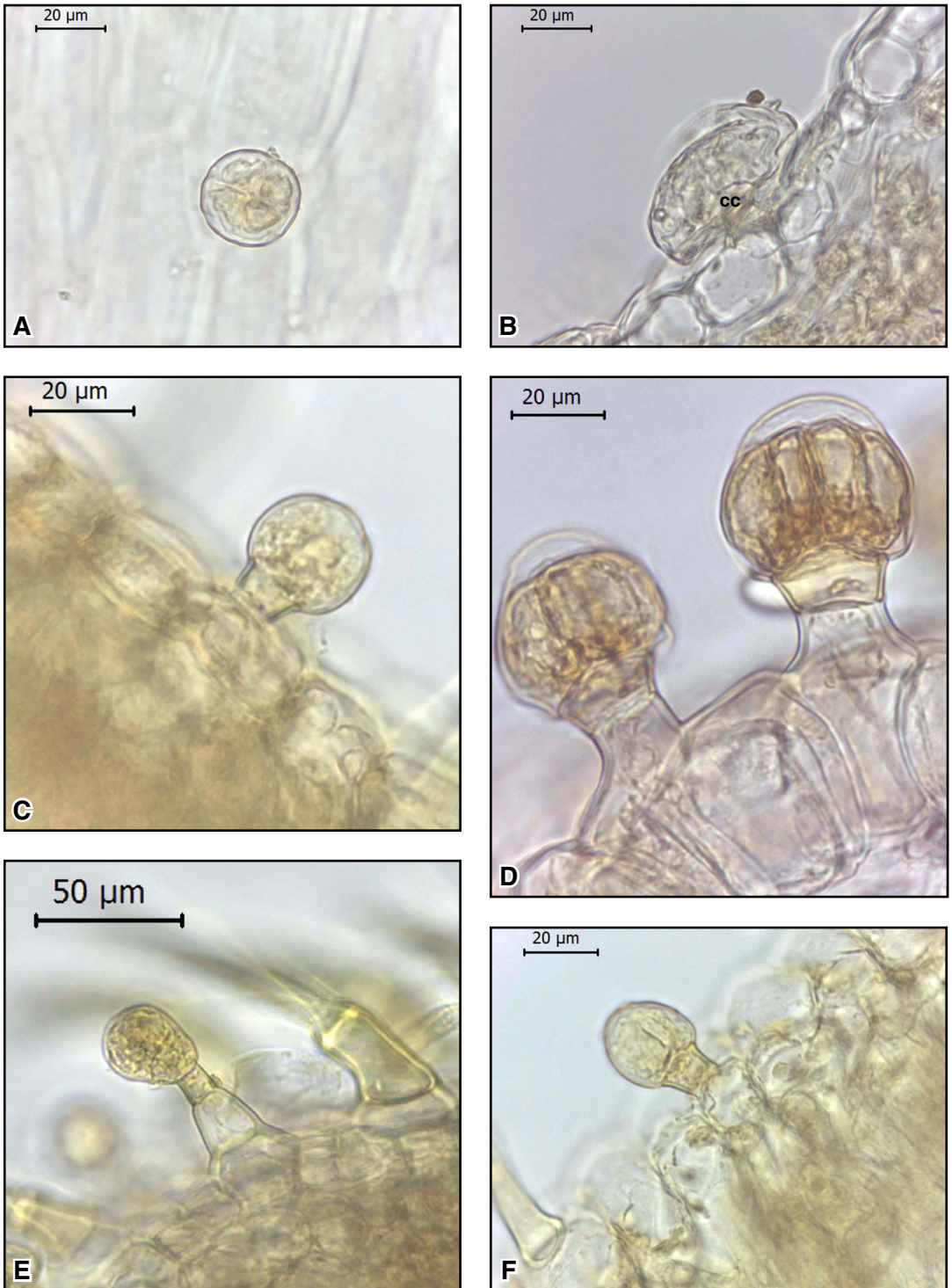


Fig. 1. Different types of glandular trichomes of *Lamium orientale*. Peltate glandular trichomes on the leaf (A, B). Capitate glandular trichomes type A on calyx (C) and corolla (D). Capitate glandular trichomes type B on corolla (E) and calyx (F). cc – center cell.

Plant organ	Capitate glandular trichomes				Peltate trichomes
	Type A		Type B		
	1 stalk cell	2 stalk cells	1 stalk cell	2 stalk cells	
Stem	+	+	-	-	++
Adaxial leaf surface	-	-	-	-	+++
Abaxial leaf surface	-	-	-	-	+++
Petiole	-	-	-	-	++
Calyx	+++	+	++	++	+
Corolla	+++	+	++	++	-

Table 2. Distribution of glandular trichomes on different organs of *Lamium orientale*. Presence of trichomes: - - absent; + - low; ++ - moderate; +++ - numerous.

Secretory materials of such trichomes together with an outer layer of the cell walls are secreted from the head into a space formed in result of elevation of their common cuticle (Özdemir & Altan 2005). Peltate trichomes of other Lamiaceae representatives usually have multicellular secretory head consisting of up to 16 cells, a monocellular stalk and a basal epidermal cell (Corsi & Bottega 1999; Hallahan 2000; Kamatou *et al.* 2006, 2007). In this study it was also found that peltate trichomes are more abundant on the vegetative organs and rare on the reproductive organs of *L. orientale* (Tab. 2), what confirms some previous findings for other species (Serrato-Valenti *et al.* 1997; Corsi & Bottega 1999).

Capitate glandular trichomes are the most common in Lamiaceae family, but they are more variable in stalk length and head shape. Presence of such trichomes is a significant taxonomic character playing also important role for pollination (Navarro & El Oualidi 2000). Such trichomes are composed of a basal epidermal cell, unicellular to multicellular stalk and a large unicellular, bicellular or multicellular secretory head. Capitate trichomes are subdivided into two types – capitate type A and capitate type B (Tab. 1). Capitate type A trichomes have unicellular, bicellular or multicellular head and stalk of one to two cells (Fig. 1 C, D). A high percentage of these trichomes have one roundish head cell. Capitate type B trichomes

have an oblong unicellular head and a short unicellular stalk. However, sometimes occur capitate glandular trichomes of type B with bicellular stalk (Fig. 1 E, F). This type of capitate trichomes was only observed on calyx and corolla (Tab. 2).

Presence of glandular trichomes, especially on the flowers of *L. orientale* is noteworthy. Such trichomes on reproductive organs of some Lamiaceae (*e.g.*, *Salvia* L.) have also been reported by Werker *et al.* (1985a, 1985b), but only a small number of Lamiaceae species has been already studied.

Conclusions

Features of glandular trichomes are useful tool for distinguishing species in *Lamium* genus. Presence of capitate type A, capitate type B, and peltate glandular trichomes on reproductive organs of *L. orientale* can be used for further taxonomic investigations in *Lamium*.

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RESEARCH ARTICLE

The features of leaf anatomical structure of some *Rhododendron* species from section *Ponticum*

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Abstract

The article represents a comparative anatomical analysis of leaves of *Rhododendron makinoi*, *R. degronianum*, *R. callimorphum* and *R. brachycarpum*. It was shown that leaves of *R. makinoi* have most pronounced xerophytic features. *R. degronianum* and *R. makinoi* are similar by most of morphometric characteristics, while *R. callimorphum* and *R. brachycarpum* are well distinguishable. However, some quantitative parameters of the leaf with statistically significant difference in *R. makinoi* and *R. degronianum* have been detected and can be used as additional criteria for their taxonomic distinguishing. In general, investigated species differ mostly by type and number of trichomes. The presence of small idioblasts in the studied species was ascertained.

Keywords: *Rhododendron*, *Ponticum*, leaf, anatomy, morphology, idioblasts

Вступ

Рододендрони є надзвичайно цінними декоративними рослинами, що широко використовуються в ландшафтному дизайні. Одними з найбільш цікавих є дослідження таксономічної різноманітності та місцезростань цих рослин. Вивчення представників роду *Rhododendron* L. скероване в першу чергу на вдосконалення агротехнічних підходів до вирощування

цих рослин та дозволяє впроваджувати в інтродукцію нові цінні види та сорти.

Дослідження особливостей будови листка, як одного з найбільш поліфункціональних і пластичних органів рослин дозволяють виявити адаптаційні особливості як окремих видів, так і родів в цілому. Існує чимало наукових напрацювань щодо анатомічних особливостей листків окремих видів рододендронів (Nilsen & Scheckler 2003;

Bondar 2012; Bondar & Zerkal 2013), однак для багатьох представників роду це питання все ще залишається відкритим.

Для нашого дослідження було обрано 4 види роду *Rhododendron* – *R. makinoi* Tagg ex Nakai et Koidz., *R. degronianum* Carrière, *R. callimorphum* Balf. f. & W.W. Sm. та *R. brachycarpum* D. Don ex G. Don., оскільки анатомічна будова листків цих видів в літературі описана недостатньо. Окрім того, існують суперечливі дані в систематиці цих рослин. Зокрема, *R. makinoi* розглядається як самостійний вид (Chamberlain et al. 1996; Walter & Gillett 1998; Goetsch et al. 2005) або підвид *R. yakushmanum* subsp. *makinoi* (Tagg ex Nakai) D.F. Chamb. (Cullen et al. 2011), інколи ж його відносять до складу *R. degronianum* (Krüssmann 1978). Додаткові анатомічні дослідження могли б допомогти з'ясувати дане питання. Отримані дані також можуть бути використані для розробки рекомендацій щодо вирощування цих рослин, а також можуть допомогти у з'ясуванні їх адаптаційного потенціалу.

Матеріали і методи досліджень

Об'єктами дослідження були *R. makinoi*, *R. degronianum*, *R. callimorphum* та *R. brachycarpum* з секції *Ponticum* G. Don. підроду *Hymenanthes* (Blume) K. Koch. *R. makinoi*, *R. degronianum* та *R. brachycarpum* належать до підсекції *Pontica* Sleumer, а *R. callimorphum* – до підсекції *Campylocarpa* Sleumer (Chamberlain et al. 1996). Ці види у природі ростуть у різних умовах. Зокрема, *R. brachycarpum* представлений на кам'янистих ділянках серед змішаних лісів Далекого Сходу Росії, в Кореї та Японії, зустрічається на Курилах (Ітуруп, Кунашир), є рідкісним реліктом Приморського краю. *R. makinoi* представлений в горах на висоті 200–700 м н.р.м. на острові Хонсю, Японія. *R. degronianum* родом з північної частини острова Хонсю (Японія), де ці рослини зростають на висоті біля 1800 м н.р.м. Батьківщиною *R. callimorphum* є Китай (Zarubenko 2006).

Для досліджень використовували листки однорічних сіянців рододендронів, котрі зростають у колекційних експозиціях Ботанічного саду імені О.В. Фоміна. Дослідження проводились на однорічних рослинах, що перезимували за ідентичних умов в теплиці та були висаджені в ґрунт навесні. В червні відбирали по два листки середнього ярусу з п'яти рослин кожного виду (в загальному було проаналізовано по 10 листків для кожного з видів). Для кожного морфометричного параметра було проведено по 10 вимірювань на листок (тобто в загальному було здійснено по 100 вимірювань для кожного з параметрів кожного з видів). Додатково було проаналізовано якісні ознаки листків (в першу чергу трихоми) на дорослих екземплярах.

Для анатомічних досліджень брали середню частину листкової пластинки, фіксували в суміші формалін:етилловий спирт:оцтова кислота, заливали її в желатин (Romeys 1954) та за допомогою заморожуючого мікротому виготовляли поперечні зрізи завтовшки 15–20 мкм, які забарвлювали сафраніном. Додатково листки мацерували для детального вивчення адаксіальної та абаксіальної епідерми. При описі епідерми листкової пластинки використовували методики Zakharevich (1954) і Baranova (1985). Вимірювання проводили за допомогою програми ImageJ та окуляр-мікромметра на мікроскопі XSP-146TR.

Статистичну обробку даних проводили за допомогою програми Statistica 8.0, достовірність результатів визначали за *t*-критерієм Стьюдента. Фотографії виготовляли за допомогою цифрової камери Canon Power Shot A630.

Результати та їх обговорення

Однорічні листки досліджених видів в цілому мають подібну анатомічну будову: дорзовентральні, гіпостоматичні, вкриті одношаровою епідермою, продиховий апарат аномоцитного типу оточений переважно

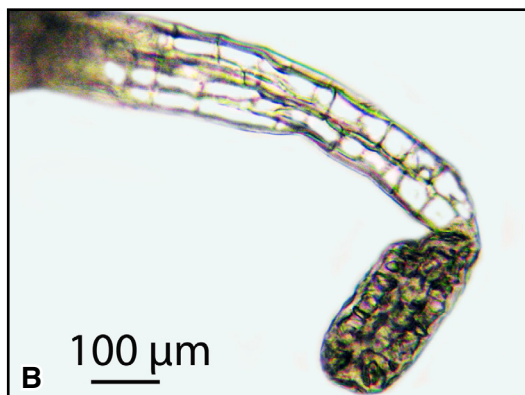
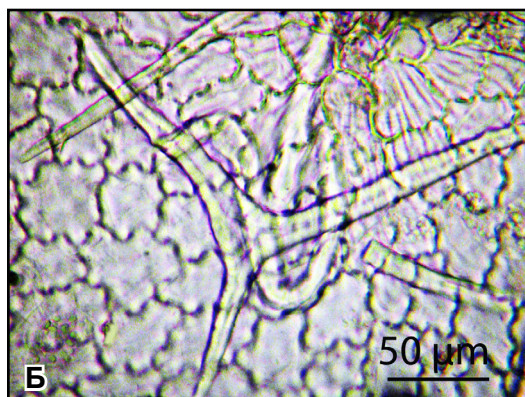
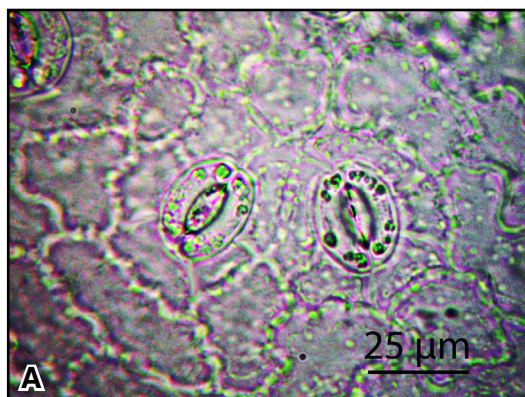


Рис. 1. Епідерма листка: **А** – продихи абаксiальної епiдерми *Rhododendron degronianum*; **Б** – незалозиста трихома *R. callimorphum*; **В** – залозиста трихома *R. callimorphum*.

Fig. 1. Leaf epidermis: **A** – stomata of the abaxial epidermis of *Rhododendron degronianum*; **Б** – non-glandular trichome of *R. callimorphum*; **В** – glandular trichome of *R. callimorphum*.

п'ятьма клітинами (Рис. 1 А). Продихи дещо припідняті над поверхнею епiдерми. Обриси епiдермальних клітин абаксiальної сторони звивисті та звивисто-хвилясті, з адаксiальної сторони – хвилясті. Проекція площі епiдермальних клітин розпластана.

За типом трихом та їх розміщенням дані види iстотно відрізняються. Листкова пластинка *R. takinoi* з абаксiального боку густо вкрита довгими одноклітинними та розгалуженими незалозистими трихомами (Рис. 2 А, Б), тоді як з адаксiального боку лише на крупних жилках можна побачити одноклітинні прості трихоми. Листки *R. degronianum* мають поодинокі одноклітинні короткі трихоми з абаксiальної сторони та окремі трихоми вздовж крупних жилок (Рис. 2 В). Листки *R. callimorphum* вкриті довгими розгалуженими трихомами з обох боків (Рис. 1 Б). На листках *R. callimorphum* присутні залозисті

трихоми з багатоклітинною ніжкою та багатоклітинною голівкою з адаксiального боку та по краю листкової пластинки, в меншій кількості – з абаксiального боку (Рис. 1 В; Рис. 2 Г). Такі залозисті трихоми характерні лише для даного виду з-посеред досліджених. За літературними даними, такі залозисті та розгалужені незалозисті трихоми характерні видам саме підроду *Hymenanthes* (Нуам 2010). Листки *R. brachycarpum* вкриті довгими нитчастими одноклітинними трихомами лише вздовж центральної жилки з обох боків листка та по краю листкової пластинки (Рис. 2 Д).

Варто відмітити, що у дорослих рослин щільність трихом дещо збільшується відносно однорічних екземплярів. Найбільші відмінності виявлені у *R. degronianum*, в дорослому віці їх листки мають поодинокі довгі трихоми з адаксiального боку та щільний покрив з довгими розгалуженими та

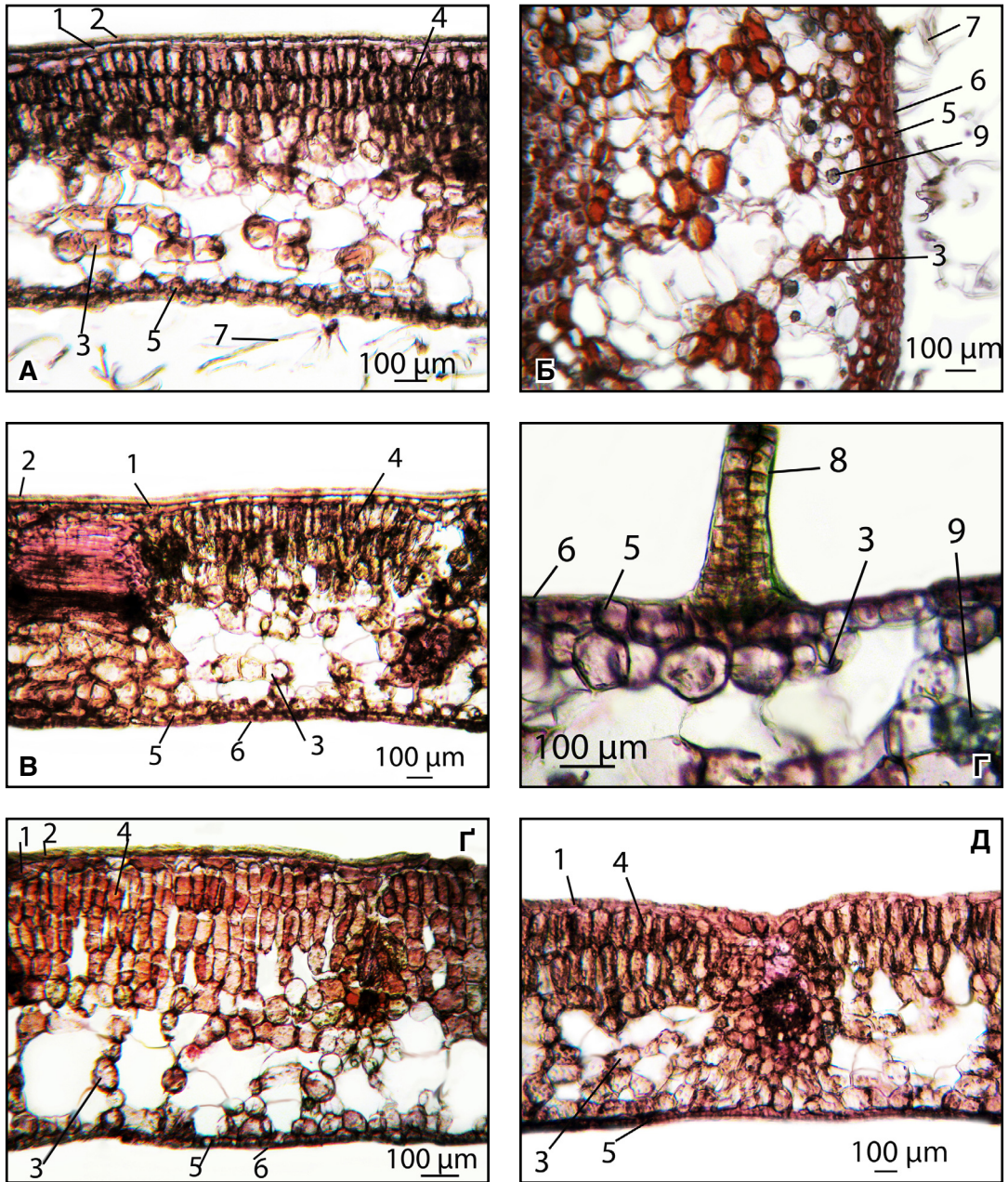


Рис. 2. Поперечний переріз листової пластинки: **А** – *Rhododendron makinoi*; **Б** – в районі центральної жилки *R. makinoi*; **В** – *R. degronianum*, **Г, Г** – *R. callimorphum*; **Д** – *R. brachycarpum*. 1 – адаксіальна епідерма; 2 – кутикула адаксіальної епідерми; 3 – губчастий мезофіл з клітинами-ідіобластами; 4 – стовпчастий мезофіл; 5 – абаксіальна епідерма; 6 – кутикула абаксіальної епідерми; 7 – проста трихома; 8 – залозиста трихома; 9 – включення оксалату кальцію.

Fig. 2. Cross-section through the leaf blade: **A** – *Rhododendron makinoi*; **Б** – в районі центральної жилки *R. makinoi*; **В** – *R. degronianum*, **Г, Г** – *R. callimorphum*; **Д** – *R. brachycarpum*. 1 – adaxial epidermis; 2 – cuticle of adaxial epidermis; 3 – idioblasts in spongy mesophyll; 4 – palisade mesophyll; 5 – abaxial epidermis; 6 – cuticle of abaxial epidermis; 7 – simple trichome; 8 – glandular trichome; 9 – inclusion of calcium oxalate.

нерозгалуженими трихомами з абаксіального боку. У дорослих екземплярів *R. makinoi* опушення листків з абаксіального боку теж значно збільшується і є найгустішим серед розглянутих видів. Такі зміни вказують на подібність цих видів. Інші ж якісні ознаки епідерми листків дорослих особин залишаються подібними до таких у однорічних рослин.

У всіх досліджених видів стовпчаста паренхіма складається з 2–3 шарів витягнутих клітин (Рис. 2 А, В, Г, Д). У *R. makinoi* в області центральної жилки у листків однорічних рослин з'являється один шар гіподерми з потовщеними стінками. Губчаста паренхіма складається з тонкостінних великих клітин та розміщених тяжами більш дрібних ідіобластів з пористими стінками, в області центрального провідного пучка такі ідіобласти містять більше вклучень (Рис. 2 Б).

Слід відмітити, що Nilsen & Scheckler (2003) виявили особливі гігантські ідіобласти присутні здебільшого у адаксіальній частині листової пластинки у представників секції *Vireya* (Blume) H.F. Copel. Однак звичні ідіобласти широко поширені серед решти представників роду. Зокрема, за нашими даними, всі досліджені види секції *Ponticum* мають дрібні ідіобласти, які розміщені групами-тяжами в губчастій паренхімі. Дослідження Nilsen & Scheckler (2003) спростували функціональну роль ідіобластів в регулюванні проникнення світла та механізмів скручування листка для представників секції *Vireya*, оскільки ідіобласти в цих видів розміщені переважно з адаксіальної сторони листка, тоді як листок закручується донизу (Nilsen 1991, 1992). Така фізіологічна особливість листків позитивно корелює з холодостійкістю цих рослин (Nilsen & Tolbert 1993). Також, ідіобластам деяких рододендронів властива секреторна функція (Nilsen & Scheckler 2003). Ми схилиємося до думки Orcutt & Nilsen (2000), згідно якої основною роллю ідіобластів все ж таки є запасання полімеризованих сполук з метою формування оборонного механізму від травоядних тварин. Наявність ідіобластів

саме на абаксіальному боці листків (хоча і не в епідермі) у рослин секції *Ponticum* не дозволяє повністю відкидати можливість значення ідіобластів у скручуванні цих листків.

У *R. makinoi* та *R. callimorphum* в губчастій паренхімі присутні друзи оксалату кальцію у більшій кількості, порівняно з іншими двома видами (Рис. 2 Б, Г). Кристали в друзах розміщені переважно радіально. З літературних джерел відомо, що накопичення кристалів кальцію є адаптивною відповіддю на втрату води шляхом регулювання внутрішньоклітинного рН (Ayala-Cordero et al. 2006). Також накопичення оксалату кальцію в тканині поруч з продирами та в ксилемі сприяє закриттю продихів протягом дня і, відповідно, зниженню транспірації (Ruiz & Mansfield 1994; Monje & Baran 2002). Більша кількість оксалатів, на нашу думку, є задатком більш вищої посухостійкості для *R. makinoi* та *R. callimorphum*.

Для кращого розуміння здатності рододендронів адаптуватися до різних кліматичних умов додатково було проведено ряд морфометричних вимірювань (Табл. 1). Як видно, за багатьма морфометричними ознаками представники *R. degronianum* та *R. makinoi* (природний ареал обидвох видів – острів Хонсю в Японії) достовірно не відрізняються між собою, тоді як *R. callimorphum* та *R. brachycarpum* відрізняються за більшістю досліджених ознак. Разом з цим, однорічні листки *R. degronianum* мають товщу листову пластинку за рахунок потовщення губчастої паренхіми та значно більшу площу адаксіальних епідермоцитів порівняно з листками *R. makinoi*. Тоді як останні вужчі та значно щільніше вкриті трихомами.

Найбільша кількість продихів поряд з великими їх розмірами спостерігається у *R. degronianum*, що вказує на кращу транспірацію, а отже охолодження листової пластинки, тоді як у *R. callimorphum* продихів найменше, а у *R. brachycarpum* їх розміри найменші водночас з малою кількістю. Такі показники поряд з

Табл. 1. Морфометричні параметри листка деяких видів роду *Rhododendron* (M ± m).Table 1. Morphometric parameters of leaf of some *Rhododendron* species (M ± m).

Параметр	<i>R. brachycarpum</i>	<i>R. callimorphum</i>	<i>R. degronianum</i>	<i>R. makinoi</i>
Довжина продохів, мкм	23,6 ± 1,7 ^{**#}	26,5 ± 2	25,4 ± 2	25,9 ± 2,3
Ширина продохів, мкм	20,6 ± 1,8 ^{**#}	26,7 ± 2 ^{*^}	21,7 ± 1,9	21,9 ± 1,9
Кількість продохів шт./мм ²	44,9 ± 9,3 [#]	23,5 ± 5,9 ^{*^}	53,7 ± 6,2	49,3 ± 7,7
Площа адакс. епідермоцитів, мкм ²	1142 ± 275 ^{**#}	1375 ± 220 ^{*^}	971 ± 181 [*]	598 ± 109
Площа абакс. епідермоцитів, мкм ²	727 ± 182 ^{**#}	619 ± 136	649 ± 248	534 ± 211
Товщина адакс. епідерми, мкм	82 ± 12,6	86 ± 16,1	85 ± 14,2	81 ± 12,1
Товщина зовнішньої клітинної стінки адакс. епідерми, мкм	27 ± 6,3 ^{**#}	36 ± 12,5 ^{*^}	46 ± 8,7	44 ± 6,9
Товщина стовпчастої паренхіми, мкм	555 ± 99	577 ± 123,3 [*]	525 ± 82	480 ± 107
Товщина губчастої паренхіми, мкм	713 ± 154 [^]	703 ± 112 [^]	848 ± 165 [*]	725 ± 87
Товщина абакс. епідерми, мкм	80 ± 12,2 ^{*^}	75 ± 12,2 ^{*^}	64 ± 8,5	59 ± 9,1
Товщина зовнішньої клітинної стінки абакс. епідерми, мкм	25 ± 5,4 ^{**#}	21 ± 4,8	21 ± 7,8	18 ± 4,2
Товщина листка, мкм	1453 ± 159 [^]	1508 ± 152 [*]	1573 ± 158 [*]	1345 ± 141

* – P < 0,05 порівняно з *R. makinoi*; ^ – порівняно з *R. degronianum*; # – порівняно з *R. callimorphum*.

* – P < 0,05 in comparison with *R. makinoi*; ^ – in comparison with *R. degronianum*; # – in comparison with *R. callimorphum*.

відносно тонкою зовнішньою клітинною стінкою епідерми та кутикулою, ймовірно зумовлюють більше перегрівання листкової пластинки в останніх двох видів, тобто обумовлюють їх низьку спекостійкість. Разом з цим, велика кількість вклячень оксалата кальцію та мала кількість продохів у *R. callimorphum* є ознаками посухостійкості даного виду. Порівняно тонка абаксальна епідерма у *R. makinoi* компенсується дуже густим опушенням довгими трихомами, що також значно зменшує негативний вплив температури чи підвищеної інсоляції. Ознаками більшої ксерофітності *R. degronianum* та *R. makinoi* є дрібніші епідермальні клітини, особливо з адаксальної сторони, та найтонша листкова пластинка у *R. makinoi*.

Nilsen & Tolbert (1993) показали, що *R. brachycarpum* (природний ареал Далекий Схід Росії, Курили, Приморський край) найбільш морозостійкий вид, що витримує морози до -27°C . *R. makinoi* та *R. degronianum* децю менш морозостійкі і витримують морози до -24°C . Можливо посилену морозостійкість у *R. brachycarpum* зумовлює потовщення абаксальної епідерми поряд з малими розмірами продохів, що в свою чергу знижує інтенсивність дихання та, відповідно, транспірації, а отже, висихання при низьких температурах.

Висновки

Таким чином, найбільше виражені ксерофітні ознаки виявлені у представників *R. makinoi*.

За багатьма морфометричними ознаками види, природним ареалом яких є острів Хонсю (*R. degronianum* та *R. makinoi*), достовірно не відрізняються між собою, що може свідчити про спрямовані в одному напрямку адаптивні механізми даних рослин, хоча вони й зростають на різній висоті над рівнем моря. Існування достовірної відмінності серед таких показників, як товщина листкової пластинки, товщина губчатої паренхіми, площа адаксіальних епідермоцитів та кількість трихом з абаксіального боку може бути додатковим таксономічним критерієм для розмежування *R. makinoi* та *R. degronianum*, хоча, ймовірно, не є достатньою підставою для виділення *R. makinoi* як самостійного виду. Для з'ясування цього питання необхідні додаткові дослідження.

Найбільш суттєво досліджені види відрізняються за типом та кількістю трихом. Характерною ознакою *R. callimorphum* є багатоклітинні залозисті трихоми.

Виявлено, що досліджені види секції *Ponticum* мають ідіобласти в губчатій паренхімі листків.

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CORRESPONDENCE

***Pyrus demetrii* (Rosaceae), a new record for Turkey, with observations on micromorphology of leaves**

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Abstract

The Caucasian species, *Pyrus demetrii* Kuth. has been reported from Turkey for the first time. The species was collected by the authors from both Georgia and Turkey. Leaf epidermis characters were studied by scanning electron microscopy to evaluate cuticle ornamentation, waxes and other relevant features of micromorphology.

Keywords: *Pyrus demetrii*, new record, epidermis micromorphology, Georgia, Turkey

Introduction

Rosaceae is widespread over the world but has diversified predominantly in the Northern Hemisphere; it includes approximately 3000 species in 100 genera (Kalkman 2004). *Pyrus* L. is the genus of woody trees with number of species vary from 41 to 73 (Robertson *et al.* 1991; Browicz 1993). For a long time it was treated under the subfamily Maloideae C. Weber (synonym of Malaceae Small), however according to further phylogenetic investigations it was nested under the subfamily Spiraeoideae C. Agardh, supertribe Pyrodae C.S. Campbell, R.C. Evans, D.R. Morgan &

T.A. Dickinson, tribe Pyreae Baill. subtribe Pyrinae Dumort. (Campbell *et al.* 2007; Potter *et al.* 2007). Nevertheless, later nomenclatural changes resulted in priority of Maloideae over Spiraeoideae and Malinae Reveal over Pyrinae. Since, it was proposed to consider *Pyrus* under subfamily Maloideae, tribe Maleae Small, subtribe Malinae (Reveal 2012a; 2012b).

Pears are native only for Europe, Asia and some mountainous regions in North Africa (Browicz 1993), and Caucasus is one of remarkable diversity centers for this genus (Rubtsov 1944). Turkey is an important region linking Europe and Caucasus, and several new taxa of *Pyrus* have been recently recorded for the

country (Uğurlu Aydın & Dönmez 2015). In this paper, *P. demetrii* Kuth. is reported as new species for the Turkish flora previously known from the Caucasian region.

According to Stace (1965), cuticular ornamentation can be used as important source of knowledge for taxonomic research. Despite the importance for taxonomy, only few studies (Ganeva 2009; Ganeva & Uzunova 2010; Zamani et al. 2015) are focused on epidermal structure of Malaceae and its taxonomic implementation. Therefore, data on leaf epidermal structure are presented here to evaluate the taxonomic relevance of micromorphological characters.

Material and methods

The type specimen and the other specimens of *P. demetrii* were examined at TBI (Herbarium of Georgian Academy of Science, Tbilisi, Georgia) and LE (V.L. Komarov Botanical Institute, Saint Petersburg, Russia). All herbarium acronyms are indicated in text according to Thiers (2017). Additional specimens of *P. demetrii* were collected by authors from Sagaredzho, Georgia, during field trips in 2012. For SEM studies, leaves of taxa were washed with 70% alcohol and coated with a gold-palladium mixture. SEM photographs were taken with a Zeiss EVO 50 EP electron microscope.

Results and discussion

Pyrus demetrii Kuth., *Zametki Sist. Geogr. Rast.* 13: 25. 1947. (Fig. 1)
= *Pyrus georgica* Kuth. var. *glabra* Kuth., *Zametki Sist. Geogr. Rast.* 8: 16. 1939.

Lectotype: GEORGIA. Gare-Kakhethia. 25.10.1938, S. Kuthatheladze s.n. TBI 1025828!, (Fig. 1 A). **Isolectotype:** LE! (lectotype selected by Uğurlu Aydın & Dönmez (2016) in Kew Bull. 71 (3): 37).

Description. Tree up to 8 m, crown globose, stem bark grey; young branches glabrous, spiny. Leaves (3–) 4–6 × 1.5–2 cm, lanceolate to oblanceolate, acute and mucronate at apex,

cuneate at base, margin serrate or slightly serrate, ± undulate, bilaterally tomentose in both side at flowering stage, finally sparsely pubescent below, glabrous above, with ciliate margins. Petioles 1.5–2 cm long, glabrous, ± thick. Stipules 9–10 × 2 mm, linear-lanceolate, deciduous. Corymb of 4–10 flowers. Bracts (5–) 8–10 × 0.5–1 mm, subulate, pubescent, brownish, orange. Pedicels 0.5 (–1) cm long, ± thick. Hypanthium concave, cupuliform, densely tomentose outside, glabrous inside. Sepals 3–5 × 1–2 mm, triangular, acute, reflexed, tomentose outside, whitish pubescent inside, persistent in fruiting stage. Petals 10–12 × 6–8 mm, white, from oblong-ovate to broadly ovate, apex rounded or rarely emarginate, with short claw. Stamens in two rows, 15–20 (–25), unequal, anthers pink before opening. Styles 3–5, minutely pubescent at base. Fruits 1.2–1.5 × 1.5–2 cm, yellow, greenish-yellow, globose, flattened globose or globose-pyriform, with lenticels, slightly juicy. Seeds 3–6 × 2–4 mm, ovate, apex acute, pale brown or dark brown.

Flowering and fruiting. From April – May till August – September.

Distribution. Middle part of Turkey, Georgia, Armenia.

Ecology. Grows on dry open hills, seldom on forest edges and in shrubs of lower mountain belts at altitudes of 800–1300 m a.s.l. Mostly individually, rarely – in groups.

Specimens examined. TURKEY: Sivas, 3.3 km from Sincan to Zara, steppe, among deciduous scrub, 39°29'31" N, 037°55'07" E, 1275 m a.s.l., 2.10.2012, AAD 19237 (HUB!) (Fig. 1 B).

Additional specimens examined. GEORGIA: Sagaredzho, Gare-Kakhethia, Khasmi village, Davidgzejii, 41°45'22" N, 045°13'06" E, 845 m a.s.l., 9.8.2012, ZUG 373 – A.A. Dönmez & N. Lachashrili (HUB!) (Fig. 1 C). ARMENIA: Daralaghez, 6.9.1936, Pojarkova (LE!); Vayots Dzor distr., 21.9.2007, Tamamyanyan K., Fayvush G. s.n. (ERE!).

P. demetrii was collected by both authors from the type locality in Sagaredzho Georgia (Fig. 1 C) where it is widespread on steppe slopes and in open forest areas. It is also widely distributed in similar habitat conditions in the inner part of Turkey, so it is not a surprise to find specimens of *P. demetrii* there. The new record is morphologically related to *P. georgica*, which is also known from Georgia. Both these species



Fig. 1. *Pyrus demetrii*: **A** – lectotype; **B** – fruits of AAD 19237; **C** – leaves of ZUG 373.

are similar in leaf morphology, but *P. demetrii* differs from *P. georgica* in having glabrous leaves and much shorter pedicels. The newly recorded species is also well differentiated among Turkish taxa of *Pyrus* by its glabrous serrate leaves and shorter pedicels.

The cuticle of the upper epidermis generally represents reticulate ornamentation under SEM. It has weak stria covered with epicuticular waxes. The lower epidermis is smooth with granular dispersed waxes. Fine parallel striations are observed radially to the stomata (Fig. 2). The stomata are densely distributed only in lower epidermis. Stomata are generally absent on the upper surface of leaf in most of *Pyrus* species including *P. demetrii* (Fig. 2 A), and they are known only for few representatives of the genus (Zamani *et al.* 2015).

Regarding micromorphological features, leaves of *P. demetrii* have some common characters with taxa of the related genera such

as *Malus* Mill. and *Cydonia* Mill. The stomata are navicular in shape and have thicker stomatal rims for all mentioned genera (Ganeva 2009; Ganeva & Uzunova 2010). However, species of *Malus* and *Cydonia* are distinct in having dense and thicker stria on epidermal surface.

Granular waxes are often observed in most *Pyrus* taxa (Zamani *et al.* 2015), while reticulated waxes were observed only in subject of this study. Epicuticular waxes show great micromorphological diversity and are mostly correlated with ecological factors (Stace 1965).

In lower leaf epidermis *P. demetrii* has roughly striate cuticle ornamentation around the stomata. Zamani *et al.* (2015) observed this feature in xerophytic *Pyrus* species growing in dry areas. *P. demetrii* also prefers such kind of dry conditions both in Turkey and Georgia. As a result, *P. demetrii* has enough features to be distinctive from the most related genera, and, in the same time, it shows similar features

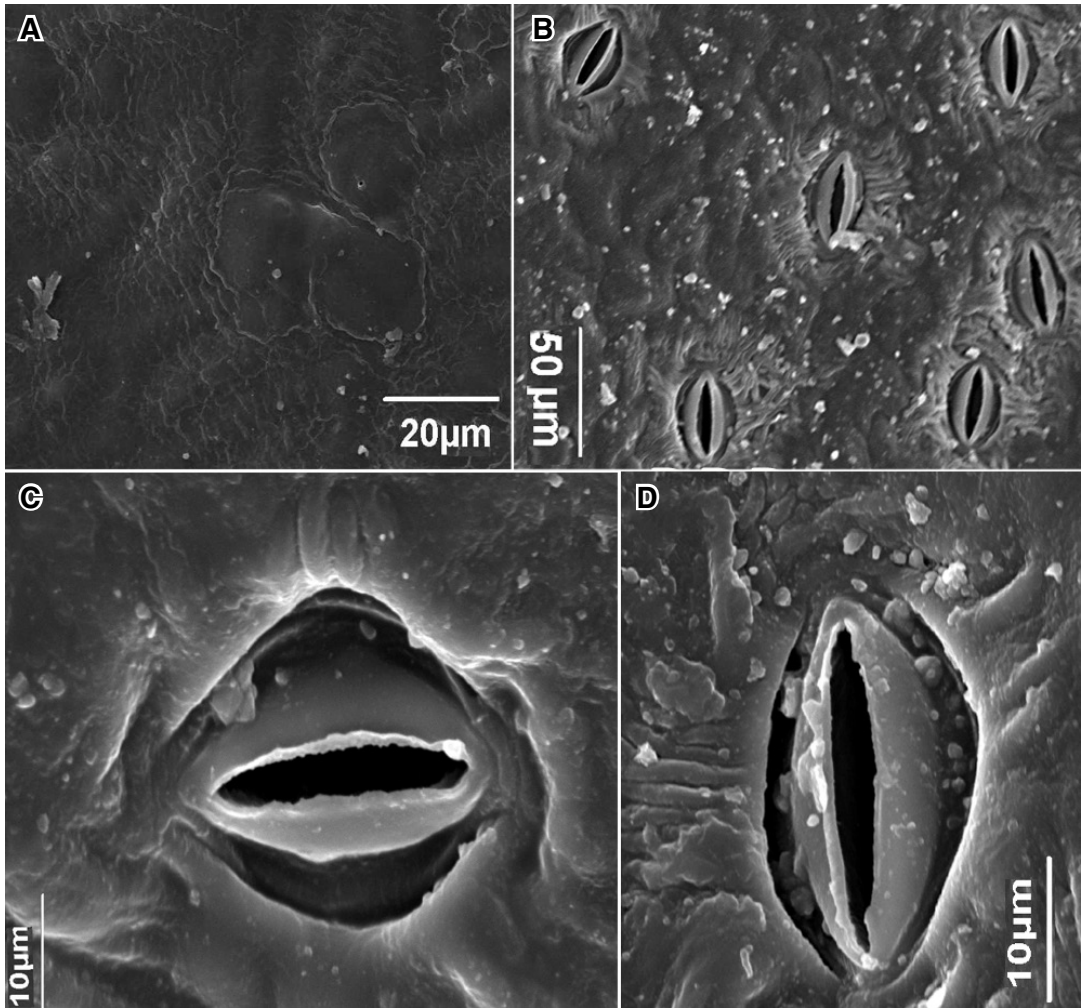


Fig. 2. Leaf micromorphology of *Pyrus demetirii* (A–C – ZUG 373; D – Tamamyan K., Fayvush G. s.n.): A – upper epidermis; B – lower epidermis showing stomata; C–D – stomata.

with xerophytic taxa of the genus. It seems that micromorphological characters support intergeneric classification of *Pyrus*. On the other side, these characters show variations and often reflect ecological conditions rather than taxonomical relationships among *Pyrus* species.

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CORRESPONDENCE

Morphological and anatomical structure of leaves of *Artemisia abrotanum* (Asteraceae) introduced in Zhytomyr Polissya

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Abstract

The upper and the lower epidermises of *Artemisia abrotanum* introduced in Zhytomyr Polissya are covered with cuticle. The leaf blades are amphistomatic, with oval anomocytic stomata. On adaxial and abaxial epidermal surfaces present indumentary trichomes of various types. In particular, there are located oval multi-cellular glandular trichomes, which are typical for Asteraceae. Secretory tissues of internal secretion are represented by essential oil containers. Leaf blade of *A. abrotanum* has isobilateral structure with collateral vascular bundles. *A. abrotanum* reveals certain xeromorphic traits: small and compact leaves, presence of covering trichomes, more developed palisade parenchyma in comparison with spongy one, and thick walls of epidermal cells. Investigated secretory structures and non-glandular trichomes are considered taxonomical characters and can be applied for identification of pharmaceutical raw material of *A. abrotanum*.

Keywords: *Artemisia abrotanum*, introduction, leaf blade, stomatal complex, trichomes, secretory structures

Вступ

Artemisia abrotanum L. (полин лікарський) належить до секції *Abrotanum* (Neck.) Besser. підроду *Artemisia* роду *Artemisia* L. родини Asteraceae Bercht. et J. Presl (Minarchenko 2005; Boiko 2011). Серед синонімів цього

виду – *A. elatior* Klokov та *A. procera* Willd. (Ostapko et al. 2010). Згідно з Greuter (2017), до гетеротипних синонімів *A. abrotanum* також належать *A. herbacea* Willd., *A. paniculata* Lam. та *A. proceriformis* Krasch.

Полин лікарський належить до євразійської групи ареалів південно-східно-

європейсько-кавказько-ірано-туранського типу (Boiko 2011) і розповсюджений по всій території України (Minarchenko 2005). Це напівкущ із стрижневою кореневою системою (Boiko 2002). Стебла підведені, 60–150 см заввишки, в нижній частині з бурою корою, в верхній ділянці вкриті восковою поволокою, голі, розгалужені. Стеблові листки черешкові, без вушок при основі, двічіпірчаторозсічені з вузьколінійними загостреними кінцевими частками; зверху сизуваті, голі, зісподу сіруваті від дрібного опушення. Верхівкові листки трійчасті або суцільні, вузьколінійні. Спільне суцвіття волотисте, рідкувате, густо уліснене; кошики майже кулясті, дрібні, більш-менш пониклі; обгортка коротковолосиста, зовнішні листочки її довгасто-ланцетні, загострені, з півчастою облямівкою, внутрішні – оберненояйцевидні, тупі, майже цілком півчасті, білуваті, квітки жовті. Паїд – сім'янка (Visjulina 1962).

Будова листків багатьох видів роду *Artemisia* детально досліджена в працях Hayat et al. (2010) та Noorbakhsh et al. (2008). Проте відомості щодо *A. abrotanum* є фрагментарними. Відомо, що клітини верхнього і нижнього епідермісу листків цього виду характеризуються звивистими обрисами, аномоцитні продихи присутні на абаксіальній та адаксіальній поверхнях, наявні неспецифічні паренхімні клітини з ефірною олією (Soyunova 2012) та залозисті трихоми (Suresh et al. 2007).

A. abrotanum – це перспективна для фармацевтичної промисловості та медицини фітонцидно-лікарська ефіроолійна культура, що містить різноманітні біологічно активні сполуки: ефірну олію, фенолкарбоніві сполуки та їх похідні, а також флавоноїди і кумарини (Kowalski et al. 2007; Suresh et al. 2012; Ivashchenko et al. 2015). Рослина виявляє спазмолітичну, глістогінну, діуретичну, гемостатичну, потогінну, антифунгіцидну, бактерицидну, ранозагоювальну, а також знеболювальну та протизапальну дії (Cubukcu et al. 1990; Kowalski et al. 2007; Kovaliova et al. 2011; Suresh et al. 2012; Ivashchenko et al. 2014; Ivashchenko 2015). Здавна в народній

медицині *A. abrotanum* застосовували як засіб, що стимулює діяльність травних органів, при судамах, тахікардії, риніті, пухлинах шлунка й печінки; зовнішньо – при маститі, опіках, обмороженні та фурункулах (Sokolov 1993; Remberg et al. 2004; Minarchenko 2005). Результати досліджень також свідчать про ефективність застосування *A. abrotanum* проти збудника малярії (Cubukcu et al. 1990).

В зоні Житомирського Полісся *A. abrotanum* не культивують, тому інтродукційне вивчення цієї культури, в тому числі морфолого-анатомічних особливостей, з метою подальшого використання в фармацевтичній промисловості, медицині та парфумерії є актуальним. Окрім того, дослідження анатомічної будови листків мають важливе значення для виявлення адаптаційних можливостей виду у різних екологічних умовах, при визначенні та ідентифікації лікарської рослинної сировини, уточненні питань систематики, вивченні філогенетичних зв'язків тощо (Hayat et al. 2009). Тому метою нашої роботи було дослідити морфолого-анатомічну будову листків *A. abrotanum* за умов інтродукції в Житомирському Поліссі.

Матеріали і методи досліджень

Об'єктом дослідження були листки *A. abrotanum*, інтродукованого в умовах ботанічного саду Житомирського національного агроекологічного університету (Рис. 1). Вихідний матеріал полину лікарського отримали із Національного ботанічного саду ім. М.М. Гришка НАН України. Збір матеріалу та його обробку проводили у період цвітіння. Дослідження анатомічної будови листків інтродуцента здійснювали в Інституті ботаніки ім. М.Г. Холодного НАН України. Фіксацію зразків проводили сумішшю 2,5% глутарового альдегіду на кокодилатному буфері рН 7,2 з 1% розчином параформальдегіду на воді. Зневоднення проводили у серії спиртів зростаючої

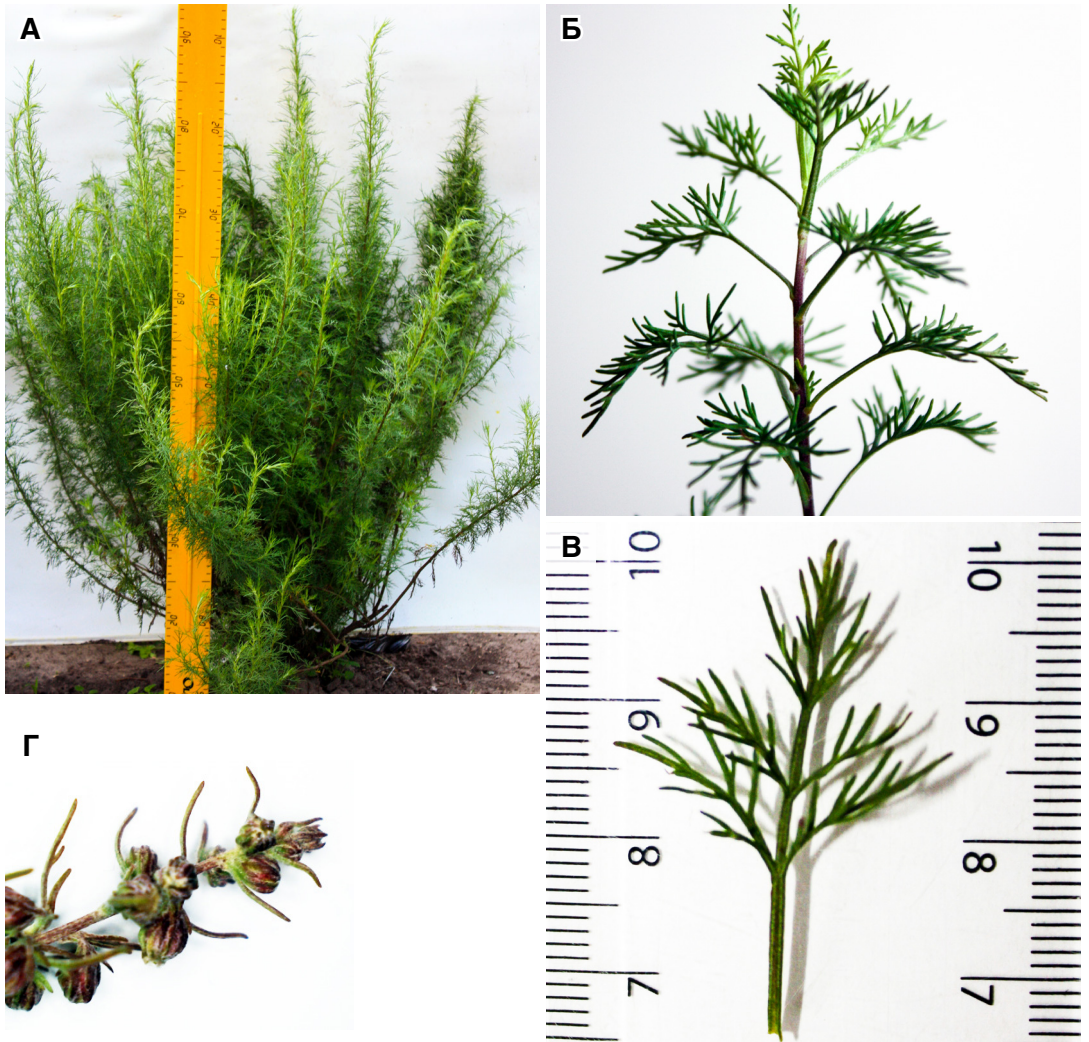


Рис. 1. *Artemisia abrotanum*: **А** – загальний вигляд рослини; **Б** – верхня частина пагону (вегетативна фаза); **В** – листок серединної формації; **Г** – верхня частина генеративного пагону (фаза бутонізації, $\times 10$).

Fig. 1. *Artemisia abrotanum*: **A** – general view of plant; **B** – shoot apex (vegetative stage); **B** – middle leaf; **G** – apex of generative shoot (budding stage, $\times 10$).

концентрації і в ацетоні. Заливку у суміш епоксидних смол (епон-аралдит) проводили за загальноприйнятою методикою (Gayer 1974; Carde 1987). Для світлової мікроскопії виготовляли зрізи завтовшки 1,5 мкм на ультрамікротомі RMC MT-XL (США), які зафарбовували 0,25 % толуїдиновим синім. Зразки вивчали під мікроскопом Axioskop-40 (об'єктиви фірми Zeiss, Germany). Анатомо-морфологічні дослідження проводили із

застосуванням стандартної мікроскопічної техніки (Barykina *et al.* 2004).

Фотофіксацію результатів здійснювали за допомогою цифрової фотокамери Canon DC 8.1 V та цифрової фотонасадки Levenhuk D50L NG. Описи проводили з використанням загальноприйнятої термінології (Esau 1980; Roshchina & Roshchin 1989; Soropudov *et al.* 2013; Novikoff & Barabasz-Krasny 2015).

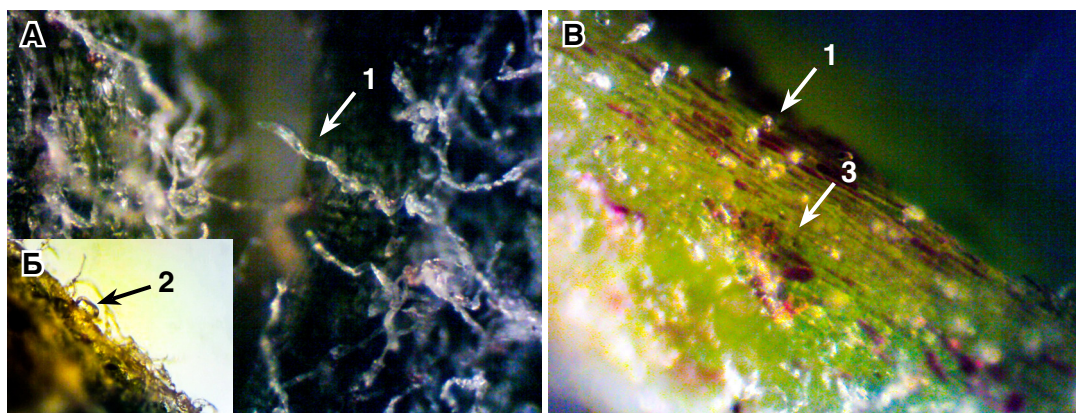


Рис. 2. Фрагменти абаксiальної поверхнi листкової пластинки (А, Б) та черешка (В) *Artemisia abrotanum*: 1 – криючі трихоми; 2 – залозиста трихома; 3 – ендогенне ефиролiйне вмістище (×160).

Fig. 2. Fragments of abaxial surface of the leaf blade (А, Б) and petiole (В) of *Artemisia abrotanum*: 1 – covering trichomes; 2 – glandular trichome; 3 – endogenic essential oil container (×160).

Результати та їх обговорення

Стеблові листки *A. abrotanum* низової та серединної формацій черешкові, двічі пірчато-розсічені з лінійчатими сегментами, за краєм цільні; верхівкої – трійчато-розсічені або цільнокраї; зелені, з абаксiальної сторони сіруваті від дрібного опушення (Рис. 1 Б–Г; Рис. 2 А–Б).

Епідермальні клітини листкової пластинки характеризуються звивистими обрисами та витягнутими або розпластаними проєкціями. Зовнішні стінки клітин верхнього епідермісу потовщені і вкриті добре розвиненою кутикулою, нижній епідерміс має слабо виражену кутикулу (Рис. 3 Б–Г).

Листкова пластинка амфістоматична. Продихи овальні, аномоцитного типу, розміщені на адаксiальній та абаксiальній поверхнях листкової пластинки, проте їх більше на нижній поверхні листка. Між клітинами епідермісу виразно видно замикаючі клітини продихів овальної форми з хлоропластами, оточені побічними клітинами, які не відрізняються від основних епідермальних клітин. Hayat *et al.* (2010) описали 6 основних типів продихів для 24 представників роду *Artemisia*. Зокрема, аномоцитні продихи автори навели для

A. amygdalina Decne., *A. dubia* Wall. ex Besser, *A. moorcroftiana* Wall. ex DC., *A. roxburghiana* Wall. ex Besser, *A. rutifolia* Spreng., *A. tournefortiana* Rchb., *A. absinthium* L., *A. siversiana* Ehrh., *A. tangutica* Pampanini, *A. scoparia* Waldst. et Kit.

Різномітні криючі трихоми нами відмічено з обох боків листкової пластинки *A. abrotanum*: закручені, Т-подібні, циліндричні, головчасті та прості конічні волоски. Згідно Hayat *et al.* (2009), у представників роду *Artemisia* виявлено 16 типів залозистих та незалозистих трихом. Зокрема, Т-подібні трихоми характерні також для *A. vulgaris* L., *A. splendens* Wild., *A. austriaca* Jacq., *A. haussknechtii* Boiss., *A. persica* Boiss., *A. absinthium*, *A. incana* (L.) Druce, *A. armeniaca* Sam., *A. tschernieviana* Besser, *A. spicigera* C. Koch. та *A. khorassanica* Podl. (Noorbakhsh *et al.* 2008).

Відомо, що трихоми відіграють важливу роль у підтриманні водного балансу і в регулюванні температури листка, також вони виконують захисну роль, сприяють запиленню, впливають на фотосинтез тощо (Wagner 1991).

Для *A. abrotanum* характерна ізобілатеральна структура листкової пластинки (Рис. 3). Наявність палисадної

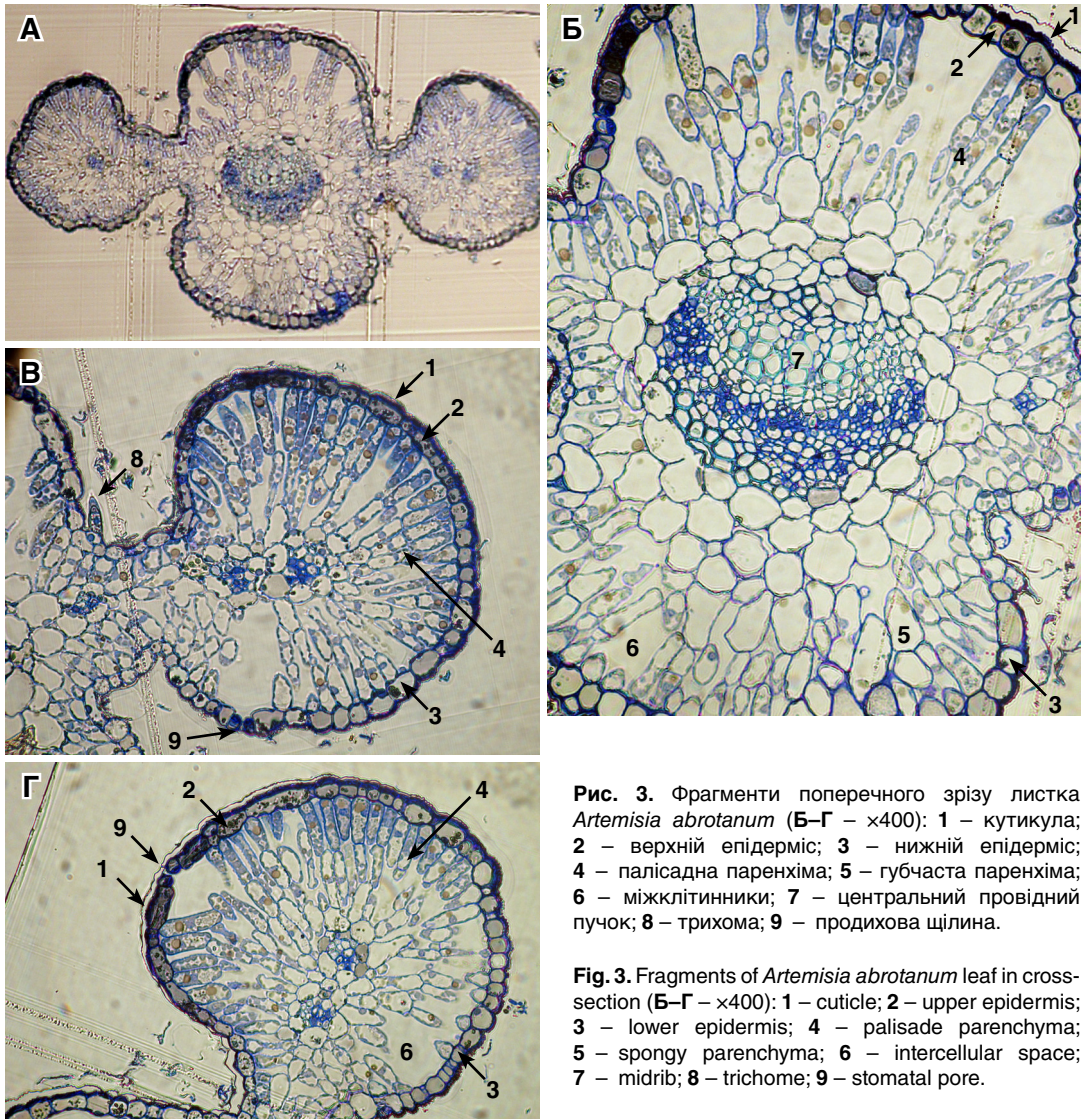


Рис. 3. Фрагменти поперечного зрізу листка *Artemisia abrotanum* (Б–Г – $\times 400$): 1 – кутикула; 2 – верхній епідерміс; 3 – нижній епідерміс; 4 – палісадна паренхіма; 5 – губчаста паренхіма; 6 – міжклітинники; 7 – центральний провідний пучок; 8 – трихома; 9 – продихова щілина.

Fig. 3. Fragments of *Artemisia abrotanum* leaf in cross-section (Б–Г – $\times 400$): 1 – cuticle; 2 – upper epidermis; 3 – lower epidermis; 4 – palisade parenchyma; 5 – spongy parenchyma; 6 – intercellular space; 7 – midrib; 8 – trichome; 9 – stomatal pore.

паренхіми з обох боків листової пластинки – ксероморфна ознака (Esau 1980). Згідно з Noorbakhsh *et al.* (2008), ізобілатеральна структура листової пластинки притаманна багатьом видам роду *Artemisia*: *A. chamaemelifolia* Vill., *A. biennis* Willd., *A. annua* L., *A. splendens*, *A. austriaca*, *A. haussknechtii*, *A. persica*, *A. absinthium*, *A. incana*, *A. armeniaca*, *A. tschernieviana*, *A. scoparia*, *A. marschalina* Sprengel, *A. deserti* Ktasc., *A. santolina* Schrenk., *A. turanica* Krasch., *A. diffusa* Krasch. ex Poljak., *A. kopetdaghensis*

Krasch., *A. turcomanica* Grand., *A. sieberi* Besser, *A. olivieriana* J. Gay ex DC., *A. gypsacea* Poljak., *A. khorassanica* Podl., *A. aucheri* Boiss., *A. ciniformis* Krasch et M. Pop. ex Poljak., *A. fragrans* Willd., *A. spicigera* C. Koch.

Палісадна паренхіма *A. abrotanum* складається з 1–3 рядів клітин циліндричної, конусоподібної чи грушоподібної форми, які відрізняються за розмірами; є міжклітинники. Клітини витягнуті перпендикулярно до поверхні листка, містять значну кількість хлоропластів, розташовані вільно. Губчаста

паренхіма складена округлими, овальними й кутастими клітинами, розташованими нещільно. У ній також присутні міжклітинники. Палісадна тканина більше розвинена порівняно з губчастою, що є ознакою ксероморфності. Центральний провідний пучок колатерального типу.

Особливої уваги заслуговують секреторні структури, виявлені на препаратах листка *A. abrotanum*. Їх будова і розташування є діагностичними ознаками і використовуються при визначенні і ідентифікації лікарської рослинної сировини. На епідермісі листків виявлено округлі багатоклітинні залозисті трихоми, типові для родини Asteraceae. Видільні тканини внутрішньої секреції представлені ефіроолійними вмістищами (Рис. 2 В), вміст яких яскраво малинового або бурштинового забарвлення.

Абаксіальна поверхня подібна до адаксіальної за характером і складом опушення, а також характером обрисів епідермальних клітин. Деякі відмінності стосуються, головним чином, кількісних показників. Так, з нижнього боку листків більше незалозистих трихом і продихів.

Згідно з Soyunova (2012), серед основних анатомо-діагностичних ознак листків *A. abrotanum*, культивованих у різних регіонах Росії є амфістоматичний продиховий апарат аномоцитного типу та неспецифічні паренхімні клітини з ефірною олією, що, загалом, узгоджується з результатами наших досліджень.

Таким чином, виявлені ксероморфні ознаки *A. abrotanum* такі: дрібні і компактні листки, наявність трихом, більший розвиток палісадної тканини порівняно з губчастою, товстостінні клітинні оболонки епідерми. Ознаки ксероморфності, характерні для *A. abrotanum*, втім не завжди присутні у інших представників поліморфного роду *Artemisia*, які розповсюджені у різних екологічних умовах (Haraim 2007). Серед них є і ксерофіти, і гігрофіти. Відомо, що екологічні фактори також впливають на анатомічну будову листкової пластинки. Зокрема, встановлено, що зразки листків *A. absinthium* з різних місць зростання

суттєво відрізняються за будовою мезофілу та епідерми (Ochirova et al. 2015). Можливо аналогічні зміни можуть бути відстежені і у *A. abrotanum*, проте для цього необхідні подальші дослідження.

Висновки

В результаті проведених досліджень описано структуру листкової пластинки *A. abrotanum* за умов інтродукції в Житомирському Поліссі. Верхній і нижній епідерміси вкриті кутикулою, продиховий апарат аномоцитного типу. Криючі трихоми відмічені з обох боків листкової пластинки; серед них присутні закручені, Т-подібні, циліндричні, головчасті та прості конічні волоски. Секреторні структури представлені округлими багатоклітинними залозистими трихомами та ефіроолійними вмістищами. Для полину лікарського характерна ізобілатеральна структура листкової пластинки та провідні пучки колатерального типу.

Відмічено такі ксероморфні ознаки *A. abrotanum*: дрібні і компактні листки, наявність трихом, виразніший розвиток палісадної тканини, товстостінні клітинні оболонки епідерми.

Секреторні структури та незалозисті трихоми можуть слугувати таксономічними ознаками і використовуватись при визначенні лікарської сировини *A. abrotanum*. Присутність у значній кількості ефіроолійних вмістищ та залозистих трихом свідчить про можливість використання рослин *A. abrotanum* як ефективних продуцентів ефірної олії в умовах Житомирського Полісся.

Подяка

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CORRESPONDENCE

Biological and morphological features of erems of *Hyssopus officinalis* L. (Lamiaceae Lindl.)

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Abstract

The paper describes morphology of erems, laboratory germination capacity and germination rate of the of *Hyssopus officinalis* plants grown under conditions of Zhytomyr Polissya. The research revealed insignificant differences between investigated hyssop varieties with respect to external structure, color, mass, and biometric indices of their erems. The mean mass index of 1000 erems for *H. officinalis* 'Markiz' was 1.09 g, for 'Atlant' – 1.13 g, and for 'Vodograj' – 1.10 g. The length of erems was slightly higher (2.91 ± 0.06 mm) in 'Atlant', while the width and thickness of erems (1.09 ± 0.05 and 0.84 ± 0.03 mm respectively) were highest in 'Vodograj'. Laboratory germination capacity of *H. officinalis* erems showed tendency to decrease during a storage period of 5 years, however the germination rate was stable for the first 4 years. 'Vodograj' demonstrated the highest germination rate (84%) and germination capacity (98.5%) in general.

Keywords: *Hyssopus officinalis*, biological features, morphological features, seed, germination capacity, germination rate

Вступ

Гісоп лікарський (*Hyssopus officinalis* L.) – цінна багаторічна ефіроолійна, ароматична й лікарська рослина, батьківщина якої Середземномор'я і Західна Європа. В Україні зустрічається у Криму, вздовж верхньої і середньої течії Дніпра, на крутих

степових схилах, виходах материкових порід Донецької області (Rabotiagov *et al.* 2003; Boyko *et al.* 2012; Kotyuk 2016). У даний час сировинні ресурси виду недостатні для ведення промислової заготівлі; гісоп зростає розсіяно, хоча й вирощується в культурі у різних регіонах України (Minarchenko 2005).

Широке впровадження і використання культури передбачає перш за все вивчення її біологічних особливостей в умовах інтродукції, особливостей її росту й розмноження.

Досить простим і економічно вигідним способом розмноження рослин є насінневий, а якість посівного матеріалу є запорукою успішної інтродукції. Життєздатне та якісне насіння є невід'ємною умовою відтворення рослин, розширення їхнього ареалу та можливості вирощування в нових умовах існування (Rakhmetov *et al.* 2004). При аналізі адаптивних особливостей інтродуцентів досить важливим є вивчення біології проростання та особливостей зберігання насіння, що дає можливість оцінити якість посівного матеріалу, передбачити швидкість і дружність сходів. Показники якості насіння є також основою для розрахунку раціональної норми висіву культури (Totskaya *et al.* 2013; Aghilian *et al.* 2014). Такі показники насіння, як швидкість і дружність проростання дозволяють розрахувати раціональну норму висіву культури, оцінити здатність майбутніх рослин конкурувати з бур'янами, отримати однорідні вирівняні сходи і, як наслідок, досягти високих і якісних врожаїв (Shibko 2011).

У зв'язку з тим, що відомості про особливості насінного розмноження гісопу лікарського дуже обмежені, метою наших досліджень було вивчити якісні показники насінневого матеріалу трьох його сортів за умов інтродукції на Житомирському Поліссі. Зокрема, завданням дослідження було встановити морфометричні показники еремів, масу 1000 штук, а також енергію проростання і схожість.

Матеріали і методи досліджень

У дослідженнях використано посівний матеріал трьох сортів: синьоквіткового сорту Маркіз (*H. officinalis* 'Markiz'), білокіткового сорту Водограй (*H. officinalis* 'Vodograj') і рожевокіткового сорту Атлант (*H. officinalis*

'Atlant') селекції Національного ботанічного саду ім. М.М. Гришка НАН України. Інтродукційні дослідження здійснювали у ботанічному саду Житомирського національного агроекологічного університету. Насіння висівали у останню декаду квітня – першу декаду травня на глибину 1 см за схемою 50 × 30 см. Збір насіння здійснювали у вересні-жовтні.

Плоди гісопу лікарського – ценобії, формуються у чашечці квітки з ценокарпного (синкарпного) двочленного гінецею, що є характерним для рослин родини Lamiaceae. Ценобій (cenobium) – двоплодолистковий схізокарпій, у кожному плодолистку якого формується вертикальна псевдосепта, внаслідок чого після дозрівання паїд розпадається не на два, а на чотири поздовжні однонасінні сегменти, які називають еремами (erem) (Рис. 1 А–В) (Totskaya *et al.* 2013; Novikoff & Barabasz-Krasny 2015; Kotyuk 2015a). Таким чином, посівною одиницею гісопу лікарського є ереми.

Вивчення якісних показників насіння здійснювали упродовж 2008–2014 років: лабораторні – на кафедрі загальної екології, польові – на колекційних ділянках ботанічного саду Житомирського національного агроекологічного університету. Показники енергії проростання та схожості насіння встановлювали за загальноприйнятими методиками згідно ДСТУ 7160–2010 (DSTU 7160–2010). Насіння пророщували на зволоженому фільтрувальному папері у чашках Петрі при температурі 25 °С у чотириразовій повторності по 100 насінин у кожній. Масу 1000 штук визначали зважуванням двох проб по 500 еремів (Grytsaenko *et al.* 2003). Статистичну обробку даних здійснювали з використанням програми Microsoft Excel 10.

Макро- і мікроморфологію еремів вивчали за допомогою мікроскопів МБС-10 та Біолам-70. Зовнішній вигляд і ультраструктуру поверхні насіння фіксували за допомогою фотокамер DSC-W40 і MCDC Levenhuk D SOL NG.

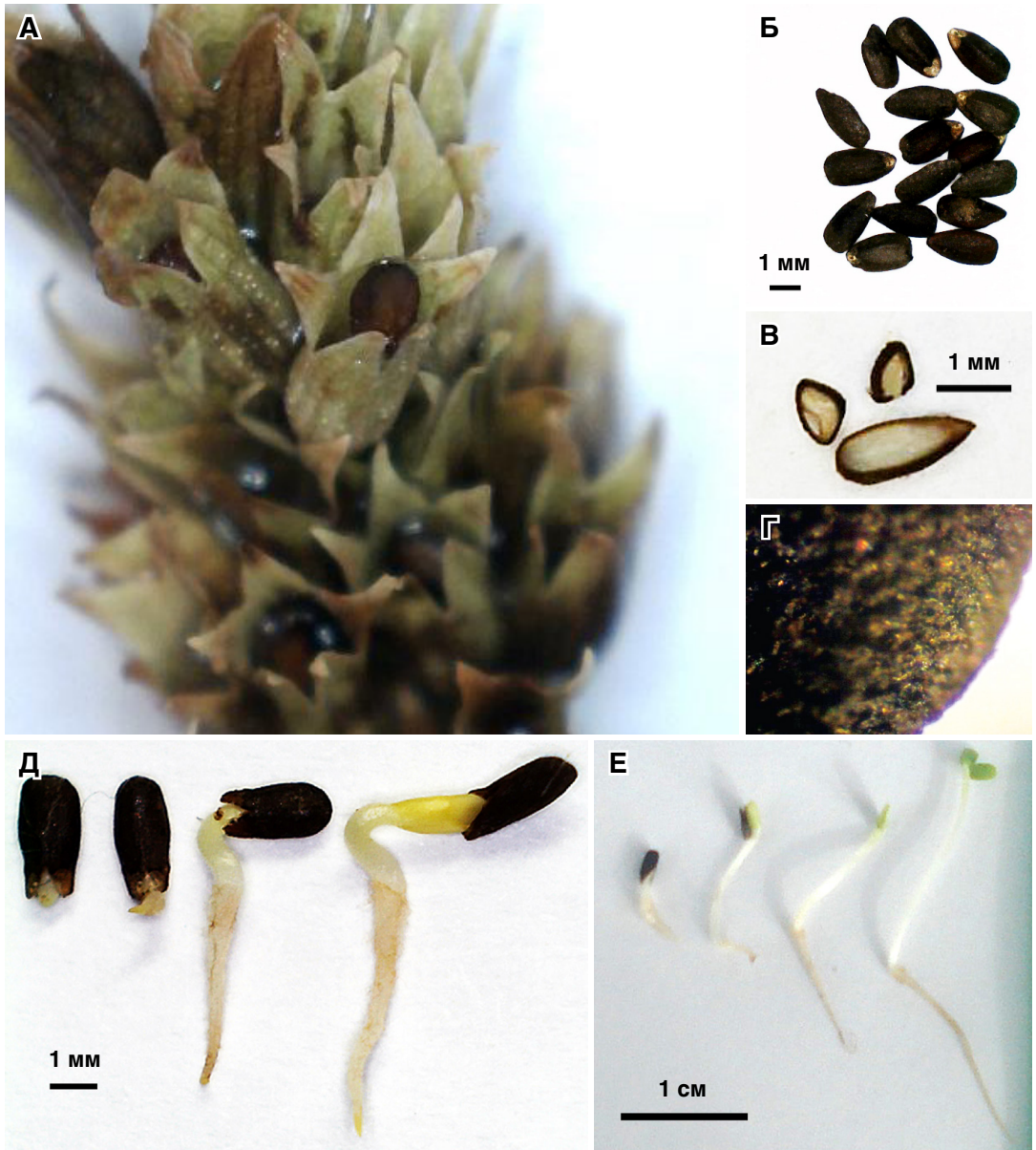


Рис. 1. Морфологічні особливості *Hyssopus officinalis*: А – плоди; Б – окремі плодики (ереми); В – ереми в перерізі; Г – поверхня ерема; Д, Е – проростки.

Fig. 1. Morphological features of *Hyssopus officinalis*: А – fruits; Б – fruitlets (erems); В – erems in cross- and longitudinal sections; Г – surface of erem; Д, Е – seedlings.

Результати та їх обговорення

Інтродукційні дослідження показали, що при посіві гісопу лікарського у третій декаді

квітня, період плодоношення розпочинався у третій декаді вересня першого року життя та третій декаді серпня – першій декаді вересня другого і наступних років життя. При чому у

Табл. 1. Маса 1000 еремів *Hyssopus officinalis*, г.Table 1. The mass of 1000 erems of *Hyssopus officinalis*, g.

Сорт	2008	2009	2010	2011	2012	2013	2014	Середнє
'Markiz'	1,17 ± 0,03	1,12 ± 0,02	1,14 ± 0,25	1,08 ± 0,13	1,04 ± 0,11	1,06 ± 0,11	1,00 ± 0,07	1,09 ± 0,10
'Atlant'	1,20 ± 0,05	1,17 ± 0,14	1,15 ± 0,04	1,20 ± 0,02	1,07 ± 0,06	1,05 ± 0,06	1,05 ± 0,02	1,13 ± 0,06
'Vodograj'	1,18 ± 0,06	1,15 ± 0,14	1,07 ± 0,06	1,19 ± 0,01	1,02 ± 0,01	1,05 ± 0,02	1,03 ± 0,02	1,10 ± 0,05

сорту Водограй цей період сповільнювався у середньому на 7 діб у порівнянні з сортами Маркіз і Атлант. При дозріванні насіння самостійно висипалося із розпадних плодів, огорнутих трубчастою чашечкою (Рис. 1 А), поширюючись механохорно. Вже через 14–20 діб спостерігали появу сходів, тобто, фізіологічний спокій для насіння був не дуже тривалий. Самосів гісопу лікарського у зимовий період гинув лише частково, що свідчить про можливість підзимнього посіву.

Ереми гісопу лікарського продовгасто-оберненояцеподібні, тригранні, мають вентральну грань на округлій поверхні. Між дорзальною і вентральною поверхнею ерема є округле ребро. Базальна частина ерема округла, апікальна – загострена. Поверхня шорстка, темно-бурого або чорного забарвлення (Рис. 1 Б–Г). Плодовий рубчик серцеподібної форми, світло-коричневий, з білуватим борошністим нальотом, розміщений у базальній частині вентральної поверхні ерема. У центрі рубчика помітний білий горбок із темною крапкою в центрі (Рис. 1 Б) (Shibko 2011; Kotyuk 2015b). Перикарпій ерема складається з екзо-, мезо- і ендокарпії. Екзокарпій утворений змертвілими клітинами, щільний, шорсткий, темно-бурого або чорного забарвлення. Мезокарпій складається із двох-трьох шарів тонкостінних клітин з міжклітинниками, ендокарпій – з ізодіаметричних нездерев'янілих клітин. Сортові відмінності у зовнішній будові і забарвленні еремів гісопу лікарського відсутні. Насінини гісопу без ендосперму (Abidkulova et al. 2009).

Виявлено, що зі збільшенням віку рослин маса 1000 еремів несуттєво зменшується – у 1,06–1,07 разів. Мінімальну масу еремів усіх досліджених сортів відмічено у врожаї сьомого року життя (2014 р.), максимальну – у врожаї першого року життя (2008 р.) (Табл. 1). Суттєвих відмінностей у масі еремів між сортами не виявлено.

Ереми гісопу лікарського дрібні (Табл. 2). При цьому прослідковується зниження розмірів еремів із збільшенням тривалості життя рослин: довжини в 1,1 рази у сортів Маркіз та Водограй і в 1,3 рази у сорту Атлант; а також товщини – в 1,4 рази у сортів Маркіз та Атлант і в 1,6 разів у сорту Водограй. Максимальні розміри еремів відмічено у врожаї 2008 року, мінімальні – переважно у врожаї 2014 року (Табл. 2). Очевидно, це зумовлено старінням рослин і зниженням їх резистентності до несприятливих умов довкілля.

Помітної різниці у біометричних параметрах еремів між сортами не виявлено. Найбільші показники за довжиною еремів виявляє сорт Атлант ($2,91 \pm 0,06$ мм), за шириною і товщиною – сорт Водограй ($1,09 \pm 0,05$ та $0,84 \pm 0,03$ мм відповідно).

Нечисленні дані про насінневе розмноження гісопу лікарського в умовах Передгірської зони Криму висвітлено у роботах Shibko (2011) і Kurbatova et al. (2009). При культивуванні синьоквіткового, рожевоквіткового і білоквіткового сортів гісопу лікарського в Криму, середня маса 1000 еремів складала $1,161 \pm 0,2412$ г, довжина ерема становила $2,64 \pm 0,125$ мм,

Табл. 2. Біометричні параметри еремів *Hyssopus officinalis* в умовах інтродукції, мм.Table 2. Biometric parameters of erems of *Hyssopus officinalis* under introduction, mm.

Сорт	Параметр	2008	2009	2010	2011	2012	2013	2014	Середнє
'Markiz'	довжина	2,54 ± 0,09	2,47 ± 0,12	2,23 ± 0,07	2,18 ± 0,07	2,18 ± 0,06	2,24 ± 0,07	2,22 ± 0,09	2,30 ± 0,08
	ширина	1,08 ± 0,04	1,03 ± 0,05	1,01 ± 0,05	0,97 ± 0,05	1,05 ± 0,04	1,04 ± 0,03	1,05 ± 0,05	1,03 ± 0,04
	товщина	0,83 ± 0,03	0,81 ± 0,03	0,77 ± 0,05	0,72 ± 0,05	0,64 ± 0,04	0,60 ± 0,05	0,59 ± 0,03	0,71 ± 0,04
'Atlant'	довжина	2,91 ± 0,06	2,88 ± 0,05	2,28 ± 0,05	2,24 ± 0,04	2,22 ± 0,05	2,20 ± 0,04	2,16 ± 0,05	2,46 ± 0,05
	ширина	1,07 ± 0,05	1,05 ± 0,04	1,05 ± 0,04	1,02 ± 0,03	1,03 ± 0,05	1,02 ± 0,04	0,99 ± 0,04	1,03 ± 0,04
	товщина	0,78 ± 0,04	0,77 ± 0,05	0,74 ± 0,05	0,68 ± 0,05	0,57 ± 0,04	0,54 ± 0,04	0,54 ± 0,03	0,66 ± 0,04
'Vodograj'	довжина	2,58 ± 0,07	2,51 ± 0,10	2,41 ± 0,09	2,39 ± 0,08	2,38 ± 0,11	2,34 ± 0,07	2,28 ± 0,09	2,41 ± 0,09
	ширина	1,09 ± 0,05	1,07 ± 0,06	1,06 ± 0,06	1,04 ± 0,05	1,04 ± 0,06	1,01 ± 0,05	1,01 ± 0,05	1,05 ± 0,05
	товщина	0,84 ± 0,03	0,81 ± 0,04	0,75 ± 0,05	0,70 ± 0,042	0,61 ± 0,04	0,57 ± 0,03	0,54 ± 0,04	0,69 ± 0,04

ширина – $1,14 \pm 0,112$ мм (Shibko 2011). При вирощуванні рослин у Казахстані маса 1000 еремів гісопу лікарського складала 1,105 г, довжина ерема становила 2,72 мм, а ширина – 1,16 мм (Kurbatova et al. 2009). В умовах Московської області маса 1000 еремів становила 0,9–1,0 г (Bespalyko et al. 2016). На нашу думку, незначну відмінність у біометричних параметрах дослідженого насіння гісопу лікарського можна пояснити ґрунтово-кліматичними умовами зони Полісся України.

В польових умовах відмічено епігеальне (надземне) проростання насіння гісопу лікарського. Першим з'являвся корінь, гіпокотиль при основі витягувався й викривлявся, а вирівнюючись, виносив сім'ядолі на поверхню ґрунту. Перикарпій опав, сім'ядолі відділялись одна від одної і розправлялись.

У лабораторних умовах при температурі 25 °С насіння гісопу лікарського починало проростати уже на третю добу. Зародок руйнував оболонку ерема (перикарпій),

з'являвся корінь, а на четверту добу – сім'ядолі (Рис. 1 Д, Е).

Відомо, що насіння *H. officinalis* має високі показники схожості упродовж перших трьох років зберігання. На темпи проростання насіння, окрім оптимальних умов навколишнього середовища, впливають також структура і фізико-хімічні особливості оболонки, активність ферментів, які призводять до виснаження запасів поживних речовин за тривалого зберігання (Shibko 2011).

З метою встановлення оптимальних термінів зберігання насіння гісопу лікарського у березні 2015 року вивчено показники енергії проростання та лабораторної схожості насіння, зібраного у вересні 2008–2014 років, яке зберігали від 6 місяців до 6,5 років відповідно. Доведено, що зібране в умовах культури насіння гісопу лікарського має високу життєздатність. Так, через 6 місяців після збирання урожаю лабораторна схожість насіння становила 97,5% для *H. officinalis* 'Markiz', 97,3% – для *H. officinalis* 'Atlant' і

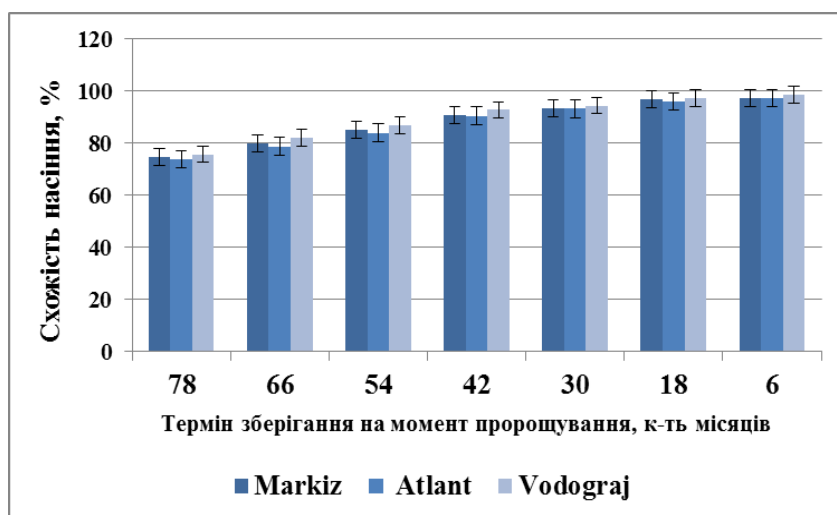


Рис. 2. Схожість насіння *Hyssopus officinalis* залежно від тривалості зберігання і сорту.

Fig. 2. The germination capacity of erems of *Hyssopus officinalis* depending from storage duration and cultivar.

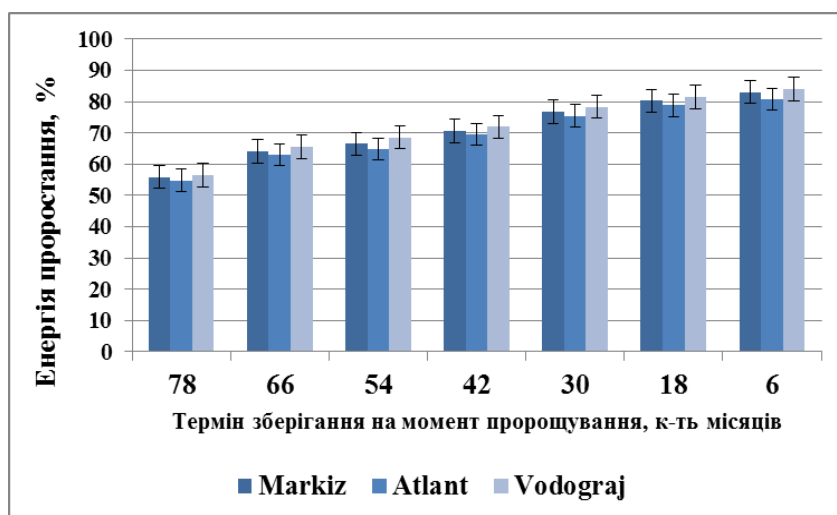


Рис. 3. Енергія проростання насіння *Hyssopus officinalis* залежно від тривалості терміну зберігання і сорту.

Fig. 3. The germination rate of erems of *Hyssopus officinalis* depending from storage duration and cultivar.

98,5% – для *H. officinalis* ‘Vodograj’, й істотно не знижувалась упродовж чотирьох років зберігання. Лабораторна схожість насіння, зібраного 2008 року, становила 74,8% для *H. officinalis* ‘Markiz’, 73,8% – для *H. officinalis*

‘Atlant’ і 75,8% – для *H. officinalis* ‘Vodograj’, тобто знизилась у 1,3 рази (Рис. 2). Енергія проростання насіння досліджуваних сортів упродовж 6,5 років зберігання знижувалась у 1,5 разів, при чому суттєвим зниженням

показника відрізнялось насіння, яке зберігали 4,5 роки (Рис. 3). Найвищу лабораторну схожість і енергію проростання насіння відмічено в сорту Водограй.

Висновки

Істотних сортових відмінностей зовнішньої будови, забарвлення, маси, біометричних параметрів еремів гісопу лікарського, вирощеного за умов інтродукції у ботанічному саду Житомирського національного агроекологічного університету не виявлено. Маса 1000 еремів *H. officinalis* 'Markiz' у середньому становила 1,09 г, *H. officinalis* 'Atlant' – 1,13 г, а *H. officinalis* 'Vodograj' – 1,10 г.

Зібране в умовах інтродукції насіння гісопу лікарського характеризувалося високою життєздатністю. Лабораторна схожість насіння досліджених сортів на шостий місяць зберігання становила більше 97% й істотно не зменшувалася упродовж 4,5 років зберігання, сягаючи в середньому 84%. В той же час, енергія проростання насіння досліджених сортів через 6,5 років зберігання у середньому зменшувалася на 27%.

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RESEARCH ARTICLE

Genus *Aconitum* in Slovakia: a phenetic approach

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Abstract

Comprehensive investigation on herbarium material was realized and taxonomical structure of the genus *Aconitum* in Slovakia has been ascertained on the base of phenetic analysis. Three new taxa (i.e. *A. degenii* subsp. *degenii*, *A. moldavicum* subsp. *hosteanum*, and *A. moldavicum* nothosubsp. *confusum* stat. nov.) were rediscovered for this region, and for one of them the new status was proposed. Morphological variation of *A. anthora* and its subdivision onto infraspecific taxa (subsp. *anthora* and subsp. *jacquinii*) are discussed. The new key for identification and maps of distribution are represented.

Keywords: *Aconitum*, Slovakia, morphology, phenetics, distribution, taxonomy

Introduction

In spite of long history of investigation, taxonomy of the genus *Aconitum* L. is still much discussed and remains unresolved. Many taxonomic problems arise from the notorious hybridity within the genus and subsequent origin of number of putative hybridogenic taxa, which form a morphological continuum between the parental taxa (Kadota 1987; Starmühler 2001). Another problem is that

different morphological traits of monkshoods were considered for genus taxonomy by different authors, and therefore very often taxonomic ranges vary significantly for the same taxonomic units (Götz 1967; Kmetová 1982; Skalický 1990; Kadota 1987; Voroshilov 1990; Tamura 1995; Tzvelev 2001). Recent revisions based on morpho-geographical concept of species (Davis & Haywood 1963) and concept of infraspecific differentiation (Skalický 1982) led to unification and progress

in taxonomy of the genus *Aconitum* in countries of Carpathian region (Mitka 2003, 2008a; Starmühler & Mitka 2001; Starmühler 2002; Mitka & Szajna 2009; Boroń 2010; Boroń *et al.* 2011; Novikoff & Mitka 2011; Sutkowska *et al.* 2013; Mitka *et al.* 2013, 2016; Novikoff *et al.* 2016; Waclawska-Ćwiertnia & Mitka 2016).

Accordingly to Flora of Slovakia (Kmeťová 1982), genus *Aconitum* here includes 7 taxa (*A. lycoctonum* L. em. Koelle, *A. moldavicum* Hacq., *A. variegatum* L., *A. lasiocarpum* (Rchb.) Gayer, *A. firmum* Rchb. subsp. *firmum*, *A. firmum* subsp. *moravicum* Skalický, and *A. anthora* L.). Kmeťová (1982) noted that in flora of Slovakia four subspecies of *A. lycoctonum* (subsp. *vulparia* (Rchb.) Schinz et Thell., subsp. *puberulum* (Ser.) Gayer, subsp. *thalianum* (Wall.) Gayer, and subsp. *penninum* (Ser.) Gayer) are sometimes distinguished mostly on the base of variation in pubescence. For example, subdivision of *A. lycoctonum* on subsp. *vulparia* and subsp. *puberulum* was accepted by Domin (1935), and subdivision of *A. lycoctonum* on subsp. *vulparia*, subsp. *puberulum*, and subsp. *penninum* – later by Dostál (1958). But Kmeťová (1982) did not accept such partition, arguing that considered traits make a morphological continuum in *A. lycoctonum*. She also noted that sometimes *A. lasiostomum* Rchb. is reported from adjacent regions, perhaps this species does not occur in Slovakia. For *A. moldavicum*, Kmeťová (1982) reported var. *australe* (Rchb. ex Baumg.) Grinț with villous carpels (“s chlpatými mechúrikmi”) from Eastern part of Slovakia. In the same time, she suggested that *A. moldavicum* subsp. *hosteanum* (Schur) Graebner et P. Graebner in Aschers. et Graebner most probably does not occur here, although it was reported for Czechoslovakia by Domin (1935) and Dostál (1958).

Domin (1935) and Skalický (1966) distinguished 3 species of aconites from *Variiegatum* group (*i.e.* *A. variegatum*, *A. gracile* Rchb. and *A. dominii* Sillinger), which also were accepted by Dostál (1958), however in range of subspecies – subsp. *variegatum*, subsp. *gracile* (Rchb.) Gayer, and subsp. *dominii* (Sillinger) Dostál. consequently. Moreover,

in Skalický (1982) only *A. variegatum* is mentioned. Kmeťová (1982) mentioned in Flora of Slovakia three subspecies of *A. variegatum* (subsp. *gracile*, subsp. *dominii*, and subsp. *kotulae*), but concluded that they make morphological intermediations.

Kmeťová (1982) also mentioned that besides of *A. firmum*, for Slovakia *A. tauricum* Wulf. (Domin 1935; Skalický 1966) or *A. napellus* L. subsp. *tauricum* (Wulf.) Gayer (Dostál 1958) are sometimes considered. Finally, she noted that *A. anthora* is highly variable taxon and delimitation of var. *jacquinianum* G. Beck has poor taxonomic background.

Starmühler (2002) published the key for identification of Slovakian aconites with 14 taxa including 6 hybrids (*A. lycoctonum* subsp. *lycoctonum*, *A. moldavicum* subsp. *moldavicum*, *A. anthora* subsp. *anthora*, *A. variegatum* subsp. *variegatum* var. *variegatum*, *A. × pawlowskii* Mitka et Starmühl., *A. lasiocarpum* subsp. *kotulae* (Pawl.) Starmühl. et Mitka, *A. × lengyelii* Gayer nothosubsp. *lengyelii* (= *A. × berdaui* Zapał. nothosubsp. *berdaui* in accordance to Mitka (2003)), *A. × lengyelii* nothosubsp. *walasii* Mitka (= *A. × berdaui* nothosubsp. *walasii* (Mitka in Starmühler et Mitka) Mitka in accordance to Mitka (2003)), *A. firmum* subsp. *firmum*, *A. firmum* subsp. *moravicum*, *A. firmum* subsp. *maninense* (Skalický) Starmühl., *A. firmum* subsp. *firmum* × *A. firmum* subsp. *maninense*, *A. firmum* nothosubsp. *paxii* Starmühl., and *A. firmum* nothosubsp. *zapolowiczii* Starmühl.). In addition, Mitka (2003, 2008a) later reported for Slovakia 5 more taxa – *A. × cammarum* L. em. Fries, *A. × triste* (Rchb.) Gayer (also mentioned for Czechoslovakia by Domin (1935)), *A. lasiocarpum* subsp. *lasiocarpum*, *A. × gayeri* Starmühl. and *A. × hebegynum* DC.

Taking into account mentioned publications, the genus *Aconitum* in flora of Slovakia could be represented at least by 19 taxa. In this paper I provide results of investigations on morphological variability and taxonomic structure of this genus in Slovakia on the base of analysis of herbarium material and published works.

Material and methods

Herbarium investigations were conducted in 2012–2013 in project frames of National Scholarship Programme of the Slovak Republic entitled “Taxonomy and chorology of the genus *Aconitum* L. (Ranunculaceae) in Slovakian flora”. Preliminary results of this study (supposed taxonomic structure of the genus in Slovakia) were published in 2013 (Novikoff 2013) and deposited online at <http://slovaconitum.myspecies.info/> (Novikoff 2013–2017). However, in 2017 obtained data were revised and reevaluated, all of taxonomic and phenetic studies were redone.

In general more than 3000 herbarium vouchers were analyzed from 7 main repositories (Append. 1): Institute of Botany SAS (SAV, Bratislava), Comenius University in Bratislava (SLO), Slovak National Museum (BRA, Bratislava), Slovak University of Agriculture in Nitra (NI), Technical University in Zvolen (ZV), Pavol Jozef Šafárik University (KO, Košice), and Museum of Tatra National Park (TNP, Tatranská Lomnica). From total number, 970 specimens were included into chorological database; 450 specimens were included in phenetic database and tested with 115 morphological traits (Tab. 1). For different taxonomic groups (*Lycotconum*, *Anthora*, *Cammarum* and *Aconitum*) inside the genus, different combinations of treats were applied for advanced partial analyses (Tab. 1). Additionally, main recent literature sources (Mitka & Starmühler 2000; Mitka 2003, 2008a) were analyzed for mention of *Aconitum* specimens from Slovakia nested in herbaria abroad. Finally, the maps from Flora of Slovakia (Kmeťová 1982) were digitized and extracted landmarks were applied in our research too (Append. 2).

For statistical analysis the Past 3.14 was used (Hammer *et al.* 2001). For correlation statistics linear r (Pearson’s) was applied. For multivariate analysis UPGMA and Ward’s clustering methods were applied in combination with Euclidian index of similarity and $N = 100$ bootstrap support. For ordination non-metric multidimensional scaling was

applied in combination with Euclidian index of similarity.

Distribution maps were built in QGIS 2.18 (QGIS Development Team 2017) with WGS84 (EPSG: 4326) geographical coordinate system and after that transformed in J-TSK_Krovak (EPSG: 102065) projection system, which is commonly applied in Slovakia for better visualization (Annoni *et al.* 2001). Maps from Kmeťová (1982) were imported to QGIS as independent geotiff layers with Polygonal 1 WGS 84 transformation mode, and after that separated layers with landmarks corresponding to points on that maps were generated.

For taxonomic verification IPNI (2017) and The Plant List (2017) databases were used. For developing of website, the free CMS Scratchpads 2.0 was applied (Smith *et al.* 2012).

The raw datasets of treats applied for phenetic analyses (S20, S21, S22, S23, S24, S25, S26, S27, S28) and database of analyzed specimens with generated coordinates of locations (S29 & S30) are freely available in Zenodo and I kindly welcome researchers to use them for any investigation purposes.

Results

General analysis was performed for 450 samples and full dataset of 115 morphological traits (Tab. 1). Cluster analysis (both UPGMA and Ward’s methods) showed good delimitation of main taxonomic groups (*i.e.* sections and subgenera) in the genus *Aconitum*, however in most cases did not allow discrimination of taxa on species and subspecies levels, as well as of some hybrid taxa (Figs. 1 & S1). Multidimensional scaling showed good delimitation of group *Lycotconum* by first two axes however did not show any good limits between this and other groups on second scatterplot. Groups *Anthora*, *Cammarum*, *Acomarum* and *Aconitum* were significantly overlapped (Fig. S2). Therefore I provided further statistical analyses on separated taxonomic groups representing sections with exclusion of uninformative traits.

Table 1. Initial set of analyzed morphological traits. For partial analyses: + – analyzed for full set of treats of separated taxonomic groups; * – analyzed for limited set of traits selected for each of separated taxonomic groups.

Nr	Group of traits	Trait	<i>Lycotctonum</i>	<i>Anthora</i>	<i>Cammarum</i>	<i>Aconitum</i>
1	Helmets	glabrous	+*		+*	+*
2		sparsely pubescent	+*		+	+
3		moderately pubescent	+*		+	+
4		densely pubescent	+*		+	+
5		with short curved trichomes	+*		+	+*
6		with protruding straight trichomes			+	+*
7		with mixed trichomes			+	+*
8	Carpels	glabrous	+*		+*	+*
9		ventral pubescent			+*	
10		dorsal pubescent	+*		+*	+*
11		sparsely pubescent	+*		+	+
12		densely pubescent	+*		+	+
13		with short curved trichomes	+*		+	+*
14		with protruding straight trichomes	+*		+	
15	Stamens	glabrous	+		+	+
16		sparsely pubescent	+		+	+
17		densely pubescent	+		+	
18		All pubescent	+			
19		Part of them pubescent	+			+
20	Upper part of pedicels	glabrous			+*	+*
21		pubescent only at receptacle				
22		sparsely pubescent				
23		moderately pubescent			+	+
24		densely pubescent			+	+
25		with short curved trichomes				+*
26		with protruding straight trichomes			+	+*
27		with mixed trichomes			+	+*
28	Lower part of pedicels	glabrous			+*	+*
29		sparsely pubescent				+
30		moderately pubescent			+	+
31		densely pubescent			+	+
32		with short curved trichomes				+*
33		with protruding straight trichomes			+	+*
34		with mixed trichomes			+	
35		Bracteoles	glabrous			+
36	ciliate (pubescent along margins only)					+
37	sparsely pubescent				+	+

Table 1. Continued.

Nr	Group of traits	Trait	<i>Lycocotnum</i>	<i>Anthora</i>	<i>Cammarum</i>	<i>Aconitum</i>
38	Bracteoles	moderately pubescent			+	+
39		densely pubescent			+	+
40		with short curved trichomes		+		+*
41		with protruding straight trichomes			+	+*
42	Inflorescence axes	glabrous			+*	+*
43		sparsely pubescent			+	+
44		moderately pubescent	+		+	+
45		densely pubescent	+		+	+
46		with short curved trichomes		+	+	+*
47		with protruding straight trichomes			+	+*
48		with mixed trichomes		+		
49	Stems	glabrous	+		+	+
50		sparsely pubescent	+		+	+
51		moderately pubescent	+	+	+	+
52		densely pubescent	+	+		+
53		with short curved trichomes	+			+
54		with protruding straight trichomes	+		+	+
55		with mixed trichomes	+			
56	Petioles	glabrous	+		+	+
57		sparsely pubescent	+			+
58		moderately pubescent	+	+	+	
59		densely pubescent	+	+		+
60		with short curved trichomes	+			+
61		with protruding straight trichomes	+		+	+
62		with mixed trichomes	+			
63	Adaxial surface of leaf laminae	glabrous	+		+	+
64		pubescent along veins	+			
65		sparsely pubescent	+		+	+
66		moderately pubescent	+			
67		densely pubescent	+			
68		with short curved trichomes	+			+
69		with protruding straight trichomes	+		+	
70	Abaxial surface of leaf laminae	glabrous	+		+	+
71		pubescent along veins	+		+	+
72		sparsely pubescent	+			+
73		with short curved trichomes	+			+
74		with protruding straight trichomes	+		+	
75	Perianth color	pale yellow		+		
76		yellow	+*	+		

Table 1. Continued.

Nr	Group of traits	Trait	<i>Lycocotnum</i>	<i>Anthora</i>	<i>Cammarum</i>	<i>Aconitum</i>
77	Perianth color	dirty blue	+			
78		violet	+			
79		blue				+
80		variegate				+
81	Helmet shape	bean-shaped		+		
82		crescent				+
83		hemispherical		+		+
84		conical				+
85		elongated conical				+
86		cylindrical				
87	Nectaries	not reaching the top of helmet			+	+
88		reaching the top of helmet			+	+
89		spurs ecapitate / capitate				+
90		spurs semi-spiral / curvedbackward bent				
91		spurs coiled				
92		spurs deflected bent				
93		claws adjoined				+
94		claws outstanding				+
95	Carpels	3			+	+
96		5			+	+
97	Stamens	without protuberances			+	+
98		part of them with protuberances			+	+
99		all of them with protuberances				+
100	Bracteoles	reaching the receptacle			+	+
101		narrower than pedicels			+	+
102		as wide as pedicels			+	+
103		wider than pedicels			+	+
104		entire				+
105		divided				+
106	Pedicels	longer than flowers			+	+
107	Inflorescences	simple	+	+	+	+
108		condensed compound	+	+	+	+
109		ramified compound	+	+	+	+
110	Shoots	orthotropic	+		+	
111		semirosulate				
112		nonrosulate				
113	Leaves	leaf to internode index > 3			+	+
114	Perianth	deciduous after blooming				
115	Life forms	polycyclic monocarpous				

***Lycocotnum* group**

152 specimens and 50 morphological traits selected among total 115 (Tab. 1, pluses) were analyzed. Neither cluster analysis (Figs. S3 & S4) nor multidimensional scaling (Fig. S5) did not show appropriated results, randomly mixing specimens from *A. moldavicum*, *A. lycocotnum* and *A. × triste* (*A. lycocotnum* × *A. moldavicum*). This explains by fact that these taxa are mainly differentiated by color of perianth, which is from dirty-blue to purple-violet in *A. moldavicum*, from whitish to yellow in *A. lycocotnum*, and yellowish-bluish in *A. × triste* (Skalický 1990; Mitka 2008a). In all three species, helmet is usually covered by short curved trichomes. But density of such pubescence significantly varies in *A. moldavicum*, where helmet can be even entirely glabrous or with only few sparsely distributed trichomes. While in studied specimens of *A. × triste* and *A. lycocotnum* helmets were always densely covered by curved trichomes.

In *A. lycocotnum* and *A. × triste* carpels can be either glabrous or covered by short curved trichomes. In *A. moldavicum* character of carpels' pubescence plays the key role for delimitation of subspecies. In *A. moldavicum* subsp. *moldavicum* carpels are glabrous. In *A. moldavicum* subsp. *hosteanum* carpels are entirely covered by short curved trichomes.

There are also several other hybrids distinguished on the base of perianth color and pubescence of carpels. For example, in *A. moldavicum* nothosubsp. *simonkaianum* (Gáyer) Starmühl. (*A. lasiostomum* × *A. moldavicum* subsp. *hosteanum*) carpels are villous – entirely covered by protruding long trichomes (Starmühler 1998; Mitka 2008a). In *A. × porcii* Starmühl. (*A. moldavicum* subsp. *moldavicum* × *A. moldavicum* nothosubsp. *simonkaianum*) helmet is purple-violet; carpels are with mixed (villous at the base and curved at upper part) pubescence (Starmühler 1998; Mitka 2008a). In *A. × baumgartenianum* Simonk. (*A. lasianthum* Simonk. × *A. moldavicum*) helmet is yellowish-bluish, glandular pilose and eglandular pubescent; carpels are entirely villous, with long

protruding trichomes (Mitka 2008a). A hybrid between *A. moldavicum* subsp. *hosteanum* and *A. moldavicum* subsp. *moldavicum* with “carpels at the backside and/or on the suture pilose” is also mentioned by Mitka (2008a).

However in recent personal communication, W. Rottensteiner (formerly W. Starmühler) noticed that he unsuccessfully tried to differentiate subtaxa among *A. moldavicum* on the base of carpels pubescence. So he concluded that “the pubescence of the carpels, which is very precious in other species, has no taxonomical value in *A. moldavicum*”. In particular, he treats *A. moldavicum* subsp. *hosteanum* as an endemic of Southern Carpathians distributed only in the highest altitudes and distinguished from *A. moldavicum* subsp. *moldavicum* by different pubescence of tepals. However, *A. moldavicum* subsp. *hosteanum* was found in Poland and Ukraine even in lower altitudes of subcarpathian region and growing in mixed populations with *A. moldavicum* subsp. *moldavicum* (Mitka 2003, 2008a, 2008b; Novikoff & Mitka 2011; Novikoff et al. 2016). Zapałowicz (1908) mentioned *A. hosteanum* Schur from Czywczyny and Gorgany Mts. in Ukraine. Tzvelev (2001) also applied type of carpels pubescence for differentiation of *A. hosteanum* among other taxa from *Lycocotnum* group and noted that it is distributed in Eastern Carpathians. So there is different understanding of taxonomic limits in *A. moldavicum*.

It is important to note that both Starmühler (2002) and Mitka (2008a) reported for Slovakia *A. lycocotnum* subsp. *lycocotnum*, although Kmeťová (1982) and some other researchers (Domin 1935; Dostal 1958; Skalický 1966, 1985; Tutin et al. 1993) mentioned for this region *A. lycocotnum* subsp. *vulparia*. In preliminary published results (Novikoff 2013) I used the name *A. lycocotnum* subsp. *vulparia*, however here for uniformity this taxon is considered as *A. lycocotnum* subsp. *lycocotnum* following Starmühler (1997, 2002) and Mitka (2003, 2008a). Moreover, Starmühler (1997) neotypified *A. lycocotnum* subsp. *vulparia* and chose the illustration from Reichenbach (1823–1827) with straight pubescence of the pedicel and the tepals. Such straight type of pubescence

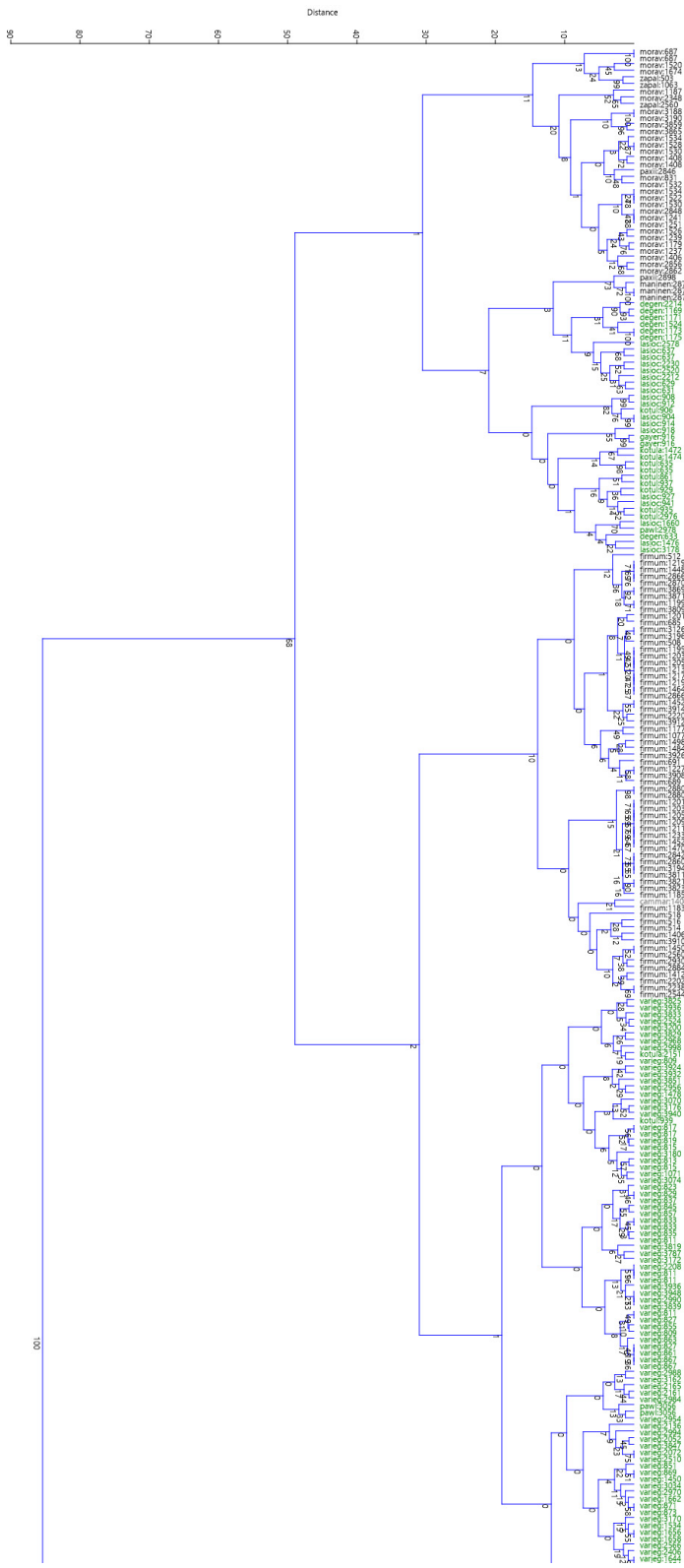


Fig. 1. Ward's dendrogram in combination with Euclidian distances for full dataset of treats and samples. **Blue** – group *Lycocotnum*; **black** – group *Aconitum*; **grey** – group *Acomarum*; **green** – group *Cammarum*; **red** – group *Anthora*.

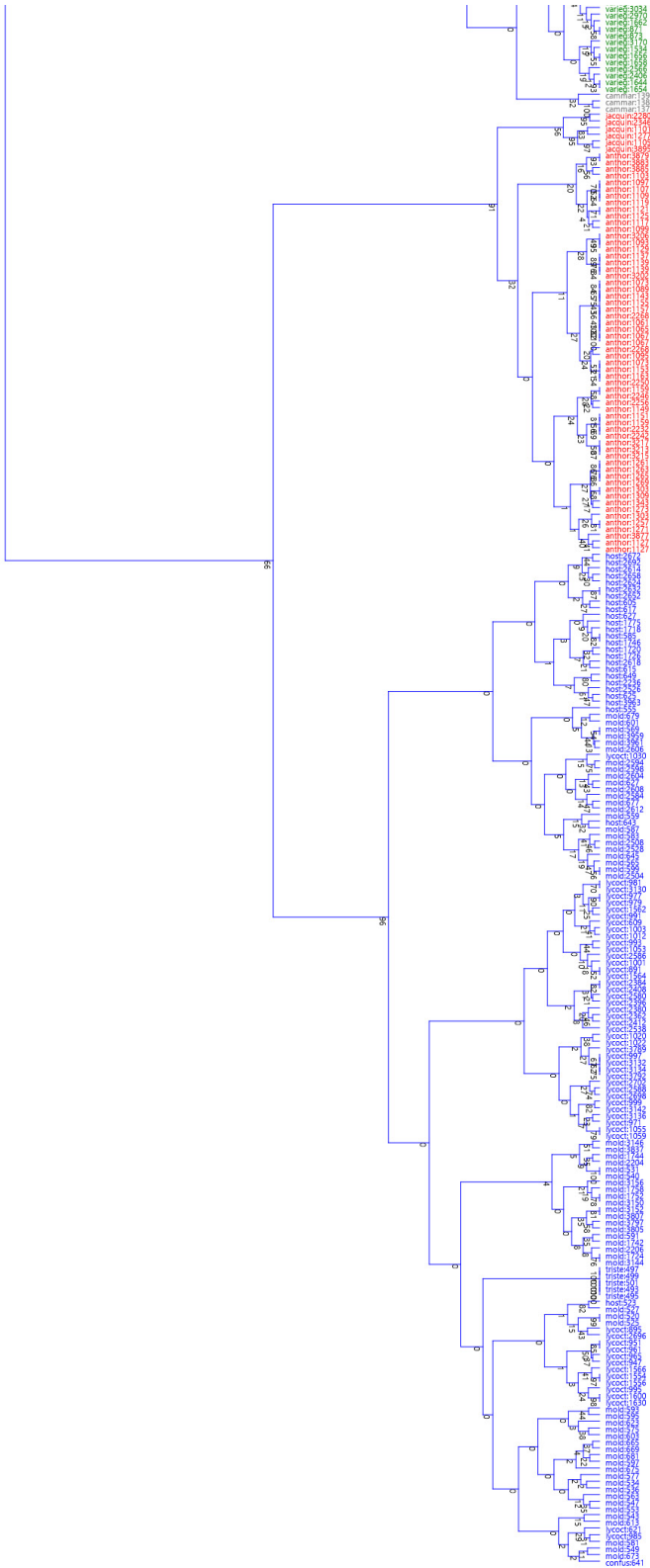


Fig. 1.
Continued.

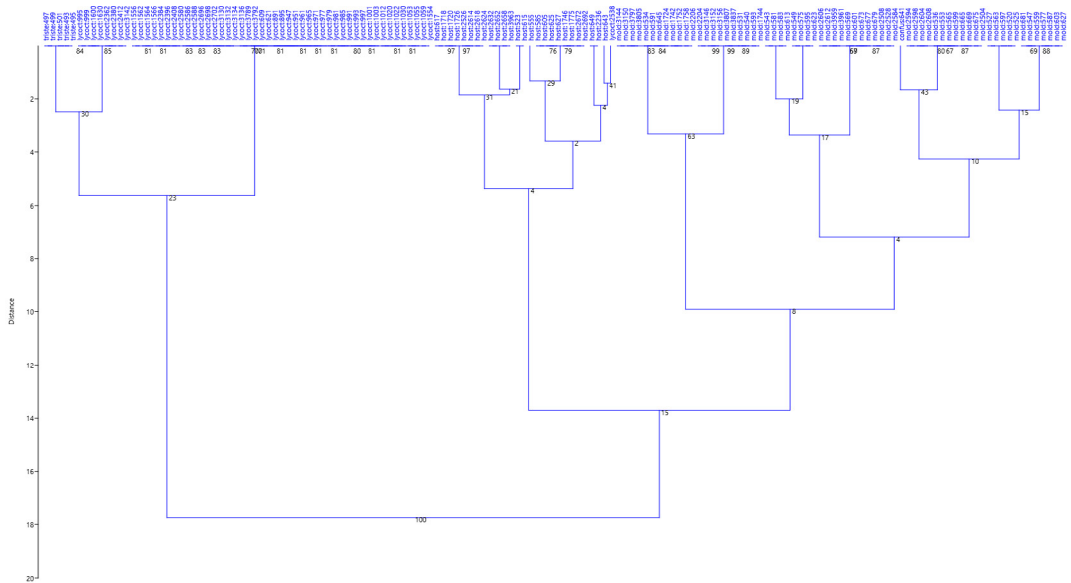


Fig. 2. Ward's dendrogram in combination with Euclidian distances for *Lycoctonum* group with dataset of 14 diagnostic traits.

is also seen in Reichenbach (1840), however contradicts with taxonomic interpretation of Czech and Slovakian researchers (Domin 1935; Dostal 1958; Skalický 1966, 1985) as well as revision of Tzvelev (2001). In particular, Tzvelev (2001), in opposition to Starmühler (1997), characterized *A. vulparia* by short curved trichomes covering inflorescence axes and pedicels, and *A. lycoctonum* – by straight trichomes.

Hence, I performed cluster analysis for 14 separated traits mentioned as diagnostic (Tab. 1, asterisks), which gave good results, but with low bootstrap support. In case of UPGMA, cluster *moldavicum* has been joined with cluster *lycoctonum* (Fig. S6), but in case of Ward's method of clustering (Fig. 2), the cluster *moldavicum* was attached with cluster *hosteanum*. In both cases, cluster *triste* was joined with cluster *lycoctonum*, and their samples were mixed on plot of multidimensional scaling (Fig. S7). Cluster *lycoctonum* was divided on two subclusters different by pubescence of carpels (glabrous in samples *lycoct: 1554–lycoct: 1556* or curved pubescent in samples *lycoct: 3142–lycoct: 995*), however with low bootstrap support (Figs. 2

& S6). Cluster *moldavicum* is subdivided on several subclusters on the base of intensity of pubescence of helmet and variation of perianth color (dirt-blue or violet). Simultaneously, cluster *hosteanum* is divided on the base of variation in helmet and carpels pubescence, and coloration of perianth too.

At SAV herbarium I found one specimen of *A. moldavicum* with carpels covered on dorsal sides by protruding villous trichomes and reported also from Romania (Grințescu 1953). Preliminary I identified this specimen as *A. moldavicum* nothosubsp. *simonkaianum* (Novikoff 2013), however *A. moldavicum* nothosubsp. *simonkaianum* is characterized by protruding villous pubescence of entire carpels. Taking into account reports of Grințescu (1953) and Mitka (2008a) representing diagnostic features of mentioned taxa, name proposed by Grințescu (1953), and following current taxonomic hierarchy in *Lycoctonum* group, I believe that this specimen should be described as *A. moldavicum* nothosubsp. *confusum* (Grinț.) A. Novikov. Due its hybrid origin and low number of analyzed material, this specimen does not occupy any stable position neither

on dendrogram (Figs. 2, S3, S4, S6) nor on the multidimensional scatterplots (Figs. S5 & S7).

Correlation analysis reveals the only very strong dependence (Pearson's $r = 0.85$) – between yellow coloration of perianth and dense pubescence of helmet, what characterizes the most of specimens from cluster *lycoctonum*.

Hence, *Aconitum* subgen. *Lycoctonum* (DC.) Peterm. sect. *Lycoctonum* DC. in flora of Slovakia is represented by 5 taxa: *A. lycoctonum* subsp. *lycoctonum*, *A. moldavicum* subsp. *moldavicum*, *A. moldavicum* subsp. *hosteanum*, *A. moldavicum* nothosubsp. *confusum* and *A. × triste*.

A. lycoctonum subsp. *lycoctonum* is well represented in herbaria of Slovakia (150 vouchers revealed). It is mostly distributed in mountain regions in western and central parts of Slovakia, and also has one documented location from eastern region near the border with Ukraine (Fig. 7). *A. moldavicum* subsp. *moldavicum* is represented by 195 herbarium vouchers. It is scattered predominantly in eastern and central mountain regions of Slovakia, however it has also several locations from western part of country (Fig. 8). Similarly, *A. moldavicum* subsp. *hosteanum* (74 investigated vouchers) is represented in eastern and central regions of Slovakia, however it is not going so far to the west (Fig. 8). *A. moldavicum* nothosubsp. *confusum* was collected just once and more than 50 years ago (Spišská Nová Ves; leg. Májovský J., 23.08.1955; SAV). Collector indicated on the label just a city Spišská Nová Ves, which is located in Hornádska Kotlina, however did not indicate any other details (Fig. 3). *A. × triste* was also collected only once in 2005 (Meliata, vlhke lesy; leg. Karasová E., 16.06.2005; SAV), and it is represented only by 5 vouchers in SAV herbarium (Fig. 8). Before this, *A. × triste* was reported for Slovakia by Skalický (1990) and whereupon by Mitka (2008a), however without indication of strict locations. Both hybrids, *A. moldavicum* nothosubsp. *confusum* and *A. × triste*, seem to be very rare for Slovakia and require further field expeditions.

***A. moldavicum* nothosubsp. *confusum* (Grinț.) A. Novikov, stat. nov. (Fig. 3)**

(*A. moldavicum* subsp. *hosteanum* × *A. moldavicum* subsp. *moldavicum*)

Basionym: *A. moldavicum* var. *confusum* Grinț. in Săvulescu, Flora Repub. Pop. Române 2: (677), 1953.

Typus: Reg.: Craiova: in pratis ad pagum Baia de Fier. (Grințescu 1953).

Diagnostic characters: Perianth from dirty-blue to violet, carpels villous (with long protruding trichomes) only on dorsal side or/and suture.

Geographical distribution: In Carpathians and adjacent regions. Rarely in the Western Carpathians and slightly often in the Eastern Carpathians; often in the Małopolska Upland (Poland), Opillya and Voronyaky (Ukraine) (Mitka 2008a).

***Anthora* group**

Aconitum subgen. *Anthora* (DC.) Peterm. sect. *Anthora* DC. is represented in flora of Slovakia by single species, *A. anthora*, which is however characterized by high morphological variation. In general I had analyzed 152 specimens of *A. anthora* s.l., from which 66 specimens were included in further phenetic analysis. Among 115 total features I defined 26, which had variation for *A. anthora* and used them for next data processing (Tab. 1, pluses). Cluster analysis on the base of Ward's method revealed two clear groups – *anthora* and *jacquinii* (Fig. S8) with high bootstrap support. However UPGMA analysis showed that group *anthora* is not homogenous and consists from two main subclusters – *jacquinii 1* and *jacquinii 2* (Fig. 4). Cluster *anthora* is characterized by curved short trichomes covered helmet, pedicels, bracteoles, and inflorescence branches. Cluster *jacquinii 1* is represented by samples with protruding villous trichomes on helmet, bracteoles and pedicels (in some observations pedicels in lower part had mixed cover consisting from curved and protruding trichomes), and with mixed trichomes on inflorescence axes. Cluster *jacquinii 2* (samples *jacquinii 2280*



Fig. 3. *A. moldavicum* nothosubsp. *confusum* (Grintz.) A. Novikov, stat. nov. in SAV herbarium.

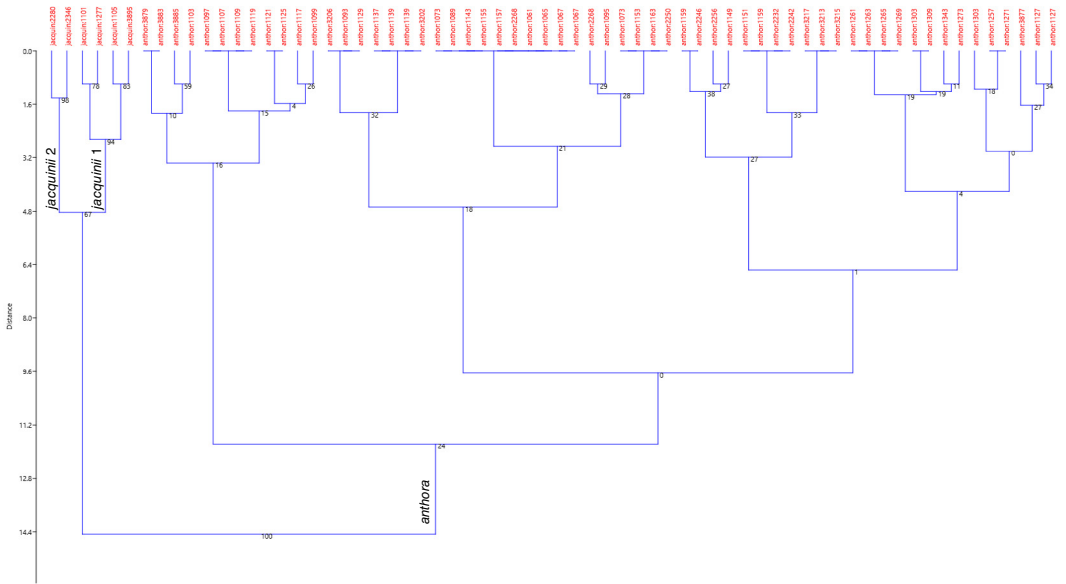


Fig. 4. Ward's dendrogram in combination with Euclidian distances for *Anthora* group with full dataset of traits.

and *jacquin*: 2346) is characterized by mixed pubescence of helmet and pedicels, protruding pubescence of bracteoles, and exclusively curved pubescence of inflorescence axes. Outstanding position of the cluster *jacquinii* 2 in Slovakia, while the shape of helmet and perianth colour (I do not consider here *A. pseudanthora* Błocki ex Pacz. with bluish or variegate perianth) are uninformative traits. Moreover, in cluster *jacquinii* 1 the variation from entire protruding to mixed pubescence of pedicels was observed, but there was no such variation in group *anthora* with always and entirely curved pubescent pedicels. Even on generalized dendrograms (Figs. 1 & S1), *jacquinii* samples are well delimited from other *anthora* samples with bootstrap support 96 (UPGMA) or 91 (Ward's method), with only two samples *jacquin*: 2280 and *jacquin*: 2346 occupying intermediate position between two main groups. Cluster *jacquinii* 2 (samples *jacquin*: 2280 and *jacquin*: 2346) most probably represent putative hybrid between *A. anthora* subsp. *anthora* and *A. anthora* subsp. *jacquinii*, which, however, requires detailed studies.

Correlation analysis revealed that curved pubescence of helmet is very strongly related to curved pubescence of pedicels (Pearson's $r=1$), bracteoles (Pearson's $r=1$), and inflorescence axes (Pearson's $r=0.80$). In the same time, protruding pubescence of the helmet is strongly correlated with protruding pubescence of pedicels (for pedicels above bracteoles Pearson's $r=1$, and for pedicels below bracteoles Pearson's $r=0.86$), protruding pubescence of bracteoles (Pearson's $r=0.80$), and mixed pubescence of inflorescence axes (Pearson's $r=1$). Mixed pubescence of helmet is very strongly correlated with mixed pubescence of pedicels (above bracteoles Pearson's $r=1$, and below bracteoles Pearson's $r=0.81$). Pubescence type of carpels, stems and petioles do not show any (even moderate) correlations, as well as the shape of helmet, colour (yellow or pale yellow) of perianth and structure of inflorescence.

Therefore, I can conclude that pubescence type of helmet, pedicels, bracteoles and inflorescence are the most significant features illuminating 3 main morphotypes of *A. anthora*. Cluster *jacquinii* 1 the variation from entire protruding to mixed pubescence of pedicels was observed, but there was no such variation in group *anthora* with always and entirely curved pubescent pedicels. Even on generalized dendrograms (Figs. 1 & S1), *jacquinii* samples are well delimited from other *anthora* samples with bootstrap support 96 (UPGMA) or 91 (Ward's method), with only two samples *jacquin*: 2280 and *jacquin*: 2346 occupying intermediate position between two main groups. Cluster *jacquinii* 2 (samples *jacquin*: 2280 and *jacquin*: 2346) most probably represent putative hybrid between *A. anthora* subsp. *anthora* and *A. anthora* subsp. *jacquinii*, which, however, requires detailed studies.

Taking into consideration that these plants grow in mixed populations (e.g., *jacquin*: 2280 was collected by J. Májovský in

1955 together with *anthor*:2276, *anthor*:2278 and *anthor*:2282), they can be considered as subspecies. Hence, cluster *jacquinii* 1 can be recognized as *A. anthora* subsp. *jacquinii*, and cluster *anthora* – as *A. anthora* subsp. *anthora*. Before, due to the taxonomic confusion, I erroneously reported for Slovakia *A. anthora* subsp. *eulophum* (Novikoff 2013), which does not occur here. *A. eulophum* Rchb. was reported by Reichenbach (1819, 1820) from Alps region including Liguria (France) and Helvetia (part of modern Switzerland), and is also often mentioned for Ukraine as independent taxon (Paczoski 1927; Visjulina 1953; Chopyk 1978; Andrienko & Peregrym 2012) or synonym of *A. anthora* (Tzvelev 2001; Didukh *et al.* 2004). Division of *A. anthora* onto subspecies for flora of Czechoslovakia was reported by Domin (1935), who recognized *A. anthora* subsp. *eu-anthora* and *A. anthora* subsp. *jacquinii* (Rchb.) Domin.

The problem with classification of *A. anthora* and related taxa has a long history starting from description by Linnaeus (1753). Reichenbach (1819, 1820, 1838, 1840) recognized several taxa in section *Anthoroideae* Rchb. viz *A. anthora*, *A. jacquinii* Rchb., *A. decandolii* Rchb., *A. anthoroideum* Rchb., *A. eulophum* Rchb. and *A. nemorosum* Rchb. He applied complex morphological analysis for delimitation of taxa, including pubescence of floral parts and shape of helmet. In particular, for Carpathians Reichenbach (1820) mentioned *A. jacquinii*, which, as he noted, differs by unique shape of helmet with elongated rostrum. In this paper Reichenbach (1820) also mentioned that *A. jacquinii* has glabrous helmets, what is very unusual for *Anthora* representatives from Carpathian region, and what I saw just once, in isolated refugial population in Stinka (Ukrainian Carpathians). However later Reichenbach (1840) published monograph containing illustration of *A. jacquinii* with villous helmet (Lám. LXXVII) and illustration of *A. anthora* γ *Jacquinianum* Rchb. (Lám. C) with glabrous flowers. Some of later researchers accepted level of species (Zapalowicz 1908; Paczoski 1927; Visjulina 1953) or level of subspecies Domin (1935) for this taxon, but in all cases authors

mentioned plants with helmet covered by protruding hairs contrasting with *A. anthora* (or *A. anthora* subsp. *anthora*) having curved pubescence of helmet. Same division is also accepted by Tzvelev (2001), who recognized *A. anthora* with pedicels and inflorescence axes covered exclusively by short curved trichomes, and *A. jacquinii* – by straight and mixed trichomes.

In our research, helmet shape showed high variation, however type of helmet pubescence seems to be a good discriminative trait for recognition of infraspecific taxa in *Anthora* group.

In general, *A. anthora* subsp. *anthora* is more widely represented in flora of Slovakia. I found 139 vouchers of *A. anthora* subsp. *anthora* in Slovakian herbaria sparsely collected from almost all territory of country (Fig. 9). *A. anthora* subsp. *jacquinii* there is represented only by 9 vouchers and occurs only in two main sites, one of which (Dreveník, obec Levoča) it shares with putative hybrid (represented by only 2 specimens in Slovakian herbaria) and other parental taxon – *A. anthora* subsp. *anthora* (Fig. 9).

Cammarum group

Aconitum subgen. *Aconitum* sect. *Cammarum* DC. is one of the most diverse group in sense of morphological variability and taxonomic representativeness in Slovakia. I used 121 specimens for analysis of 68 morphological traits (Tab. 1, pluses). UPGMA clustering did not showed any appropriated results (Fig. S10). Clustering by Ward's method separated two main clusters representing mostly *A. variegatum* and *A. lasiocarpum* samples. However *A. degenii* Gayer and *A. × pawlowskii* were randomly nested in these two main clusters (Fig. S11) and scattered on multidimensional plot (Fig. S12). Therefore I performed analysis on limited number of 15 traits, which are considered diagnostic for this group (Tab. 1, asterisks). This allowed obtaining better results, but some samples of *A. lasiocarpum* subsp. *lasiocarpum* were nested among *A. degenii* specimens, while samples of

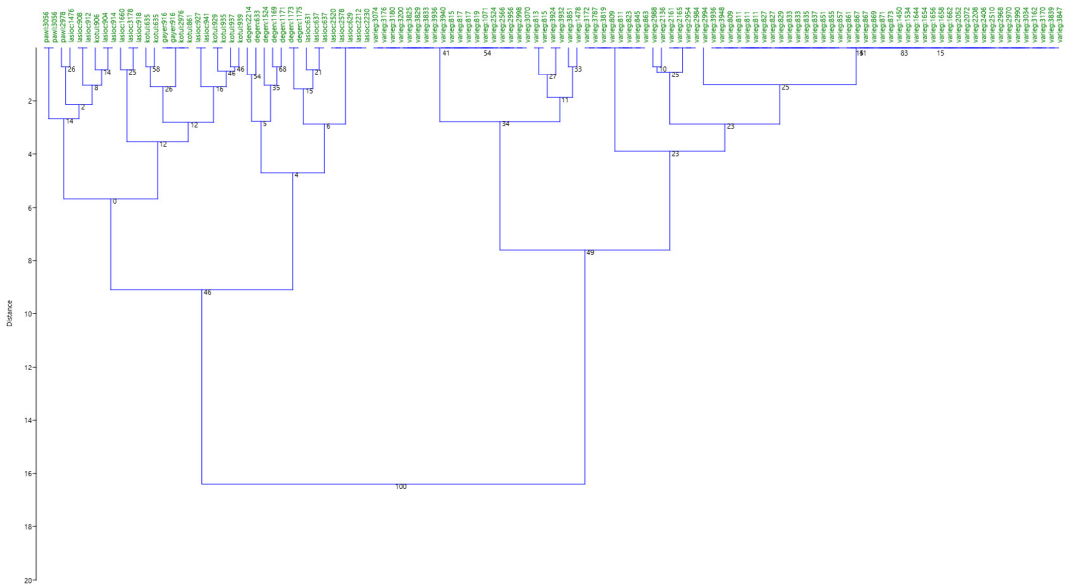


Fig. 5. Ward's dendrogram in combination with Euclidian distances for *Cammarum* group with dataset of 15 diagnostic traits.

A. × pawlowskii – among *A. lasiocarpum* subsp. *lasiocarpum* (Figs. 5 & S13). On the plot of multidimensional scaling, *A. × pawlowskii* was placed between its parental taxa *A. variegatum* and *A. lasiocarpum*, while *A. degenii* occupied marginal position (Fig. S14). The reason of such divergence of *A. degenii* samples was illuminated later – only cluster of *degen*: 1173 and *degen*: 1175 containing samples from Eastern part of Slovakia truly represents *A. degenii*, while other samples were misidentified and belong to *A. firmum* subsp. *maninense*.

Correlation analysis showed dependence of glabrous helmet with sutural pubescence of carpels (very strong, Pearson's $r=0.94$), glabrous pedicels (strong, Pearson's $r=0.84$ for upper and Pearson's $r=0.75$ for lower part of pedicels), glabrous inflorescence axes (strong, Pearson's $r=0.65$), what is typical for *A. variegatum*. There was also moderate positive correlation (Pearson's $r=0.59$) between glabrous helmet and nectarines not reaching the helmet top, what also characterizes *A. variegatum*. *A. variegatum* has elongated conical helmet, while the shape of helmet in *A. lasiocarpum* varies. In the same time, *A. degenii* has often hemispherical or

slightly elongated helmet and glabrous carpels, what was reflected in strong positive correlation (Pearson's $r=0.7$) between hemispherical helmet and glabrous carpels in my analysis too (Tab. 1).

Sect. *Cammarum* in flora of Slovakia is represented by 6 taxa: *A. variegatum* subsp. *variegatum*, *A. lasiocarpum* subsp. *lasiocarpum*, *A. lasiocarpum* subsp. *kotulae*, *A. × pawlowskii* (*A. lasiocarpum* × *A. variegatum*), *A. degenii* subsp. *degenii* and *A. × gayeri* (*A. degenii* × *A. lasiocarpum*).

A. degenii subsp. *degenii* is reported here for the first time for flora of Slovakia. All other taxa were already reported before (Mitka & Starmühler 2000; Starmühler 2002; Mitka 2003). Mitka (2003) also reported *A. × hebegynum* (*A. degenii* × *A. variegatum*) for flora of Slovakia, however this taxon was later rediscovered and nested in *A. degenii* subsp. *degenii* as var. *intermedium* (Zapař.) Mitka (Ilnicki & Mitka 2011).

In total, I found in Slovakian herbaria 190 specimens of *A. variegatum* subsp. *variegatum* collected mostly from central Slovakia, however few samples also were collected from eastern

regions (Fig. 10). *A. lasiocarpum* subsp. *lasiocarpum* is represented by 18 (Fig. 11), and *A. lasiocarpum* subsp. *kotulae* – by 11 vouchers collected from central and eastern regions of Slovakia (Fig. 11). *A. × pawlowskii* was found in herbaria only twice (Fig. 12). *A. degenii* subsp. *degenii* in analyzed collections is represented only by 2 vouchers (Fig. 12), and *A. × gayeri* – by single specimen hosted in SAV (Fig. 12). However Mitka (2003) reported 1 more voucher of *A. × gayeri* from Bukovské Vrchy, which is deposited in herbarium of Jagiellonian University in Kraków (Rabia Skała, 1150 m, J. Mitka, P. Bochenek, J. Terray, 23.08.1995; KRA).

A. lasiocarpum, *A. × pawlowskii*, *A. degenii* and *A. × gayeri* are rare for flora of Slovakia. Last two taxa are distributed here on the edge of their natural range.

Acomarum group

Aconitum subgen. *Aconitum* nothosect. *Acomarum* Starmühler in flora of Slovakia is represented by cultivated intersectional hybrid *A. × cammarum* (*A. napellus* [?] × *A. variegatum*). *A. × cammarum* was reported for Czechoslovakia by Skalický (1982), and also mentioned by Domin (1935) and Dostál (1958) as *A. × stoerckianum* (= *A. napellus* subsp. *neomontanum* (Wulfen) Gayer × *A. variegatum* subsp. *euvariegatum* Dostál). I did not include this taxon neither in morphological nor in chorological analyses, because it is ornamental plant and therefore there is low number of specimens (only 17 were observed) deposited in herbaria (Fig. 13). Sometimes *A. × cammarum* can be found escaped into the wild nature.

Skalický (1966) also reported for Czechoslovakia *A. × exaltatum* Bernh. ex Rchb. (*A. plicatum* Köhler ex Rchb. subsp. *plicatum* × *A. variegatum* subsp. *variegatum*), which is morphologically close to *A. × cammarum* (Mitka 2003). However, there are no mentions of this hybrid exactly from Slovakia.

Starmühler (2002) mentioned for Slovakia two more taxa for this nothosection, however without details on their distribution – *A. × lengyelii* Gayer nothosubsp. *lengyelii*

(*A. firmum* subsp. *firmum* × *A. variegatum* subsp. *variegatum*) and *A. × lengyelii* nothosubsp. *walasii* Mitka (*A. firmum* subsp. *moravicum* × *A. variegatum* subsp. *variegatum*). Later these two nothotaxa were also mentioned in Starmühler & Mitka (2001), but only for territory of Poland and are not listed for Slovakia. In personal communication W. Rottensteiner also noted that these nothotaxa are highly possible to be found in Slovakia, since both parental taxa are present there.

In Mitka (2003) *A. × lengyelii* nothosubsp. *lengyelii* and *A. × lengyelii* nothosubsp. *walasii* were rediscovered as *A. × berdaui* Zapal. nothosubsp. *berdaui* and *A. × berdaui* nothosubsp. *walasii* (Mitka in Starmühler et Mitka) Mitka, respectively. Mitka (2003) listed only 2 specimens of *A. × berdaui* nothosubsp. *berdaui* from Slovakian Tatra Mts. (*Aconitum napellus* L., Podspady nad Jaworzynką; leg. A. Rogalski, 25.07.1878.) and Slovenský Raj (Plantae exsiccatae regni Hungariae, Comit. Szepes, in m. Hollókő pr. Sztraczena; G. Lengyel, 01.08.1901; BP) (Fig. 13). In personal communication J. Mitka also provided me with two more recently rediscovered locations – one in Tatras (Tatras, Javorova Dolina; leg. A. Sutkowska, 18.08.2009; herb. J. Mitka) and other in Malá Fatra (Malá Fatra, Terchová; leg. J. Mitka, 31.07.2009; KRA 0464764). However, Mitka (2003) does not mention *A. × berdaui* nothosubsp. *walasii* for Flora of Slovakia. During our revision of Slovakian herbaria, I did not find specimens of *A. × berdaui*, and therefore this nothospecies is provided here on the base of reports of Starmühler (2002) and Mitka (2003) only. Presence and distribution of *A. × berdaui* nothosubsp. *walasii* in Slovakia still require confirmation in field.

Aconitum group

I performed cluster analysis for 104 specimens and 82 morphological traits (Tab. 1, pluses), which revealed division of *Aconitum* subgen. *Aconitum* sect. *Aconitum* in flora of Slovakia on two main clusters – *firmum* and *moravicum* (Figs. S15 & S16). Cluster *firmum* includes

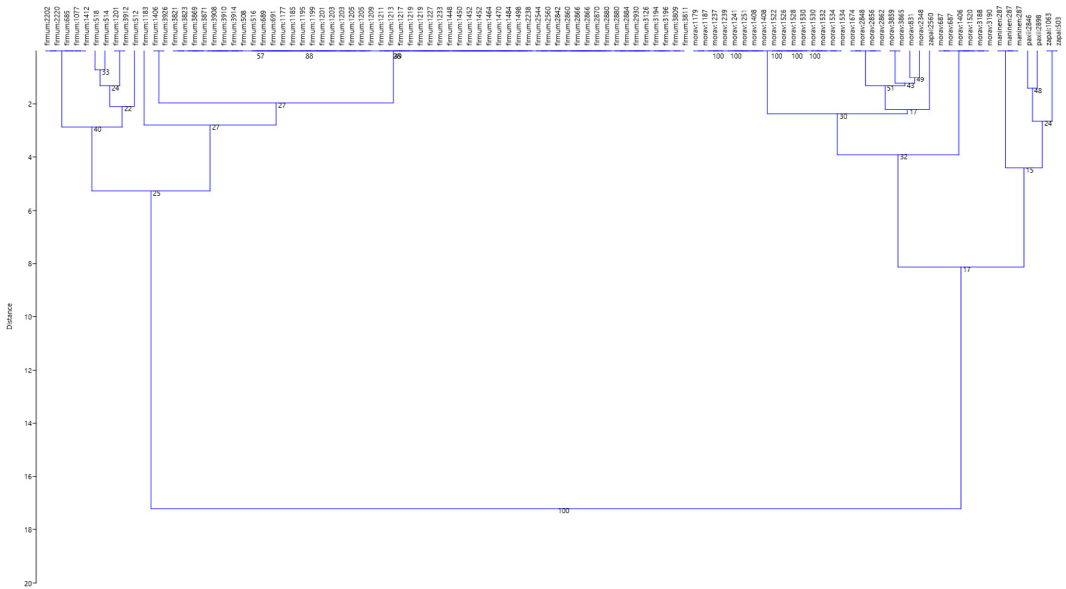


Fig. 6. Ward's dendrogram in combination with Euclidian distances for *Aconitum* group with dataset of 23 diagnostic traits.

A. firmum subsp. *firmum*. Cluster *moravicum* consisted mostly from *A. firmum* subsp. *moravicum* samples, but also included randomly dispersed samples of *A. firmum* subsp. *maninense*, *A. firmum* nothosubsp. *paxii* and *A. firmum* nothosubsp. *zapolowiczii* (Fig. S17). All samples of *A. firmum* subsp. *maninense* had localized position together, which also confirmed by multidimensional scaling, however they were nested inside the cluster *moravicum*. Therefore, I performed analysis for limited number of 23 morphological traits considered as diagnostic for *Aconitum* group and obtained trees were better fitting accepted taxonomic division – there were independent and clearly distinguished clusters of *A. firmum* subsp. *firmum*, *A. firmum* subsp. *moravicum* and *A. firmum* subsp. *maninense*, however hybrids (*A. firmum* nothosubsp. *paxii* and *A. firmum* nothosubsp. *zapolowiczii*) were still overlapped with other taxa (Figs. 6, S18 & S19).

For this limited number of 23 morphological traits I provided correlation analysis (Tab. 1, asterisks), which showed very strong positive correlation between glabrous helmet and glabrous pedicels (Pearson's $r=0.9$ for pedicels

above bracteoles and Pearson's $r=0.88$ for pedicels below bracteoles), glabrous bracteoles (Pearson's $r=0.88$) and glabrous inflorescence axes (Pearson's $r=0.88$), which are typical for *A. firmum* subsp. *firmum*. Similarly, this analysis showed very strong positive correlation between curved pubescent helmet and curved pubescent pedicels (Pearson's $r=0.93$ for pedicels above bracteoles and Pearson's $r=0.83$ for pedicels below bracteoles), curved pubescent bracteoles (Pearson's $r=0.91$) and curved pubescent inflorescence axes (Pearson's $r=0.87$), what characterizes *A. firmum* subsp. *moravicum*. Very strong positive correlation was found also for pilose pubescence of helmet and pilose pubescence of pedicels (Pearson's $r=1$ both for pedicels above and below the bracteoles), and pilose pubescence of inflorescence axes (Pearson's $r=0.86$); as well as strong positive correlation (Pearson's $r=0.64$) – for pilose pubescences of helmet and bracteoles, what in general characterizes *A. firmum* subsp. *maninense*. Finally, strong positive correlation (Pearson's $r=0.7$) was also shown for mixed pubescence of helmet and mixed pubescence of pedicels above the level of bracteoles, what

identifies *A. firmum* nothosubsp. *paxii*. This analysis did not reveal any strong or even moderate correlations for shape of helmet, which can vary from crescent to slightly conical even in the same inflorescence.

Starmühler (2002) and Starmühler & Mitka (2001) reported for Slovakia also hybrid between *A. firmum* subsp. *firmum* × *A. firmum* subsp. *maninense* with glabrous perianth and pilose pedicels, discovered on the base of single specimen deposited in herbarium of Natural History Museum in Sibiu, Romania (Hungaria, comitatus Szepes, Montes Bélaenses, in valle Drechslerhäuschen [Dolina siedmich prameňov valley] sub monte Stirnberg, alt. cca. 1400–1500 m, solo calc.; 01.09.1907; leg. E.G. Nyárady; SIB). However in Slovakian herbaria I did not found such specimens.

In total I found and analyzed 152 specimens of *A. firmum* in Slovakian herbaria. The most represented there is collection of *A. firmum* subsp. *firmum* counting 121 herbarium vouchers mostly from central Slovakia (Fig. 14). *A. firmum* subsp. *moravicum* is represented by 26 vouchers (Fig. 14). I found just few herbarium vouchers of *A. firmum* subsp. *maninense* in SLO, SAV and BRA herbaria (Fig. 14). Six more vouchers of *A. firmum* subsp. *maninense* are mentioned by Mitka (2003) from Vysoké Tatry and Stražovské vrchy, and deposited in herbaria SIB (Natural History Museum in Sibiu, Romania), PRC (Charles University in Prague, Czech Republic) and KRA (Jagiellonian University in Kraków, Poland). Moreover, Mitka *et al.* (2015) noted that *A. firmum* subsp. *maninense* is characterized by high genetic divergence and probably is a relict taxon isolated from other representatives of *Aconitum* group.

Morphologically *A. firmum* subsp. *maninense* is similar to *A. degenii* subsp. *degenii* and can be easily misidentified on the base of protruding glandular cover of floral parts.

A. firmum nothosubsp. *paxii* was represented only by 2 herbarium vouchers, and *A. firmum* nothosubsp. *zapalowiczii* – only by 3 specimens, one of which is mounted on the same herbarium sheet with *A. firmum* subsp. *firmum* (Fig. 14). Hence I can conclude that *Aconitum* subgen.

Aconitum sect. *Aconitum* in flora of Slovakia is represented by 5 *A. firmum* subspecies and one unnamed hybrid.

Key for identification

1. Helmet cylindrical, about 2.5–3 times higher than wide; nectaries do not reach the top of helmet and have coiled spurs; leaf laminas incised less than ½, with very broad segments; semirostrate plants with rhizomes 2

1*. Helmet from crescent to elongated conical, but its height does not exceed 2.5 of width; nectaries reach or not the top of helmet, their spurs from semi-spiral curved to ecapitate; leaf laminas incised more than ½, with segments from broad to narrow linear; nonrostrate plants with tubers 6

2. Perianth from white-yellowish to yellow; helmet densely covered by short curved trichomes; carpels glabrous or pubescent by short curved trichomes
A. lycoctonum* subsp. *lycoctonum

2*. Perianth has other coloration 3

3. Perianth yellowish-bluish; helmet densely covered by short curved trichomes; carpels glabrous or pubescent by short curved trichomes ***A. × triste***

3*. Perianth from dirty-blue to purple-violet; helmet more or less covered by short curved trichomes or, rarely, glabrous 4

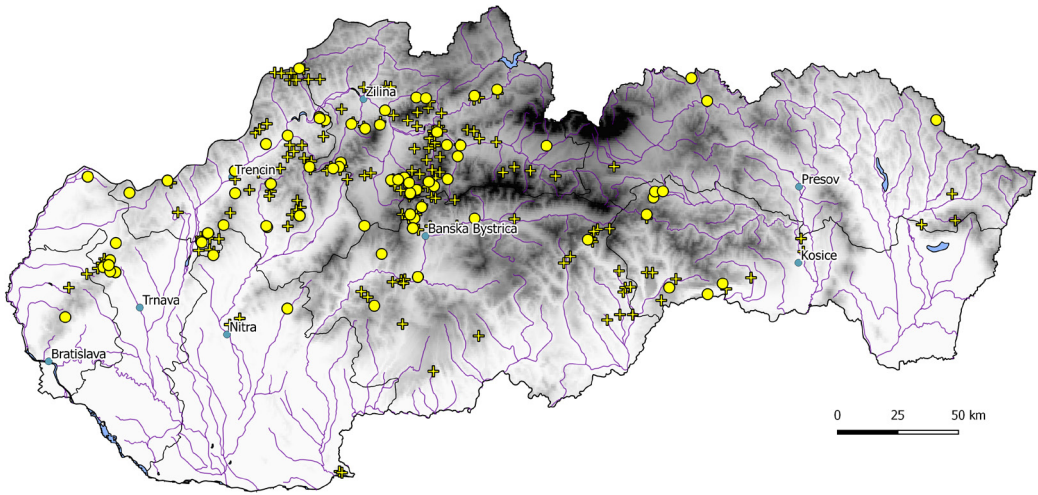
4. Carpels glabrous ***A. moldavicum* subsp. *moldavicum***

4*. Carpels pubescent 5

5. Carpels entirely covered by short curved trichomes ***A. moldavicum* subsp. *hosteanum***

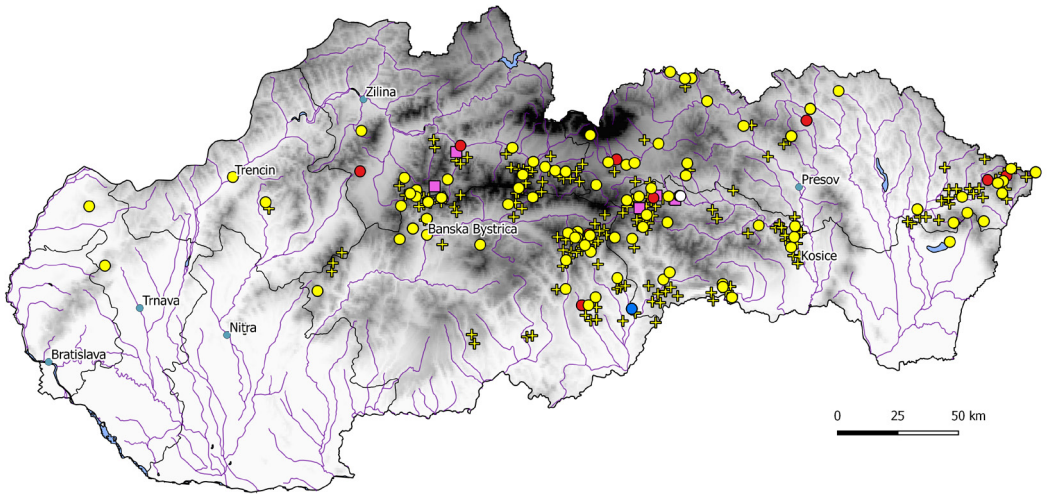
5*. Carpels have long protruding trichomes only on dorsal side or/and suture
A. moldavicum* nothosubsp. *confusum

6. Perianth from yellowish to yellow, persists after blooming; helmet hemispherical or slightly elongated, sometimes with protruded front part; nectaries reach the top of helmet, with semi-spiral curved spurs; leaves deeply incised, with very narrow linear segments; tubers from ovate to napiform, sometimes divided 7
- 6*. Perianth usually dark-blue, but sometimes violet, lilac, white or variegate, deciduous after blooming; nectaries reach or not the top of helmet, with spurs from backward bent to ecapitate; leaves incised near $\frac{1}{2}$ – $\frac{2}{3}$, with more or less broad segments; tubers from ovate to napiform 9
7. Helmet, pedicel, and bracteoles covered by short curved trichomes; carpels covered by curved or protruding trichomes; inflorescence axes covered by short curved trichomes
A. *anthora* subsp. *anthora*
- 7*. Pubescence type differs 8
8. Helmet, pedicel, and bracteoles covered by protruding trichomes; carpels covered by protruding trichomes; inflorescence axes covered by mixed curved and protruding trichomes **A. *anthora* subsp. *jacquinii***
- 8*. Helmet and pedicels covered by mixed curved and protruding trichomes; bracteoles and carpels covered by protruding trichomes; inflorescence axes covered by curved trichomes **A. *anthora* subsp. *anthora* × A. *anthora* subsp. *jacquinii***
9. Helmet from hemispherical to elongated conical; nectaries usually do not reach the top of helmet and have hooked or backward bent spurs; tubers ovate with short stolones or napiform; plants usually high with ramified inflorescences 10
- 9*. Helmet from crescent to hemispherical; nectaries reach the top of helmet and have capitate or ecapitate spurs; tubers napiform; plants usually low with compact or dense ramified inflorescences 18
10. Helmet elongated conical, often with pronounced rostrum; tubers ovate with stolones 11
- 10*. Helmet from hemispherical to slightly elongate; tubers ovate or napiform 14
11. Helmet usually glabrous, rarely covered by short solitary trichomes; pedicels and bracteoles glabrous; carpels have trichomes only along ventral sutures; inflorescence axes glabrous
A. *variegatum* subsp. *variegatum*
- 11*. Helmet, pedicels (at least above the bracteoles) and bracteoles covered by protruding glandular (pilose) trichomes 12
12. Pedicels and bracteoles entirely covered by protruding glandular trichomes; inflorescence axes covered by protruding pilose trichomes; carpels entirely pilose
A. *lasiocarpum* subsp. *lasiocarpum*
- 12*. Pedicels covered by protruding glandular trichomes only above the bracteoles, below the bracteoles they are glandular and/or eglandular pubescent, sparsely glandular pilose or even glabrous. In very rare cases pedicels are entirely glabrous. Inflorescence axes have different cover, from sparsely pilose to glabrous 13
13. Carpels entirely pilose **A. *lasiocarpum* subsp. *kotulae***
- 13*. Carpels have trichomes only along ventral sutures **A. × *pawlowskii***
14. Helmet glabrous or only sparsely covered by protruding trichomes; carpels sterile; tubers napiform 15
- 14*. Helmet entirely covered by short protruding trichomes; pedicels and bracteoles covered by short protruding trichomes (sometimes less covered or glabrous in lower part of pedicels); tubers ovate 17
15. Helmet glabrous; carpels glabrous and sterile; pedicels have trichomes just near the



Taxa observed in herbaria **Taxa by Kmet'ová (1982)**
 ● *A. lycoctonum* subsp. *lycoctonum* ✚ *A. lycoctonum*

Fig. 7. Map of *Aconitum lycoctonum* distribution in Slovakia.



Taxa observed in herbaria **Taxa by Kmet'ová (1982)**
 ● *A. x triste* ✚ *A. moldavicum*
 ○ *A. moldavicum* nothosubsp. *confusum*
 ● *A. moldavicum* subsp. *hosteanum* **Taxa by Mitka (2008)**
 ● *A. moldavicum* subsp. *moldavicum* ■ *A. moldavicum* subsp. *moldavicum*

Fig. 8. Map of *Aconitum moldavicum* and *A. x triste* distribution in Slovakia.

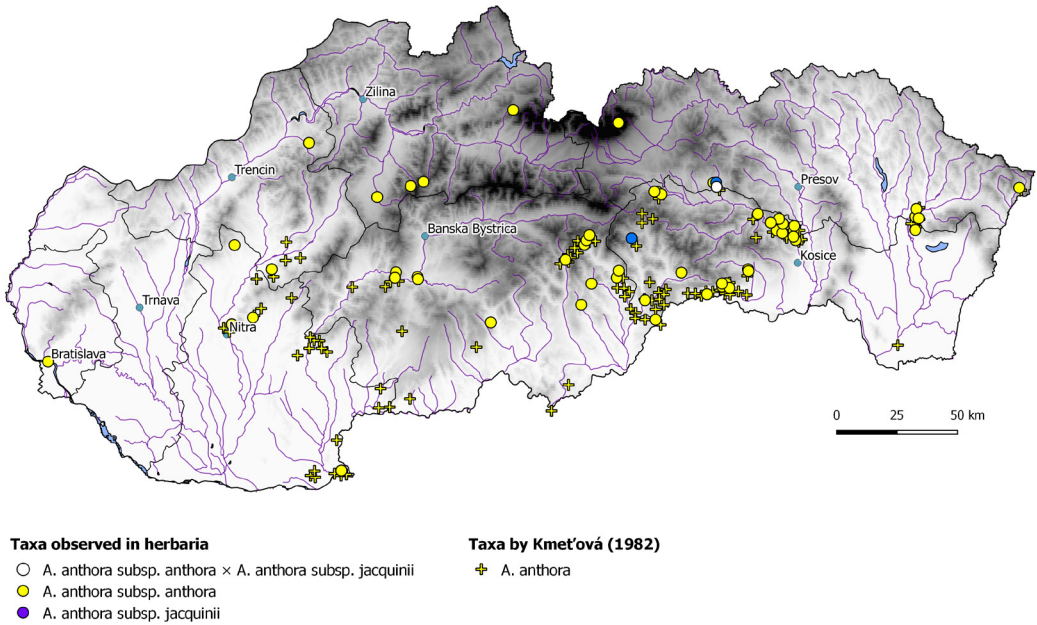


Fig. 9. Map of *Aconitum anthora* distribution in Slovakia.

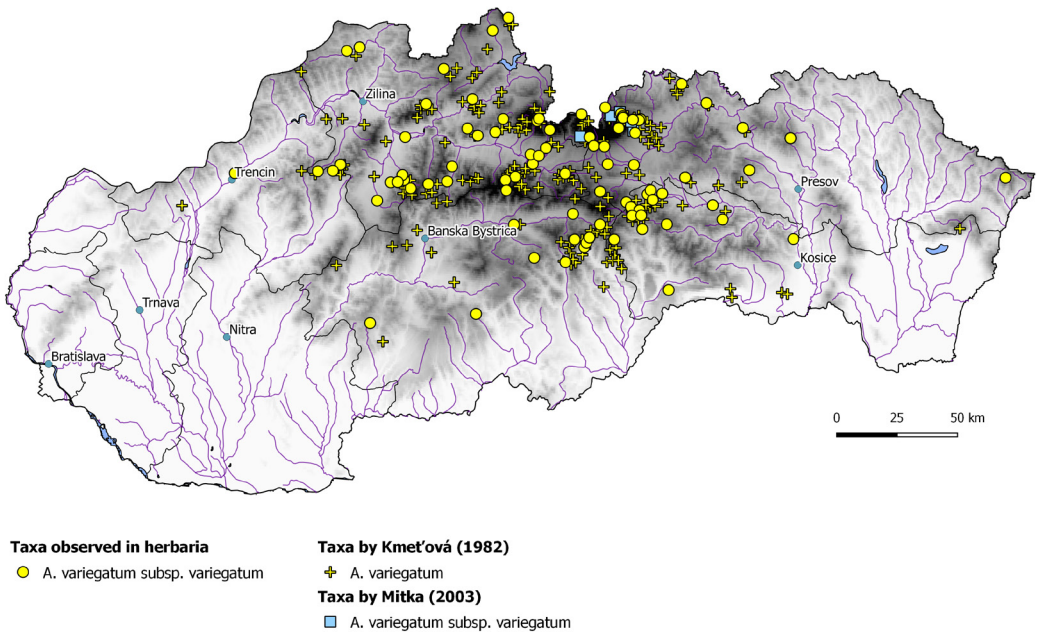


Fig. 10. Map of *Aconitum variegatum* distribution in Slovakia.

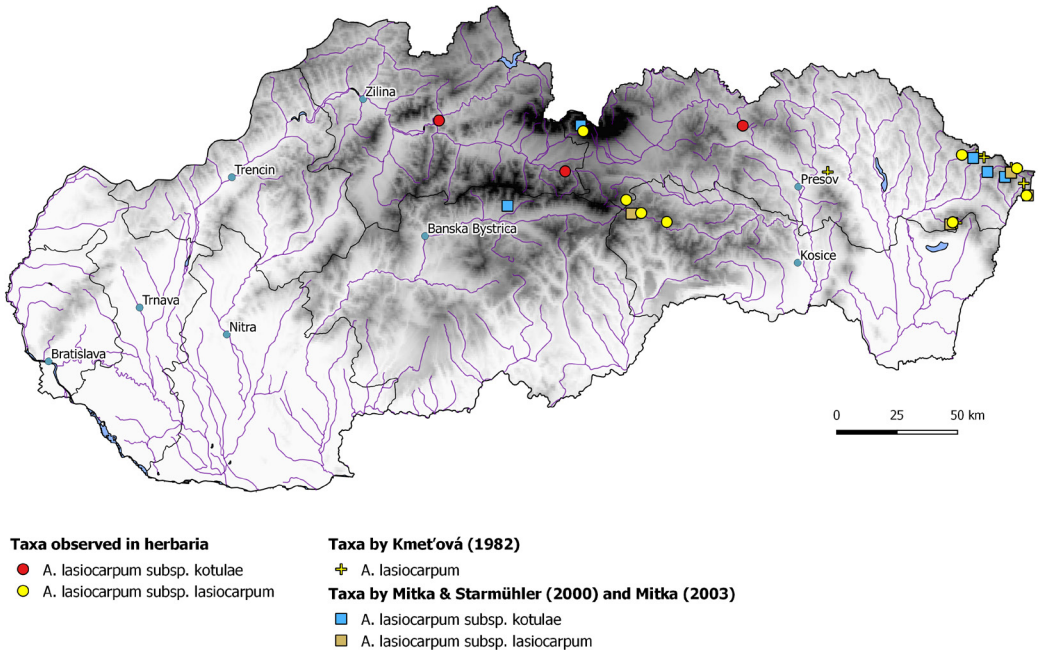


Fig. 11. Map of *Aconitum lasiocarpum* distribution in Slovakia.

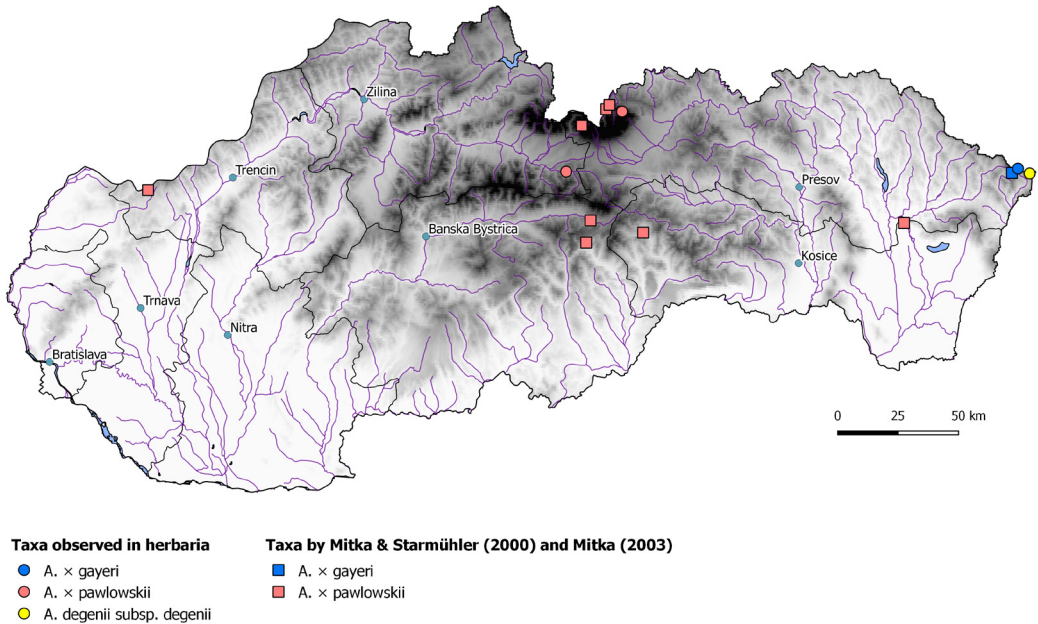


Fig. 12. Map of *Aconitum degenii*, *A. x gayeri* and *A. x pawlowskii* distribution in Slovakia.

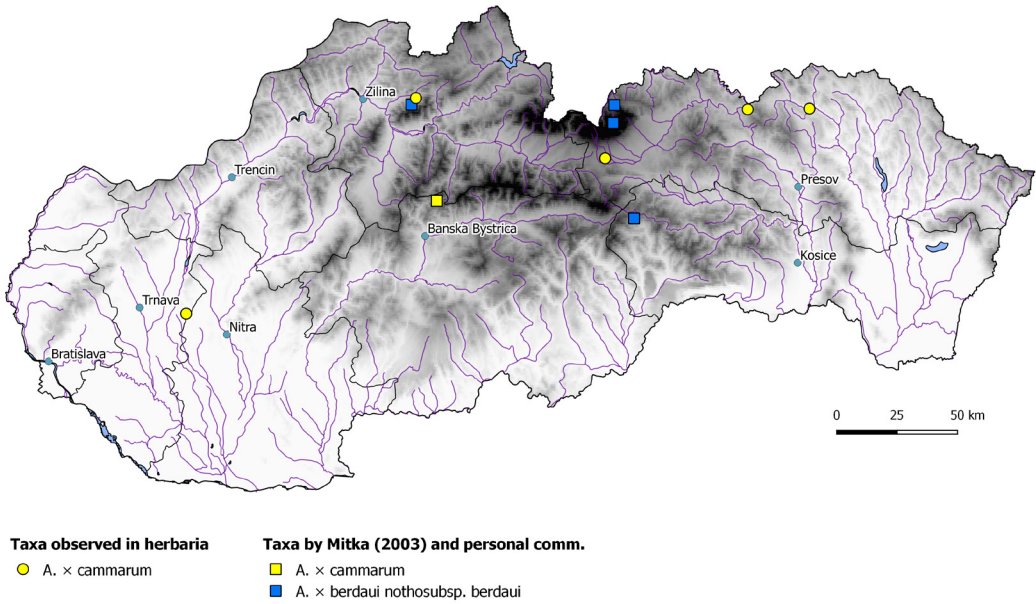


Fig. 13. Map of *Aconitum x cammarum* and *A. x berdaui* distribution in Slovakia.

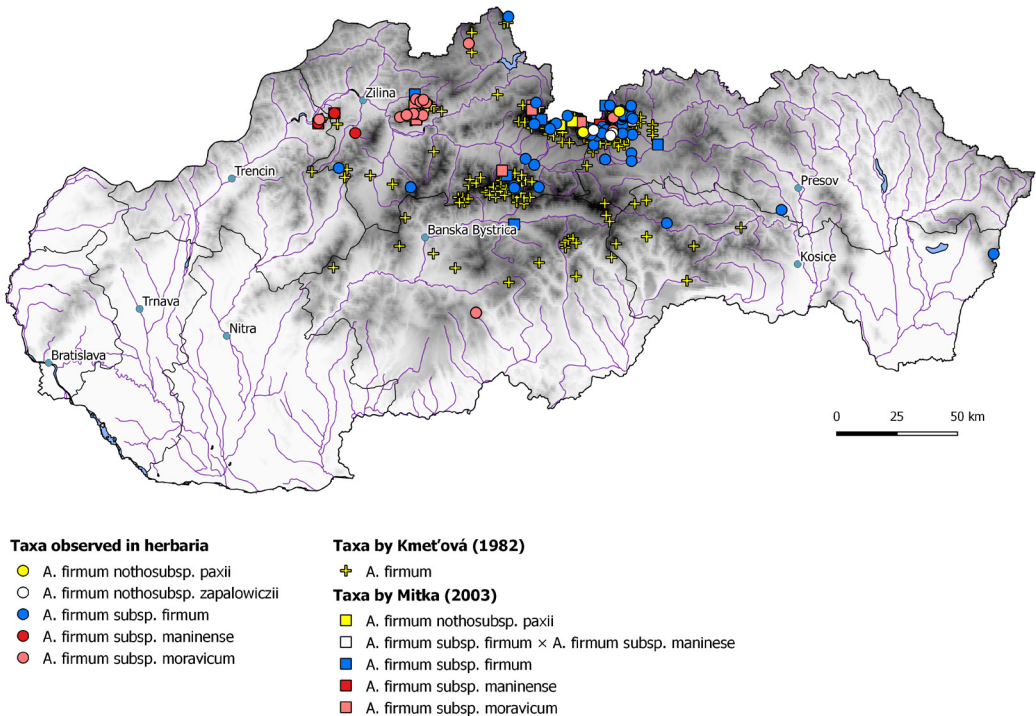


Fig. 14. Map of *Aconitum firmum* distribution in Slovakia.

receptacle; bracteoles glabrous or covered by solitary trichomes *A. × cammarum*

15*. Helmet glabrous or only sparsely covered by protruding trichomes; carpels glabrous or pilose along ventral sutures, sometimes with solitary trichomes on dorsal side **16**

16. Helmet, pedicels, bracteoles and inflorescence axes glabrous
A. × berdaui nothosubsp. berdaui

16*. Helmet glabrous or only sparsely covered by protruding trichomes; pedicels and bracteoles covered by curved trichomes; inflorescence axes covered by short curved trichomes or almost glabrous *A. × berdaui nothosubsp. walasii*

17. Carpels glabrous ... *A. degenii subsp. degenii*

17*. Carpels densely covered by protruding trichomes *A. × gayeri*

18. Helmet glabrous **19**

18* Helmet covered by trichomes **21**

19. Pedicels, bracteoles, and inflorescence axes glabrous; carpels glabrous or rarely pubescent on dorsal side *A. firmum subsp. firmum*

19*. Pedicels covered by trichomes **20**

20. Pedicels and bracteoles covered by mixed curved and protruding trichomes; inflorescence axes covered by mixed or curved trichomes; carpels glabrous or slightly pubescent
A. firmum nothosubsp. zapalowiczii

20*. Pedicels at least in upper parts are covered by protruding pilose trichomes; iflorescence axes glabrous; carpels glabrous or slightly pubescent on dorsal side *A. firmum subsp. firmum × A. firmum subsp. maninense*

21. Helmet, pedicels, bracteoles and inflorescence axes covered by short curved trichomes; carpels usually glabrous, rarely pubescent by solitary curved trichomes
A. firmum subsp. moravicum

21*. Type of pubescence differs **22**

22. Helmet, pedicels, bracteoles and inflorescence axes covered by protruding pilose trichomes; carpels usually glabrous, rarely covered by pilose trichomes
A. firmum subsp. maninense

A. firmum subsp. maninense

22*. Helmet, pedicels, and bracteoles covered by mixed curved and protruding trichomes; inflorescence axes covered by mixed or curved trichomes; carpels glabrous or slightly pubescent *A. firmum nothosubsp. paxii*

Conclusions

This investigation showed that genus *Aconitum* in Slovakia is represented by 21 taxa of species and infraspecific levels, and probably contains two more unnamed hybrids belonging in general to 3 subgenera. Three new taxa for flora of Slovakia (*A. degenii* subsp. *degenii*, *A. moldavicum* subsp. *hosteanum* and *A. moldavicum* nothosubsp. *confusum* stat. nov.) have been ascertained, for one of which the new taxonomic status has been proposed. General taxonomic structure of the genus *Aconitum* in flora of Slovakia is next:

Subgen. *Lycotconum* (DC.) Peterm.
Sect. *Lycotconum* DC.

Ser. *Lycotconia* Tamura et Lauene

A. lycotconum L. em. Koelle

subsp. *lycotconum*

A. × triste (Rchb.) Gáyér

A. moldavicum Hacq.

subsp. *moldavicum*

subsp. *hosteanum* (Schur)

Graebner et P. Graebner in
Aschers. et Graebner

nothosubsp. *confusum* (Grinč.)

A. Novikov, stat. nov.

Subgen. *Anthora* (DC.) Peterm.

Sect. *Anthora* DC.

A. anthora L.

subsp. *anthora*

subsp. *jacquinii* (Rchb.) Domin

subsp. *anthora* × subsp. *jacquinii*

Subgen. *Aconitum*Sect. *Cammarum* DC.Ser. *Variegata* Steinberg ex Starmühl.*A. variegatum* L.subsp. *variegatum*Ser. *Toxicum* (Rchb.) Mucher*A. lasiocarpum* (Rchb.) Gáyersubsp. *lasiocarpum*subsp. *kotulae* (Pawl.) Starmühl.

et Mitka

A. degenii Gáyersubsp. *degenii**A.* × *gayeri* Starmühl.Nothoser. *Toxigata* Starmühl.*A.* × *pawlowskii* Mitka et Starmühl.Nothosect. *Acomarum* Starmühl.*A.* × *cammarum* L. em. Fries*A.* × *berdau* Zapal.nothosubsp. *berdau*nothosubsp. *walasii* (Mitka in
Starmühler et Mitka) MitkaSect. *Aconitum**A. firmum* Rchb.subsp. *firmum*subsp. *moravicum* Skalickýsubsp. *maninense* (Skalický)

Starmühl.

nothosubsp. *zawalowiczii* Starmühl.nothosubsp. *paxii* Starmühl.subsp. *firmum* × subsp. *maninense*

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Appendix 1. Specimens examined in herbaria

Aconitum lycoctonum subsp. *lycoctonum*

SLOVAKIA: Muránsky hrad; leg. Hubová O., Kmetová E., 06.06.1968; (SAV). — Vrch Riečky [Mt. Vrchriečky near village Litmanová]; leg. Zahradníková K., 05.07.1972; (SAV). — Považský Inovec, Marhát, *Aceti-Fagetum*; 690 m a.s.l.; leg. Michalko J., Magic D., 03.06.1965; (SAV). — Veľká Fatra: Blatnická dolina, na svahu SE od Kečerovky [Kačárová]; 700 m a.s.l.; leg. Kováčiková, 20.09.1974; (SAV). — Veľká Fatra: Gaderská dolina; leg. Futák J., Jasičová M., 25.06.1963; (SAV). — Oravský Podzámok: vrch Skalka; 530 m a.s.l.; leg. Futák J., 08.07.1959; (SAV × 2 vouchers). — Bánovce nad Bebravou [?], *Calamagrostis*; 803 m a.s.l.; leg. Futák J., 12.07.1960; (SAV). — Manínska nížina, pri potoku, vápenc; 350 m a.s.l.; leg. Futák J., 05.06.1958; (SAV × 2 vouchers). — Orava: Podbiel, Biela skala; leg. Futák J., Jasičová M., 24.06.1963; (SAV). — Ostrý kameň; leg. Maglocky Št., 09.08.1967; (SAV). — Lubochňa; leg. Futák J., Jasičová M., 23.06.1963; (SAV). — Veľká Fatra: [Kráľova studňa], svetlina v bučine pod chatou; 1100 m a.s.l.; leg. Grebenščikov O., 02.07.1953; (SAV). — Bánovce nad Bebravou [?], *Fagetum*; 750 m a.s.l.; leg. Futák J., 15.07.1960; (SAV). — Štubn. Teplice, Kostelecká dol., svetliny; leg. Horák P., 13.07.1942; (SAV). — Smolenice – hrebeň. Malé Karpaty; leg. Krippelová T., 21.06.1960; (SAV × 3 vouchers). — Strážovská hornatina, dolomit, bučina; 540 m a.s.l.; leg. Futák J., 26.07.1961; (SAV × 4 vouchers). — Bánovce nad Bebravou [?], Kanisova skala, dolomit; 700 m a.s.l.; leg. Futák J., 15.07.1960; (SAV). — Záruby, hreben; 700 m a.s.l.; leg. Horák P., 07.1923; (SAV). — Tematínske kopce, Modriná; leg. Michalko J., 03.07.1957; (SAV). — pri Jablonici, okraj lesa; leg. Krippelová T., 07.1967; (SAV). — Strážovská hornatina; leg. Futák J., Hubová O., 21.05.1954; (SAV). — Malé Karpaty: vápencový hrebeň západne od zrúcaniny Ostrý kameň v bučine; 500 m a.s.l.; leg. Kollár J., Zahradníková K., 12.06.1968; (SAV). — Stráž. vrchy, medzi obcami Mníchova Lehota a Kostolné Mítice na hrebni Maiková a Lapkov kameň, v lese pri ceste v bučine; 600 m a.s.l.; leg. Schidlay E., 01.08.1950; (SAV). — Vlhké... pod vrcholom Javoriny; leg. ?, 19.07.1911; (SAV). — Revúca [Liptovské Revúce]. Vél. Fatra; 1000 m a.s.l.; leg. Nábelek V., 07.1936; (SAV). — Slovenský raj: Sokolia dolina, dolná časť; 600 m a.s.l.; leg. Hubová O., 16.08.1962; (SAV). — Strečno; leg. Popovič, 21.06.1959; (SAV). — Tematínske kopce, pri potoku Kališťa za obcou Modrová; 220 m a.s.l.; leg. ?, 31.05.1957; (SAV). — Lubina: Veľká Javorina [Biele Karpaty] – pri turist. chate – prenesené na pokusný pozemok Bratislava–Patrónka (kultúra 3 roky); leg. Zahradníková K., 16.07.1963; (SAV). — Považský Inovec, kopce nad Tematínskym Podhradím, vápenc, lúčina; 500 m a.s.l.; leg. Michalko J., Zahradníková K., 08.07.1959; (SAV). — Rajecké Teplice, in monte Tlstá hora, in fageto; 450 m a.s.l.; leg. Schidlay E., 10.06.1947; (SAV). — Biele Karpaty, Vrbovce, les nad žel. stanicou Vrbovce, pod vrchom Stráň (550,3 m.m.n.), pri červenej turistickej značke (cesta M. Kudeříkové); 550 m a.s.l.; leg. Perný M., 12.06.1998; (SAV). — Strážovská hornatina, Strážov (k. 1240 m), lúčne porasty pri kóte; 1150 m

a.s.l.; leg. Kollár J., 21.07.1966; (BRA × 2 vouchers). — Pohoří Rokoše: Smrkový les pod vrcholem Rokoš; 950 m a.s.l.; leg. Dvořák J., 14.06.1979; (BRA). — Pohoří Rokoše: in fageto sub cacumine montis Rokoš; 960 m a.s.l.; leg. Dvořák J., 05.06.1975; (BRA). — Považský Inovec: dolina Kališťa na V od obce Modrová, pri lesnej ceste v rekr. stredisku; 240 m a.s.l.; leg. Mucina L., 26.06.1974; (BRA). — Slovenský raj: Prielom Hornádu, vápence; 550 m a.s.l.; leg. Hajdúk J., 11.06.1959; (BRA). — Malé Karpaty: Smolenice, les pod Havraňou skalou, tmavosivý vápenc; 400 m a.s.l.; leg. Vozárová M., 10.07.1996; (BRA × 2 vouchers). — Strážovské vrchy, dolina medzi V. Málenicou (909 m) a Rohatínom (832,4 m), v bučine; 520 m a.s.l.; leg. Michalková V., 26.05.2001; (BRA). — Liptovská Osada – Podsuchá; 700 m a.s.l.; leg. ?, 16.07.1972; (BRA). — Vel. Fatra: Čierny kameň –; 1400 m a.s.l.; leg. ?, 21.08.1968; (BRA). — Malé Karpaty: prope vici Smolenice, montis Záruby in iugo, ad terra calcareum; 730 m a.s.l.; leg. Michalko J., 08.08.1965; (BRA). — Stará Lehota – Kňazný vrch; leg. Maglocký Št., 13.08.1965; (BRA). — ober Harmanec; leg. ?, 09.07.1857; (BRA). — pri obci Topoľčianky; leg. Drévlackanský Fr., 19.06.1958; (BRA × 2 vouchers). — Na Nitr. Javorine [Panská Javorina] miestami; leg. Holuby J., 07.1864; (BRA). — Malé Karpaty, Trnava: in fageto sub arce Ostrý kameň prope pag. Biksard [today Buková village]; 500 m a.s.l.; leg. Valenta V., 18.07.1940; (BRA × 2 vouchers). — Kremnica, Bartoška; leg. Zechentner, ?; (BRA). — prope Banská Štiavnica – in declivibus borealis, orientalis montis Koelberg in fruticetis – solo humoso – planta sociabilis; leg. Hlavaček A., 29.05.1936; (BRA). — Ružomberok: in saxosis calcareis m. Vysoký gruň supra pag. Lubochňa; 650 m a.s.l.; leg. Valenta V., 16.06.1971; (BRA). — Ilava: in convalle inter pagos Beluša et Mojtín; 340 m a.s.l.; leg. Valenta V., 29.06.1936; (BRA). — Prievidza: in fruticetis ad marginum silvae in m. Revan ad viam Gajdel – Fačkov; 850 m a.s.l.; leg. Valenta V., 11.07.1935; (BRA). — Západné Tatry: kataster obce Pribylina, okres Liptovský Mikuláš, vysokobylinné spoločenské okolo potôčkov v kroví; 850 m a.s.l.; leg. Horníčková J., 12.08.1982; (BRA). — Liptovské Revúce, Čierny kameň; leg. Grodkovský G., 13.07.1934; (BRA × 2 vouchers). — Veľká Fatra, Dedošova dolina, v bučine; 800 m a.s.l.; leg. Uhlířová J., 11.07.1994; (BRA). — Slovenský raj: Stratená, Dedinky, orientatio W, acclivitas 40°, substratum CaCO₃, terra rendzina; 900 m a.s.l.; leg. Hajdúk J., 31.07.1956; (BRA). — Biele Karpaty, Veľká Javorina; leg. Záborský J., 20.08.1972; (SLO × 9 vouchers). — Biele Karpaty, Veľká Javorina, v lese; leg. Záborský J., 16.09.1972; (SLO). — Zlatnícka dolina pri Skalici, v lese; leg. Záborský J., 01.08.1972; (SLO × 2 vouchers). — Sever. Biele Karpaty, Pruské; leg. ?, 19.06.1989; (SLO × 3 vouchers). — Biele Karpaty, Veľká Javorina; leg. Záborský J., 14.09.1973; (SLO). — Skalica: Zlatnícka dolina; leg. Záborský J., 24.07.1965; (SLO × 3 vouchers). — Juž. Biele Karpaty, Skalica: Zlatnícka dolina; leg. Záborský J., 27.08.1984; (SLO × 2 vouchers). — Juž. Biele Karpaty, Skalica: Zlatnícka dolina; leg. Činčura F., Záborský J., 02.09.1984; (SLO). — Násyp pri Harmanci; leg. Záborský J., 30.06.1970; (SLO). — Biele Karpaty, Veľká Javorina; leg. Záborský J., 14.09.1973; (SLO × 3 vouchers). — Slovenský raj: Kysel; leg. ?, 27.06.1971; (SLO). — Biely Potok [part of town Ružomberok]; leg. ?, 25.06.1979; (SLO). — Nemecká – Hradisko; leg. Háberová I., 05.06.1975; (SLO). — Manínska úžina; leg. Háberová I., 04.06.1976; (SLO). — Palota; leg. Majzlanová E., 10.06.1977; (SLO). — Malá Fatra – sedlo pod Klakom; lesy v okolí chaty; leg. Činčura F., 13.07.1968; (SLO × 2 vouchers). — Veľká Fatra – lesy pri chate pod Δ Úplaz, smerom od Harmanca; leg. Činčura F., 04.07.1968; (SLO). — Malé Karpaty – Ostrý kameň; leg. Filová M., 08.07.1949; (SLO). — Harmanecká dolina; leg. Michalko J., 28.06.1952; (SLO). — Neresnícka dolina pri Zvolene – v lesoch vedľa cesty; leg. Činčura F., 14.06.1967; (SLO × 4 vouchers). — Rokoš vs. od Bánovce nad Bebravou; leg. Futák J., 04.07.1931; (SLO). — Veľká Fatra: dolina Bystrice pod Kráľovou studňou; leg. Futák J., 15.06.1947; (SLO). — Veľká Fatra: Majerova skala; leg. Futák J., 15.06.1947; (SLO). — Kopce a lúky nad Lubochňou; leg. Májovský J., 06.07.1951; (SLO). — Kozel – Biela skala, les; leg. Hollá D., 11.07.1968; (SLO). — Veľká Fatra: Gaderská dolina; leg. Májovský J., 21.06.1948; (SLO). — Malá Fatra; leg. Futák J., 11.06.1947; (SLO). — Harmanecká dolina; leg. Michalko J., 28.06.1952; (SLO). — Veľká Fatra: Horný Jelenec; leg. Futák J., 14.06.1947; (SLO). — Bučiny nad Riečkou a dolina B.B. [Banská Bystrica] vodovodu; leg. Májovský J., 29.06.1952; (SLO × 2 vouchers). — Zvolen; leg. Futák J., 07.1941; (SLO). — Reváň; leg. Pantocsek J., 02.08.1902; (SLO). — Moravia Occidentalis. Ad confines Slovakiae: Javorníky, in pratis “Javornické louky”; 1056 m a.s.l.; leg. Říčan G., 07.1924; (SLO × 1 voucher, ZV × 1 voucher). — Juhoslovenský kras; leg. ?, 27.06.1964; (SLO). — Neresnícka dolina pri Zvolene – v lesoch vedľacesty; leg. Činčura F., 14.06.1967; (SLO × 2 vouchers). — Veľká Fatra: Tlstá; leg. Exkurzia, 22.06.1948; (SLO). — Veľká Fatra: Dedošová dolina; leg. Májovský J., 21.06.1948; (SLO). — Rokoš: bučina na sev. svahu; 920 m a.s.l.; leg. Futák J., 28.07.1942; (SLO × 2 vouchers). — Malé Karpaty: Záruby – Buková; leg. Medovič J., 25.08.1955; (SLO × 3 vouchers). — Malé Karpaty: Veterlín; leg. Filová M., 04.07.1948; (SLO × 2 vouchers). — Malé Karpaty: Driny – Čelo; leg. Berta J., Medovič J., 24.08.1955; (SLO). — Strážovská hornatina, Rajecké Teplice, hrebeň na Tlstú horu, les; leg. Hallonová, 04.06.1977; (SLO). — Strážovská hornatina, Lietava, severný svah pod Lietavským hradom, les; leg. Hallonová, 17.06.1978; (SLO). — Remata – Roviny; leg. Takáč, 17.07.1962; (NI). — Stráž. vrchy: Uhrovské Podhradie, sedlo Rokoš; 945 m a.s.l.; leg. Eliáš P. jun., 22.07.2003; (NI). — Stráž. vrchy: Δ Baske, v lese pod chatou; leg. Eliáš P. jun., 23.07.2003; (NI × 2 vouchers). — M. Karpaty: Δ Záruby, hojne okolo turist. chodníka; 755 m a.s.l.; leg. Eliáš P. jun., 21.07.2010; (NI). — Silická planina, 620 m, “Fujančo”, Z, 15°; leg. ?, 17.05.1946; (NI). — Podsuchá – vzáčne na úpätí stráne nad riekou pri letnom tábore; leg. ?, 10.07.1992; (NI). — Vápencové predhorie medzi Kysucou a Varínkou – Brodnianka – Tac–FQ, exp. V–Z, skl. 10–20°; 700 m a.s.l.; leg. Greštiak M., 13.06.1965; (ZV). — Homôlka; leg. Mikoláš V., 15.09.1984; (KO). — pod Tesnou rížňou [Tesná rížňa – Malá Biela]; leg. Mikoláš V., 18.08.1984; (KO). — Zádielska dolina; leg. Vojtůň A., 14.06.1968; (KO).

Aconitum moldavicum* subsp. *moldavicum

SLOVAKIA: Vernár; leg. ?; (SAV). — okres Bánovce nad Bebravou: Skupina Kňazného stola, medzi Lutovom a Timoradzou; 525 m a.s.l.; leg. Futák J., 05.07.1956; (SAV). — Zádielska dolina: na strmých skalách; 450 m a.s.l.; leg. Schidlay E., 05.08.1954; (SAV). — bukový les v doline Čierneho Váhu; 800 m a.s.l.; leg. Futák J., 12.07.1957; (SAV × 2 vouchers). — na hrebeni vrchu Rígeľ medzi Čiernym Váhom a Svarínom, dolomit, smrečina; 850 m a.s.l.; leg. Futák J., 11.07.1957; (SAV). — Slovakia ...; 800 m a.s.l.; leg. Futák J., 19.08.1955; (SAV). — Veľká Fatra; 1180 m a.s.l.; leg. Hubová O., 27.05.1975; (SAV). — Pusté pole; leg. Futák J., 21.08.1973; (SAV). — Veľká Fatra: Majerova skala; leg. Nábělek V., 15.06.1947; (SAV). — Novoselica [Nová Sedlica] – Prikry – Sužica. Rezervácia [Bukovské vrchy, NP Poloniny]; leg. Exkurzia, 16.07.1955; (SAV × 4 vouchers). — Okres Snina, Ruský Potok, Malý Bukovec; leg. Exkurzia, 16.07.1955; (SAV × 2 vouchers). — Nízke Tatry, ŠPR Ohnište, údolie potoka Šuštiacka; leg. Drábová J., Hrouda L., 16.07.1986; (SAV). — Humenská; leg. Maglocký Št., 19.05.1971; (SAV). — Studňa [Muránska planina]; leg. Futák J., Zahradníková K., 08.07.1970; (SAV). — Vihorlat, Jovsa, Jovsianska hrabina (štvorec 7198); 162 m a.s.l.; leg. Mráz P., 28.05.1998; (SAV). — Pusté pole; 1000 m a.s.l.; leg. Futák J., 13.07.1965; (SAV). — Muránska planina: Δ Vrbiarka; 740 m a.s.l.; leg. Šípošová H., Peniašteková M., 17.07.1980; (SAV). — Muránska planina; leg. Zahradníková K., 08.07.1970; (SAV). — Zádiel, Zádielska dolina; leg. Kmeťová E., 11.06.1969; (SAV). — Korytnica, okraj krovia; 800 m a.s.l.; leg. Schidlay E., 11.08.1941; (SAV × 2 vouchers). — Veľká Fatra, Horný Jelenec; leg. Futák J., 12.07.1965; (SAV). — Slizké; 410 m a.s.l.; leg. Kliment J., 10.06.1976; (SAV). — Striežovce; leg. Kliment J., 30.06.1976; (SAV × 2 vouchers). — Jelšava; leg. ?, 20.05.1934; (SAV). — Liptovská Teplička ?; 600 m a.s.l.; leg. Michalko J., 17.07.1972; (SAV). — Stratenská hornatina: roklina Suchá Belá / pri Hrabušiciach /; 600 m a.s.l.; leg. Hubová O., Tatarková G., 22.07.1966; (SAV). — Stratenská hornatina: roklina Veľký Sokol; 700 m a.s.l.; leg. Hubová O., Tatarková G., 22.07.1966; (SAV). — vrch Zámčisko pri Kvetnici, Tilio–Picetum; leg. Michalko J., 13.06.1984; (SAV). — Muránska pošina: Cigánka, JZ od obce Muráň, okraj lesa; 900 m a.s.l.; leg. Jasičová M., 19.06.1963; (SAV). — Vyšná Slaná, vrch Radzim; 920 m a.s.l.; leg. Hajdúk J., 05.08.1956; (SAV). — Studňa, Mochnatá; leg. Futák J., Zahradníková K., 08.07.1970; (SAV). — Veľká Fatra, vrch Krížna [+ sample from Čorna Ríká in Podkarpatská Rus ?]; leg. Nábělek V., 07.1936; (SAV). — dolina Lesnica [?]; 560 m a.s.l.; leg. Hubová O., 08.1962; (SAV). — Veľká Fatra: Gader, u potoka; leg. Schidlay E., 24.08.1936; (SAV). — Šturec [Veľká Fatra], južný svah pri cestárskom domčeku; leg. Futák J., 12.07.1965; (SAV). — Slovenské rudohorie – vrch Stolica – zasný Kyprov; leg. Kliment J., 11.08.1977; (SAV). — Vrch Riečky [Mt. Vrchriečky near village Litmanová]; leg. Zahradníková K., 05.07.1972; (SAV). — Liptovský Hrádok, na okraji lesa medzi krovím, dolomit, N; 700 m a.s.l.; leg. Futák J., 10.07.1957; (SAV). — Žiar [časť lúčanskej časti Malej Fatry]; 1010 m a.s.l.; leg. Futák J., 11.07.1957; (SAV). — Slovakia orientalis, in silvis caeduis supra Kavečany prope oppidum Košice, solo calcareo; 500 m a.s.l.; leg. Hlavaček A., 21.07.1950; (SAV). — Pieniny, Vysoké skalky, na SZ svahu pri hranici; leg. Michalko J., 22.08.1953; (SAV). — Slovenské rudohorie, vrch Radzim; 920 m a.s.l.; leg. Hajdúk J., 05.08.1956; (SAV). — Ďurkovec [Bukovské vrchy Mts] (okres Snina); leg. Májovský J., 05.10.1958; (SAV). — Spišská Nová Ves; leg. Májovský J., 23.08.1955; (SAV). — Riaba skala (okr. Snina); leg. Májovský J., 04.10.1958; (SAV). — Kráľova Lehota, skaly nad stanicou; leg. Futák J., Jasičová M., Zahradníková K., 22.06.1863; (SAV). — Maša [Hnúšťa]; leg. Rošetská K., 12.07.1954; (SAV). — V lese na Šturci u pasu; leg. Horák P., 13.07.1938; (SAV). — Malé Karpaty, Ostrý kameň; leg. Perný M., 23.10.1997; (SAV). — Muránske pohorie: in monte Veľká Stožka, in declivibus silvaticis; 1450 m a.s.l.; leg. Dvořák J., 23.07.1975; (BRA). — Nízke Tatry, Liptovský Hrádok, vrch Zapač – východná krovinatá stráň v lese; 700 m a.s.l.; leg. Horníčková J., 07.1983; (BRA × 3 vouchers). — Slovenský raj: Stratená, Dedinky, acclivitas 40°, substratum Ca, Mg, CO₃, terra rendzina; 1000 m a.s.l.; leg. Hajdúk J., 17.07.1956; (BRA). — Slovenský raj: Dedinky, Hnilec, orientatio J, acclivitas ca. 35°, substratum calcareus, terra rendzina, Abieto–Fagetum vegetatio; 1000 m a.s.l.; leg. Hajdúk J., 23.07.1955; (BRA). — Stratenská hornatina, pri Dobšinskom hydrostave, kóta 921; leg. Hajdúk J., 19.07.1956; (BRA). — Slovenský raj: Dobšinská Maša, collis Skala, flumen Hnilec, orientatio V (E), substratum calcit + dolomit, terra rendzina čierna, vegetatio *Picetum*; 1000 m a.s.l.; leg. Hajdúk J., 27.07.1955; (BRA). — Slovenský raj: Hrabušice, Veľký Sokol, substratum calcit carb., terra litozem, orientatio NW; 900 m a.s.l.; leg. Hajdúk J., 1956; (BRA). — Slovenský raj: Radzim, orientatio S (N), acclivitas ca. 40°, substratum calcit, terra rendzina, vegetatio *Abieto–Fagetum*; 940 m a.s.l.; leg. Hajdúk J., 13.08.1955; (BRA). — Slovenský raj: Vernár, dolomit, *Pulsatillo–Pinetum*; 1100 m a.s.l.; leg. Hajdúk J., 01.08.1955; (BRA). — Slovenské rudohorie, montes Muránska planina: in jugo saxoso ab arce Muráň ad orientem versus; 1040 m a.s.l.; leg. Vašák V., 18.07.1978; (BRA × 2 vouchers). — hrad Muráň; leg. ?, 27.06.1972; (BRA). — Brzotín – les, výstup na Silickú planinu; leg. ?, 23.06.1972; (BRA). — Bardejov; leg. Berganský J., 1923; (BRA). — Nízke Poloniny: in platis inter pag. Topoľa et Runina; 400 m a.s.l.; leg. Pišút I., 18.07.1962; (BRA × 2 vouchers). — Revúca, mont. Cigánka supra pag. Muráň; 800 m a.s.l.; leg. Valenta V., 09.07.1970; (BRA). — Nízke Tatry: Tále, na okraji riedkeho lesa pod silnicí; 750 m a.s.l.; leg. Dvořák J., 17.07.1972; (BRA). — Baba bei Lučivná; leg. Grodkovszký G., 26.06.1933; (BRA). — Tisovec, Voniaca; leg. Vraný V., 06.06.1922; (BRA). — N. Tatry, údolí Štiavnica; leg. Balthasar V., 07.1934; (BRA). — Muránska vrchovina: Javorníková dolina, vápencové svahy v horní časti doliny; 850 m a.s.l.; leg. Dvořák J., 08.08.1980; (BRA). — Zádielska dolina; leg. ?, 19.07.1956; (BRA). — Muráň; leg. ?, 04.07.1962; (BRA). — Bardejov: Zabavastrasse; leg. Berganský J., 17.06.1922; (BRA × 2 vouchers). — Bardejov: Baadgelände; leg. Berganský J., 18.08.1929; (BRA). — Bardejov: Kapitnoka; leg. Berganský J., 25.06.1927; (BRA × 3 vouchers). — Bardejov: Gresak Kert; leg. Berganský J., 10.06.1922; (BRA × 8

vouchers). — Bardejov: Teglam; leg. Berganský J., 14.05.1922; (BRA × 3 vouchers). — Bardejov: fúrdő erdő féle; leg. Berganský J., 14.06.1922; (BRA × 2 vouchers). — Bardejov: Bartfeld; leg. Berganský J., 28.06.1922; (BRA). — Bardejov: Alte Bleiche; leg. Berganský J., 30.05.1924; (BRA). — Bardejov: Moliterka; leg. Berganský J., 04.07.1927; (BRA × 3 vouchers). — Bardejov: Zabava; leg. Berganský J., 17.06.1922; (BRA). — Bardejov: vedľa potoka pri papierni; leg. Berganský J., 1924; (BRA). — Bardejov: Moliterka; leg. Berganský J., 09.06.1922; (BRA × 2 vouchers). — Bardejov; leg. Berganský J., 1925; (BRA × 3 vouchers). — Liptovský Hrádok, vrch Zapač, krovinaté úpatie, SV svah; 670 m a.s.l.; leg. Horníčková J., 20.06.1973; (BRA). — Bardejov: Rurná; leg. Berganský J., 12.06.1924; (BRA × 3 vouchers). — Bardejov: Kohlengrund; leg. Berganský J., 02.05.1924; (BRA × 3 vouchers). — Bardejov: Popova hura Gelände; leg. Berganský J., 25.05.1924; (BRA). — Slovenský raj: Dedinky, Gerava, Hnilec, orientatio W, acclivitas 25°, substratum CaCO₃, terra rendzina; 1000 m a.s.l.; leg. Hajdúk J., 17.07.1956; (BRA). — Ružomberok – Biele skaly; leg. ?, 07.1964; (BRA). — Čergov – Bystrá, v bukovom lese na vrchole; leg. Májovský J., 20.06.1947; (SLO × 2 vouchers). — Sninský kameň, v bučinách na sev. svahu, *Aceri-Fagetum*; leg. Májovský J., 10.07.1967; (SLO). — Δ Sokol, v bučinách nad dedinou, skala; leg. Májovský J., 21.05.1968; (SLO). — Vihorlat [vrch], na hrebeni na okraji lesa pri chodníku; leg. Májovský J., 23.06.1961; (SLO). — Bučiny nad Riečkou a dolina B.B. [banskobystrického] vodovodu; leg. Májovský J., 29.06.1952; (SLO × 4 vouchers). — Čergov – Bystrá, na hrebeňoch v bukovom lese (pieskovce); 900 m a.s.l.; leg. Májovský J., 20.06.1947; (SLO). — Čierny Váh: na lúke Stará Poľana; leg. Ferjanec J., 26.07.1965; (SLO). — Skalky nad Dubovou; leg. Suchá D., 29.06.1952; (SLO). — Pohorie Čergov, Δ Bystrá, v bučine na hrebeni; leg. Májovský J., 20.06.1947; (SLO). — Pieniny: Vysoké Skalky –; leg. exkurzia, 19.07.1954; (SLO). — Muráň; leg. Michalko J., 08.07.1952; (SLO). — Cestou z Dlhej Lúky do Muráňa (okr. Revúca) [Muránska Dlhá Lúka]; leg. Opluštiová T., 08.1952; (SLO). — Muráň; leg. Futák J., 10.07.1944; (SLO). — Zádiel; leg. Futák J., 27.07.1949; (SLO × 2 vouchers). — Špania Dolina, Panský diel, Baláže; leg. Michalko J., 27.06.1952; (SLO × 4 vouchers). — Čergov – Solisko, v bukovom lese; leg. Májovský J., 24.06.1947; (SLO × 2 vouchers). — Nízke Tatry: pri Váhu medzi Lipt. Hrádkom a Lipt. Jánom, vápenec; leg. Futák J., 16.08.1944; (SLO). — Spišská Nová Ves, ad stationem viae ferr. “Štiavnik”; 600 m a.s.l.; leg. Suza J., 20.06.1931; (SLO). — Flora Leutschoviensis [Levoča], Dolina; leg. Greschik V., 08.1887; (SLO). — Flora Tatrae Magnae, Leutschovie [Levoča], Dolina; leg. Greschik V., 07.1942; (SLO). — Flora tatrae Magnae, Leutschovie [Levoča], Dolina; leg. Greschik V., 06.1944; (SLO × 3 vouchers). — Flora Tatrae Magnae, Leutschovie [Levoča], in convalle “Dolina”; leg. Greschik V., 06.1910; (SLO × 2 vouchers). — Kesmark [Kežmarok], Kleinwäldchen; leg. Greschik V., 06.1886; (SLO). — Flora tatrae Magnae, Leutschovie [Levoča], in convalle “Dolina”; leg. Greschik V., 07.1920; (SLO). — Flora Tatrae Magnae, Löcse [Levoča]: Löcsefüred [Levočské Kúpele]; leg. Greschik V., 06.1888; (SLO × 2 vouchers). — Čierny Váh – Δ Cudzenica pri Malej Lehote, les; 750 m a.s.l.; leg. Vartíková E., 21.07.1972; (SLO × 2 vouchers). — Čierny Váh, lesy po hájovňu Niž. Chmelinec, S exp.; 750 m a.s.l.; leg. Vartíková E., 18.07.1972; (SLO). — Nízke Tatry: Čierny Váh, Δ Gregové; 1000 m a.s.l.; leg. Vartíková E., 03.07.1974; (SLO). — Nízke Tatry, Δ Vysoké; 900 m a.s.l.; leg. Vartíková E., 04.07.1974; (SLO). — Nízke Tatry: Čierny Váh, Δ Gregové; 950 m a.s.l.; leg. Vartíková E., 03.07.1974; (SLO × 2 vouchers). — Nízke Tatry: Δ Muránik, Čierny Váh; 850 m a.s.l.; leg. Vartíková E., 09.07.1974; (SLO). — Korytnická dolina – svetlé, trávnaté miesta na lesných svahoch vedľa cesty smerom na Donovaly; leg. Činčura F., 06.07.1968; (SLO × 5 vouchers). — Veľká Fatra: Majerova skala; leg. Futák J., 15.06.1947; (SLO × 2 vouchers). — Slovakia ...; leg. Májovský J., 16.06.1949; (SLO). — Kopec medzi Kolbasovom a Uličom (okres Snina); leg. exkurzia, 14.07.1955; (SLO × 3 vouchers). — Muránska planina; leg. Svobodová Z., 1970; (NI). — Rybník (okres Rimavská Sobota): les na vápencovom podklade v doline potoka Drienek nad dedinou [now obec Revúca]; leg. Řehořek V., 31.05.1968; (NI × 2 vouchers). — Ľubietovský Vepor: paseka smerom na Tri Vody; leg. Svobodová Z., 10.06.1968; (NI). — Slovenský kras: Rožňava, skalky; leg. Kostúl [?], 06.1936; (NI). — Spišská Nová Ves, ad stationem viae ferr. “Štiavnik”; 600 m a.s.l.; leg. Suza J., 20.06.1931; (NI). — Banskobystrická vrchovina, Radvaňská jamka – Varta, Banská Bystrica. Svahy nad Jamkou; 480 m a.s.l.; leg. Manica M., 01.06.1960; (ZV). — u Sivce; leg. Mikoláš V., 13.07.1962; (KO). — Kysak, sev. svahy Hradu; leg. Mikoláš V., 29.05.1999; (KO). — Veľká lúka [Muránska planina] – Cigánka; leg. Mikoláš V., 04.07.1984; (KO). — Turnianske sedlo – Erneho jask; leg. Mikoláš V., 19.06.1962; (KO). — Príkrá nad Spišskou Teplicou; leg. Pačlová L., 28.06.1956; (TNP). — Slovenský raj, proti Letanovskému mlynu; leg. Odložilíková L., 30.06.1957; (TNP). — bei Poprad; leg. Scherfel A., 29.06.1979; (TNP).

Aconitum moldavicum* subsp. *hosteanum

SLOVAKIA: Košická kotlina, Zádiel; leg. Krippelová, 16.06.1966; (SAV). — Humenné: svahy nad potokom; leg. Michalko J., 22.06.1959; (SAV). — Nízke Tatry: Skalky pri Ružomberku, v kroví. 2 r.; leg. Nagy G., 06.1969; (SAV). — Nízke Poloniny, Runina – brehy “Pláša potoka” pred obcou; 450 m a.s.l.; leg. JZ, 21.08.1970; (SAV). — Slovenské rudohorie – vrch Stolica – zadný Kyprv; leg. Kliment J., 11.08.1977; (SAV). — Jovsa, les pri obci; leg. Michalková E., 17.06.1992; (SAV). — Liptovský Hrádok, na okraji lesa medzi krovím [?], dolomit, N; 700 m a.s.l.; leg. Futák J., 10.07.1957; (SAV). — v lese na vrchu Sokol [vrch v Žiari]; 950 m a.s.l.; leg. Futák J., 12.07.1957; (SAV). — Novosedlica – Príkrý – Stučica. Rezervácia [Bukovské vrchy, NP Poloniny]; leg. exkurzia, 16.07.1955; (SAV). — Slovenské rudohorie: Hrušovo, vápenec; 350 m a.s.l.; leg. Hajdúk J., 21.05.1968; (BRA × 3 vouchers). — Bardejov; leg. Berganský J., 20.06.1929; (BRA). — Muránska vysočina, Muráň; leg. Skřivánek V., 06.07.1951; (BRA). — Slovenský raj: Dobšinská Maša, collis

na skalu, flumen Hnilec, substratum calcit, terra rendzine; 850 m a.s.l.; leg. Hajdúk J., 31.07.1956; (BRA). — Slovenské rudohorie, montes Muráňská planina: in jugo saxoso ab arce Muráň ad orientem versus; 1040 m a.s.l.; leg. Vašák V., 18.07.1978; (BRA). — Nízke Poloniny: brehy potoka "Plaša" pri križovatke na Runinu; 450 m a.s.l.; leg. Gallo A., 18.06.1970; (BRA). — Nízke Poloniny: in valle rivi inter pag. Topoľa et Runina, loco umbroso; 380 m a.s.l.; leg. Pišút I., 18.07.1962; (BRA). — Muráň, lesy; leg. Blatný T., 18.06.1947; (BRA). — Baba; leg. Grodkovský G., 26.06.1933; (BRA). — Bardejov; leg. Berganský J., 18.08.1929; (BRA). — Bardejov: Baadgelände; leg. Berganský J., 18.08.1929; (BRA). — Bardejov: Zabavastrasse; leg. Berganský J., 17.06.1922; (BRA). — Zádielská dolina; leg. ?, 19.07.1956; (BRA). — Hnilec, potok, vápencové skály, Z 20°; 900 m a.s.l.; leg. Marva V. [?], 06.07.1958; (BRA). — Bardejov: Zabavastrasse; leg. Berganský J., 17.06.1922; (BRA × 10 vouchers). — Bardejov: Kľušov; leg. Berganský J., 1925; (BRA). — Bardejov: Stationsgelände; leg. Berganský J., 12.06.1929; (BRA). — Bardejov: Baadgelände; leg. Berganský J., 18.08.1929; (BRA). — Bardejov; leg. Berganský J., ?; (BRA × 14 vouchers). — Bardejov; leg. Berganský J., 1927; (BRA). — Bardejov: Zabava; leg. Berganský J., 17.06.1922; (BRA). — Bardejov: Moliterka; leg. Berganský J., 20.06.1929; (BRA × 2 vouchers). — Bardejov: Kohlengrund; leg. Berganský J., 02.05.1924; (BRA). — Bardejov: Gresak Kert; leg. Berganský J., 10.06.1922; (BRA). — Slovenský raj: Hrabušice, Malý Sokol, horná časť, orientatio NW, acclivitas 40°, substratum Ca, Mg, CO₃, terra rendzina, vegetatio *Acerion*; 700 m a.s.l.; leg. Hajdúk J., 07.08.1955; (BRA). — Čergov – Bystrá, v bukovom lese na vrchole; leg. Májovský J., 20.06.1947; (SLO). — Vihorlat [vrch], na hrebeni na okraji lesa pri chodníku; leg. Májovský J., 23.06.1961; (SLO). — Kopce medzi Darou [obec Dara] a Starinou (okr. Snina); leg. Exkurzia, 14.07.1955; (SLO). — Pieniny: Vysoké skalky; leg. exkurzia, 19.07.1954; (SLO). — Čergov – Šoltýsova hora v bukovom lese na vrchole; leg. Májovský J., 23.06.1947; (SLO × 2 vouchers). — Cestou na Muráňsku planinu; leg. Opluštilová T., 08.1952; (SLO). — Muráňská vysočina: Cigánka; leg. Futák J., 11.07.1944; (SLO). — Flora Tatrar Magnae, Leutschovie [Levoča], Dolina; leg. Greschik V., 07.1890; (SLO). — Flora Tatrar Magnae, Leutschovie [Levoča], in convalle "Dolina"; leg. Greschik V., 06.1910; (SLO). — Flora Leutschoviensis [Levoča], Dolina; leg. Greschik V., 07.1926; (SLO). — Flora Tatrar Magnae, Lőcse [Levoča]: Lőcsefüred [Levočské Kúpele]; leg. Greschik V., 06.1888; (SLO). — Korytnická dolina – svetlé, trávnaté miesta na lesných svahoch vedľa cesty smerom na Donovaly; leg. Činčura F., 06.07.1968; (SLO). — Hrabušice – Podlesok; leg. exkurzia, 08.07.1955; (SLO). — Baba [vrch] nad Svitom; 750 m a.s.l.; leg. Odložilková L., 13.07.1956; (TNP).

Aconitum moldavicum* nothosubsp. *confusum

SLOVAKIA: Spišská Nová Ves; leg. Májovský J., 23.08.1955; (SAV).

Aconitum* × *triste

SLOVAKIA: Meliata, vlhké lesy; leg. Karasová E., 16.06.2005; (SAV × 5 vouchers).

Aconitum anthora* subsp. *anthora

SLOVAKIA: Bukovské vrchy, Stinská, hraničný hrebeň; leg. Marhold K., 18.08.1988; (SAV). — Šivec [vrch]; leg. Futák J., 30.08.1950; (SAV). — Biela skala; leg. Futák J., 26.08.1950; (SAV × 2 vouchers). — Sokol pri Humennom; leg. Májovský J., 27.08.1950; (SAV). — Juhoslovenský kras: Felsőhegy [Horný vrch] pri Zádieli; leg. Michalko J., Popovič, 30.07.1956; (SAV). — Zádielská dolina, na strmých skalnatých svahoch na dne, sreby na dne údolia; 350 m a.s.l.; leg. Schidlay E., 05.08.1954; (SAV). — Vihorlat: [Humenský] Sokol, váp. skalky, *Fageto-Quercetum*; 300 m a.s.l.; leg. Michalko J., 26.08.1954; (SAV). — Slovenský raj: Skrik nad Letanovským mlynom, kroviny, exp. J.; 750 m a.s.l.; leg. Hubová O., 22.08.1961; (SAV × 2 vouchers). — Slovenské rudohorie, vrch Radzim, exp. J., skaly; 945 m a.s.l.; leg. Hajdúk J., 05.08.1956; (SAV). — Vihorlat: [Humenský] Sokol, váp. skalky, *Fageto-Quercetum*; 300 m a.s.l.; leg. Michalko J., 26.08.1954; (SAV × 2 vouchers). — Skalka, Jelšava; leg. ?, 02.08.1953; (SAV). — Zádielská dolina, na skalách na dne údolia; 420 m a.s.l.; leg. Schidlay E., 05.08.1954; (SAV). — Pohornádie: Skalky pri M. Vieske severne od Košíc; leg. Michalko J., 19.09.1956; (SAV). — Zádielská rokle; 400 m a.s.l.; leg. Horák P., 14.07.1936; (SAV). — Zvolen; 450 m a.s.l.; leg. Futák J., 20.08.1961; (SAV). — Kováčovské skály, Hronská Breznica; leg. Nábělek V., 09.1936; (SAV). — Zádielská dolina; leg. Krč J., 29.07.1954; (SAV). — Slovenský raj: Tomášovský výhľad; leg. Zahradníková K., 20.08.1961; (SAV). — Zádielská dolina; leg. Fabianková K., 12.09.1972; (SAV). — Malá Vieska – Trebejov; leg. Fabianková K., 23.08.1973; (SAV). — Jelšava; 510 m a.s.l.; leg. Kliment J., 28.08.1977; (SAV). — Slovakia Orientalis, ad saxa calcarea in valle Hájska dolina prope Háj apud opp. Košice; 350 m a.s.l.; leg. Hlavaček A., ?; (SAV). — Pohornádie: váp. skalky na Šivci pri Ružine, hrebeň; 780 m a.s.l.; leg. Michalko J., 23.08.1960; (SAV). — Bratislava, pestované v záhrade p. Černého (pochádza z okolia Parkána [Štúrovo]); leg. Schidlay E., 13.09.1944; (SAV). — Juhoslovenský kras: Zádielská dolina. V spodnej časti rokle; leg. Krippelová T., Zahradníková K., 26.09.1962; (SAV). — Zobor [vrch]; leg. Jasičová M., Fabianková K., 12.10.1972; (SAV). — Nitra – Zobor [vrch]; leg. ?, 24.07.1966; (SAV). — Zádielská dolina; leg. Krippelová T., 26.08.1966; (SAV). — Slovakia media, in fruticetis iugi montis Pešianska prope Kotionem viae ferroviae Hronská Dúbrava, solo audes; 500 m a.s.l.; leg. Hlavaček A., 27.07.1956; (SAV). — Slovakia australis, distr. Plešivec, regio Slovenský kras: ad margines sylvae deciduae collis Čertova diera prope Domica, solo calcareo; 400 m a.s.l.; leg. Deyl M., 03.09.1933;

(BRA). — Gemerské rudohorie: na južnom svahu kopca Cíger na V od obce Jelšava; 320 m a.s.l.; leg. Gallo A., 30.08.1969; (BRA). — Kováčovske kopce; leg. ?, 09.09.1969; (BRA). — Humenské chrbáty: svah na Z od obce Oreské [okres Michalovce]; 220 m a.s.l.; leg. Gallo A., 03.10.1969; (BRA). — Slovenské rudohorie: Stolické vrchy, Brdárka, Vyš. Slaná, Radzim, orientatio medit, acclivitas 45°, substratum Ca, Mg, CO₃, vegetatio *Spiraeum mediae*; 930 m a.s.l.; leg. Hajdúk J., 13.08.1955; (BRA). — Slovenské rudohorie: Stolické vrchy, Brdárka, Vyš. Slaná, Radzim, orientatio SW, acclivitas 20°, substratum Ca, Mg, CO₃, terra rendzina, litozem, vegetatio *Spiraeum mediae*; 940 m a.s.l.; leg. Hajdúk J., 04.08.1957; (BRA). — Slovenský raj: Dedinky, Biele Vody – collis Geravy, Baranie rohy, orientatio J (S), acclivitas 10°, substratum calcit, terra litozem, vegetatio *Festucetum pallentis*; 1000 m a.s.l.; leg. Hajdúk J., 02.09.1956; (BRA × 2 vouchers). — Muráň; leg. Blatný T., 25.08.1950; (BRA). — Nitra, pod Zoborom; leg. ?, 20.09.1959; (BRA × 4 vouchers). — Kováčovske kopce, Kováčov; 320 m a.s.l.; leg. Valenta V., 25.08.1936; (BRA × 3 vouchers). — Revúca, Muráň; 925 m a.s.l.; leg. Valenta V., 11.08.1937; (BRA). — Hronská Breznica; leg. Nábělek V., 09.1936; (BRA). — Muráňyi fensik [Muránska planina]; leg. ?, 08.1953; (BRA). — Muráň; leg. ?, 25.08.1950; (BRA). — Slovensko juž., Nitra, v lese na Zoboru; 350 m a.s.l.; leg. Plaskavka J., 06.08.1936; (BRA). — Nitra, pod Zoborom; leg. ?, 20.09.1959; (BRA × 8 vouchers). — Gemerské rudohorie: J svahy vrchu Dúbrava na S od obce Jelšava; 420 m a.s.l.; leg. Gallo A., 24.08.1970; (BRA). — Prieva Hornádu; leg. Májovský J., 05.09.1960; (SLO). — Slovenský kras: Hrušov, vápencové svahy; leg. Májovský J., Záborský J., 08.09.1960; (SLO). — Turniarsky hradný vrch; leg. Májovský J., 15.09.1966; (SLO). — Turčianský hradný vrch; leg. Májovský J., 15.09.1966; (SLO). — Hôrky a Vápenicé (vápence južne od vrchu Ploská); leg. Eliáš P., 02.09.1968; (SLO × 2 vouchers). — Jelenec: gaštanica, v svetlom lese (dub, gaštan); leg. Májovský J., 07.10.1965; (SLO). — Tisovec, Δ Čeremošná, v bučinách hrebeňa od kameňolomu na Δ Martinová, čistinky; 850 m a.s.l.; leg. Májovský J., Magic D., 18.08.1970; (SLO × 2 vouchers). — Klátova Nová Ves, Skalka, posled. vrch na hrebeni J Δ Kostrín; 440 m a.s.l.; leg. Kováčiková, 19.09.1972; (SLO). — Veľká Lodina, vrch Babiná, *Quercetum pubescentis*; leg. Futák J., 08.09.1946; (SLO). — Kováčovske kopce [now pohorie Burda], medzi Kováčovom a Kamendinom; leg. Futák J., 22.08.1948; (SLO × 3 vouchers). — Zádielska dolina; leg. Futák J., 27.07.1949; (SLO). — Šivec, pri obci Ružín; leg. Futák J., 30.08.1950; (SLO). — Dreveník, obec Levoča; leg. Májovský J., 27.08.1955; (SLO × 3 vouchers). — Kysak, Trebejov, Malá Vieska [now Družstevná pri Hornáde]; leg. Bosáčkova E., 11.09.1956; (SLO × 3 vouchers). — Margecany: Kurtova skala; leg. Bosáčkova E., 12.09.1956; (SLO). — Kysak; leg. Futák J., 10.09.1946; (SLO). — Dvorník pri obci Spišské Podhradie; leg. Futák J., 11.09.1946; (SLO). — Sokol pri Humennom; leg. Májovský J., Michalko J., 14.09.1950; (SLO × 3 vouchers). — Zobor [vrch] pri Nitre, v lese; 350 m a.s.l.; leg. Futák J., 23.07.1942; (SLO). — medzi Budčou a Dúbravou, okres? Zvolen; leg. Futák J., 18.07.1933; (SLO). — Slovakia ...; leg. ?, 08.1941; (SLO). — Slovakia ...; leg. Futák J., 15.09.1948; (SLO). — Krásna Hôrka pri Rožňave; leg. Futák J., 24.08.1947; (SLO × 2 vouchers). — Juhoslovenský kras, Hrušov, I časť; leg. Futák J., 03.09.1949; (SLO). — Juhoslovenský kras, Hrušov, II časť; 580 m a.s.l.; leg. Futák J., 03.09.1949; (SLO × 2 vouchers). — Dreveník; leg. ?, 08.1889; (SLO). — Muránska planina, Δ Cigánka; leg. Záborský J., 25.09.1984; (SLO). — Δ Sokol – exp. S–V; leg. Hudáková, 20.08.1975; (SLO). — Δ Sokol – skaly, exp. V–J; leg. Hudáková, 29.07.1975; (SLO). — Δ Podskalka, skaly, exp. V; leg. Hudáková, 07.08.1974; (SLO). — Δ Podskalka, skaly, exp. V; leg. Hudáková, 18.08.1975; (SLO). — Δ Podskalka, skaly, exp. V; leg. Hudáková, 18.08.1975; (SLO). — Váp. skala nad Jasovom; leg. Jurko A., 20.08.1950; (SLO). — Mladina v horu nad ?; leg. Záborský J., 10.08.1959; (SLO × 3 vouchers). — Vrch pri obci Veľká Lodina; leg. Futák J., 25.08.1947; (SLO). — Ružín na záp. od Kysaku; leg. Futák J., 04.09.1946; (SLO × 3 vouchers). — Malá Lodina; leg. Futák J., 08.09.1946; (SLO). — Belá hora; leg. Jurko A., 07.08.1959; (SLO). — Vihorlat: Sokol, rúbaň po *Quercus*, vápenec, J svah; leg. Michalko J., 14.09.1950; (SLO × 6 vouchers). — Sokol pri Humennom; leg. Májovský J., Michalko J., 27.08.1950; (SLO). — Vihorlat: Dupne [Dúpná jaskyňa], v *Quercus*; 300 m a.s.l.; leg. Michalko J., 11.09.1953; (SLO). — Nitra, Δ Zobor; leg. Svobodová Z., 08.07.1955; (NI × 2 vouchers). — Pov. Inovec, Topolčany: Závada, juž. svah Δ Vinište, v riedkom poraste, pospolite asi 300 rastl.; 400 m a.s.l.; leg. Eliáš P. jun., 10.09.2004; (NI). — distr. Nitra: in locis stepposis fruticosisque monti Zobor supra opp. Nitra, solo calcareo; 550 m a.s.l.; leg. Osvačilová V., 13.09.1953; (NI). — distr. Nitra: in rupibus ad decl. septentr. sub summa montis Zobor supra opp. Nitra, solo andesitico; 550 m a.s.l.; leg. Osvačilová V., 11.07.1953; (NI). — Kozlica – Jánova Ves; leg. Takáš, 14.09.1962; (NI). — Zádiel, hor. č.; leg. Mikoláš V., 23.08.1984; (KO). — Plešivec – Hrad; leg. Mikoláš V., 12.09.1984; (KO). — Ružinské skaly; leg. Mikoláš V., 20.08.1959; (KO). — Muránska planina – Hrad; leg. Mikoláš V., 24–25.08.1959; (KO × 2 vouchers). — Turňa – pod hradom; leg. Vojtúň A., 04.08.1966; (KO). — Jasovská skala; leg. Vojtúň A., 20.08.1977; (KO). — Zádiel, prvý balkón [Zádielska tiesňava]; leg. Vojtúň A., 22.09.1967; (KO). — Kurtova skala u Margecan; leg. Pačlová L., 25.07.1957; (TNP).

Aconitum anthora* subsp. *jacquinii

SLOVAKIA: Slovenské rudohorie, vrch Radzim, exp. J., skaly; 945 m a.s.l.; leg. Hajdúk J., 05.08.1956; (SAV). — Spišské kotliny: Spišský hradný vrch; leg. Marciová, 19.08.1996; (SAV). — Slovenské rudohorie: Stolické vrchy, Brdárka, Vyš. Slaná, Radzim, orientatio medit, acclivitas 40°, substratum Ca, Mg, MgCO₃, terra rendzina, litozem, vegetatio *Spiraeum mediae*; 940 m a.s.l.; leg. Hajdúk J., 26.08.1955; (BRA). — Dreveník, obec Levoča; leg. Májovský J., 27.08.1955; (SLO). — in saxis meridionalis ad Dreveník; leg. Greschik V., 08.1910; (SLO). — in monte Dreveník; leg. Greschik V., 07.1910;

(SLO). — Dreveník; leg. Vojtúň A., 26.09.1964; (KO). — Kirchdrauf – Drewenyik [Dreveník]; leg. Scherfel A., 08.08.1924; (TNP). — in Garten, Drewenyik [Dreveník]; leg. Scherfel A., 08.1891; (TNP). — Drewenyik [Dreveník]; leg. Waldebrenner, ?; (TNP).

Aconitum anthora* subsp. *anthora* × *A. anthora* subsp. *jacquinii

SLOVAKIA: Dreveník, obec Levoča; leg. Májovský J., 27.08.1955; (SLO). — in monte Dreveník; leg. Greschik V., 07.1910; (SLO).

Aconitum variegatum* subsp. *variegatum

SLOVAKIA: Slovenské rudohorie; Galmus, okr. Sp. N. Ves, pravá strana Belej, v úžlabine; leg. Hajdúk J., 22.08.1959; (SAV). — Tatry. Faixová a Votrubova chata; leg. Horák P., 25–26.08.1937; (SAV). — Liptovská kotlina: Pribylina; leg. Futák J., 18.09.1970; (SAV × 2 vouchers). — Slovenské rudohorie – vrch Stolica – zadný Kyprov; leg. Kliment J., 11.08.1977; (SAV). — Slovenský raj: Suchá Belá, vápencová roklina; leg. Zarzycki, Zahradníková K., 14.09.1970; (SAV). — Čergov; leg. Flašíková, 21.05.1969; (SAV). — M. F. [Malá Fatra], Klak, cesta na Čičmany; leg. Zahradníková K., 23.07.1975; (SAV). — Veľká Fatra: Blatnická dolina; leg. Zahradníková K., 27.08.1972; (SAV × 2 vouchers). — Belianske Tatry, Kotlina – Skalné vráta; leg. Michalko J., Zahradníková K., 07.08.1972; (SAV). — Slovakia ...; leg. Ptačový J., 11.09.1954; (SAV). — Biela skala [in massif of Sivý vrch?], in sylve in alpestr; 1385 m a.s.l.; leg. Schidlay E., 09.09.1953; (SAV). — Pusté Pole; leg. Peniašteková M., 21.08.1973; (SAV × 3 vouchers). — Nízke Tatry, sev.-vých. Pustia, Iľanovská dolina; leg. Zahradníková K., 16.10.1962; (SAV × 2 vouchers). — Chočské pohorie, Prosiecka dolina; leg. Zahradníková K., Jasičová M., 10.08.1966; (SAV). — Nízke Tatry, vch Siná; leg. Zahradníková K., Fabianková [Goliašová] K., 15.08.1972; (SAV × 2 vouchers). — Západné Tatry, Biela skala; leg. Marhold K., 08.07.1986; (SAV). — Pilsko, Babia hora; leg. Futák J., Magic D., 10.08.1964; (SAV). — pod vrcholom Klenovského Vepra (Slovenské rudohorie); leg. Letz R., 23.08.1993; (SAV). — vrch Sitno; leg. Zahradníková K., 08.07.1972; (SAV). — Slovenský raj: Stratenská hornatina, substrat calcit; 900 m a.s.l.; leg. Hajdúk J., 09.08.1956; (BRA). — Vys. Tatry; leg. Szép R., 20.08.1904; (BRA). — Slovenský raj, Stratenské vrchy, Dobšiná, Andrejisko, hrebeň Andrejiská, Orientatio SN, acclivitas 30°, substratum calcit, terra rendzina, vegetatio *Abieto-Fagetum*; 1200 m a.s.l.; leg. Hajdúk J., 09.08.1956; (BRA). — Západné Tatry: Liptovský Hrádok, krovitý breh – Džadovica; 680 m a.s.l.; leg. Horníčková J., 25.08.1976; (BRA). — Slovenský raj: Vernár, dolomit, rendzina, Pinetum sylv.; 1000 m a.s.l.; leg. ?; (BRA). — Slovenský raj: Hnilec, orientatio V(E), substratum calcit; 850 m a.s.l.; leg. Hajdúk J., 31.07.1956; (BRA). — Stratenská hornatina, Vernár, juž. exp., vápence; 1000 m a.s.l.; leg. Hajdúk J., 16.08.1956; (BRA). — Nízke Poloniny: brehy "Plaša potoka" pri križovatke na Runinu; 450 m a.s.l.; leg. Gallo A., 21.08.1970; (BRA). — Ružomberok: in convalle rivi Lubochňanka ad dom. tenat. Vyš. Tajch; 800 m a.s.l.; leg. Valenta V., 16.08.1940; (BRA). — Bel. Tatry: Muráň; 1500 m a.s.l.; leg. Nábělek V., 07.1936; (BRA). — Bel. Tatry: in declivibus supra pag. Tatranská Kotlina; 1000 m a.s.l.; leg. Valenta V., 08.08.1936; (BRA × 2 vouchers). — Vysoké Tatry: Bielovodská dolina; 1700 m a.s.l.; leg. Drévlackanský Fr., 07.10.1962; (BRA). — Horná Nitra, cestou M. Klákk – V. Klák, na horských lúkach; 1350 m a.s.l.; leg. Drévlackanský Fr., 14.08.1963; (BRA × 3 vouchers). — Dobšiná, pri jaskyni; leg. ?, 07.08.1962; (BRA × 2 vouchers). — Slovenský raj: Stratenské vrchy, Dobšiná, Vernár, collis Kopanec, od cesty Stratená – Hrabušice ca. 250 m, orientatio J (S), acclivitas ca 30°, substratum calcit, terra rendzina, vegetatio *Cephalanthero-Fagetum*; 1100 m a.s.l.; leg. Hajdúk J., 16.08.1956; (BRA). — Slovenský raj: Stratená, Vernár, collis Havrania skala, orientatio 35° J (S), substratum calcit, terra rendzina, vegetatio *Aceretum pseudoplatani*; 1000 m a.s.l.; leg. Hajdúk J., 03.08.1955; (BRA). — Slovenský raj: Stratenské vrchy, Stratená, collis Roveň (Havrania skala), substratum CaCO₃, terra rendzina, vegetatio *Fagetum*; 1000 m a.s.l.; leg. Hajdúk J., 24.08.1956; (BRA). — Slovenský raj: Stratená, collis Andrejisko, na vrchole kopca, orientatio N, acclivitas 20°, substratum calcit, terra rendzina, vegetatio zmiešaný les; 1100 m a.s.l.; leg. Hajdúk J., 01.08.1956; (BRA). — Slovenský raj, Stratenská hornatina, Dobšinská Maša, horná časť prehodý, orientatio S (N), substratum calcit, terra rendzina, vegetatio *Abieto-Fagetum*; 900 m a.s.l.; leg. Hajdúk J., 31.07.1956; (BRA). — Slovenský raj: Dobšiná, collis Andrejisko, od ľadovej jaskyne 2,8 km, orientatio S (N), acclivitas ca 30°, substratum calcit, terra rendzina, vegetatio *Cephalanthero-Fagetum*; 1000 m a.s.l.; leg. Hajdúk J., 09.08.1956; (BRA). — Nízke Tatry: vápencové svahy nad Trangošskou dolinou pod "Kozími chrbáty"; 1750 m a.s.l.; leg. Dvořák J., 11.07.1966; (BRA). — Nízke Tatry: Prostredná dolina, na okraji olšiny poblíž Kyslé; 750 m a.s.l.; leg. Dvořák J., 15.07.1972; (BRA). — Stratená; leg. ?, 09.08.1949; (BRA). — Tisovec (Voniaca); leg. ?, 12.08.1948; (BRA). — Dobšiná, pri jaskyni; leg. ?, 07.08.1962; (BRA). — Strážovská hornatina, Strážov (k. 1214 m), lúčne porasty pri kóte; 1150 m a.s.l.; leg. Kollár J., 21.07.1966; (BRA). — Staškov, v záhrade; leg. ?, 26.08.1960; (BRA × 3 vouchers). — Slovenský raj, na pravom brehu Bieleho potoka; leg. Vyparina St. [?], 16.08.1958; (BRA). — Slovenský raj, na pravom brehu Bieleho potoka, 1,5 km južne od Sokolovej doliny; 600 m a.s.l.; leg. Vyparina St. [?], 16.08.1958; (BRA). — Horná Nitra, cestou M. Klák – V. Klák, na horských lúkach; 1350 m a.s.l.; leg. Drévlackanský Fr., 14.08.1963; (BRA × 3 vouchers). — Veľká Fatra, in convalle fluvii Lubochňanka ad dom. tenat. Vyš. Tajch; 800 m a.s.l.; leg. Valenta V., 16.08.1940; (BRA). — Brezno; leg. Zechentner, ?; (BRA). — Ružomberok – Biele skaly; leg. Hodoval V., ?; (BRA). — Cestou na Čergov; leg. Drévlackanský Fr., 09.08.1957;

(BRA × 5 vouchers). — Muránska planina: Muráň, hradný vrch, nádvorie hradu; leg. Kochjarová J., 06.08.1985; (BRA). — Liptovská Osada – Podsuhá, Vel. Brankov, pod Kuruckou skalou; leg. ?, 16.07.1972; (BRA). — Vel. Fatra – Čierny kameň; 1400 m a.s.l.; leg. [Horváthová ?], 21.08.1968; (BRA). — Nízke Tatry: Pusté Pole, na brehu menšieho rybníka poblíž hájovny; 850 m a.s.l.; leg. Dvořák J., 17.08.1976; (BRA). — Chočské pohorie: Veľký Choč; leg. ?, 06.1964; (BRA). — Tisovec, Voniaca; leg. Blatný T., 12.08.1948; (BRA). — Veľká Fatra: Gaderská dolina, in fundo vallis, ad marginae silvae; leg. Uhlířová J., 18.09.1993; (BRA × 2 vouchers). — Vysoké Tatry: Tatranské Matliare, SV od osady, dlhý les a mokryny, podmáčaná smrečina; 850 m a.s.l.; leg. Vozárová M., 23.08.1995; (BRA). — Slovenský raj: ústie doliny Veľký Sokol za hájovnou; 800 m a.s.l.; leg. Gallo A., 24.08.1971; (BRA). — Veľká Fatra: Tlstá, vrchol; leg. Bernátová [?], 02.09.1976; (BRA). — Belanské Tatry: cestou z Tatranskej Kotliny; leg. Májovský J., 04.09.1960; (SLO). — Muránska planina, Δ Cigánka (hrad); leg. Záborský J., 25.09.1984; (SLO). — Dolina Kysel (Slovenský raj); leg. Hejná, Rošetzká, 21.08.1951; (SLO). — Dolina Kysel (Slovenský raj); leg. Hejna, Rošetzká, 21.08.1951; (SLO × 3 vouchers). — Δ Solisko, na okraji lesa, pri hrebeňovom chodníku, v *Nardetum*; 1000 m a.s.l.; leg. Májovský J., 07.1955; (SLO × 4 vouchers). — Oravská Polhora: vystup na Babiu horu?; leg. Futák J., 25.08.1964; (SLO). — Západ. Tatry: Roháčske plesá; leg. Šomšák L., 08.1972; (SLO). — Veľká Fatra: Bukovina [Bukovinka]; leg. Michalková V., 05.09.1962; (SLO). — Poprad; leg. Šmarda J., Pačlová L., 11.09.1956; (SLO). — Vysoké Tatry: Zadné Medodoly; 1350 m a.s.l.; leg. Májovský J., 20.09.1967; (SLO × 5 vouchers). — Vysoké Tatry: Zadné Medodoly, pri lesnej ceste do Javoriny, v smrečine; 1350 m a.s.l.; leg. Májovský J., 20.09.1967; (SLO). — Skala na V. Fatre, vápence; 1100 m a.s.l.; leg. Futák J., 11.08.1942; (SLO). — Záp. Beskydy: Oravská Lesná, Juzikovka; leg. Murín A., 25.07.1983; (SLO × 2 vouchers). — Jastub na skalkach v lese; leg. Májovský J., 18.09.1947; (SLO). — Rozsutec [Veľký Rozsutec]; leg. Futák J., 23.08.1943; (SLO). — Tatr. Kotlina; leg. Futák J., 28.08.1954; (SLO × 2 vouchers). — Belianske Tatry: Ždiarska Vidla – Havran; leg. Májovský J., 23.08.1957; (SLO × 2 vouchers). — Turzovka; leg. Halbová I., 20.08.1962; (SLO). — Lúky pod Vys. Tatrami, cestou z Podbanska na Štrbské Pleso; leg. Pastýrik L., Májovský J., 27.08.1941; (SLO × 2 vouchers). — Flora Leutschoviensis [Levoča]; leg. Greschik V., 07.1890; (SLO). — Leutschovia [Levoča], Gr. Rehberg; leg. Greschik V., 07.1918; (SLO). — Flora Leutschoviensis [Levoča], Dolina; leg. Greschik V., 07.1889; (SLO). — Flora Tatrae Magnae, Leutschovia [Levoča]; leg. Greschik V., 07.1928; (SLO). — Flora Tatrae Magna, Leutschovia [Levoča], in silvae Kienwald; leg. Greschik V., 07.1900; (SLO). — Flora Tatrae Magnae, Leutschovia [Levoča], in silvus elesioribus; leg. Greschik V., 08.1928; (SLO). — Leutschovia [Levoča], in [Wäldern ?]; leg. Greschik V., 07.1918; (SLO). — Vysoké Tatry: Téryho chata; leg. Futák J., 18.08.1946; (SLO). — Vyšné Hágy, rašelinisko; leg. Futák J., 26.08.1954; (SLO × 2 vouchers). — Juhoslovenský kras, Silická planina; leg. Futák J., 05.09.1946; (SLO). — Tatr. Kotlina; leg. Futák J., 28.08.1954; (SLO). — Slovenský raj: na lúčke pod Lipovcom [planina Lipovec] nad potokom; leg. Ferjanec J., 04.09.1962; (SLO × 4 vouchers). — Čierny Váh: na lúke Stará Polana; leg. Ferjanec J., 26.07.1965; (SLO). — Údolie Čierneho Váhu; leg. Májovský J., 18.08.1957; (SLO). — Orava: Polhora kúpele; leg. Jurko A., 16.09.1955; (SLO × 6 vouchers). — Fatra Major: in valle “Žarnovická dolina” non procul a p. Dolná Štubňa [now Turčianske Teplice], in declivibus; 600 m a.s.l.; leg. Brižický G., Červeňová Ž., 18.08.1940; (SLO). — Veľká Fatra: Tlstá; leg. Hrivnáková, 20.08.1954; (SLO). — Čierny Váh – pri horárni Biely Potok; 830 m a.s.l.; leg. Vartíková E., 15.09.1972; (SLO). — Jamnícka Dolina – v kosodrevine; leg. ?, 09. 1981; (NI). — Lúčky [okres Ružomberok]; leg. Svobodová Z., 04.09.1973; (NI × 2 vouchers). — Muránska vysočina: Veľká Stožka; leg. Sillingner P., 11.08.1937; (NI). — Bielske Tatry: Drabina [near Kobyly vrch]; leg. Domin K., 22.08.1937; (NI). — Bielske Tatry: Ivanka; leg. Domin K., 29.08.1937; (NI). — Klak; leg. Takáč, 06.09.1962; (NI). — in monte Klak; leg. Brancsik K., 07.1900; (NI). — Pieniny (Vysoké Skalky), Stráňany; 1052 m a.s.l.; leg. Magic D., 19.08.1963; (ZV). — v doline potoka nad obcou Olcnavá; leg. Šmídt I., 20.08.1990; (KO × 2 vouchers). — úd. Kvačanské rokle; leg. Mikoláš V., 19.09.1984; (KO). — sev. a sv. svahy pod Klakom; leg. Mikoláš V., 15.08.1984; (KO). — Homôlka (Lúč. MF); leg. Mikoláš V., 15.09.1984; (KO). — str.–sp. č. úd. od haj. Vonžovec – červ./žltá; leg. Mikoláš V., 18.09.1984; (KO). — Vavrišovo; leg. ?, 19.08.1960; (KO). — Tokáreň; leg. Mikoláš V., 14.08.1958; (KO). — Muránska planina – Hrad; leg. Mikoláš V., 24–25.08.1959; (KO × 6 vouchers). — úd. Dudliavky – Kamenné Vráta; leg. Mikoláš V., 16.08.1984; (KO × 2 vouchers). — Olcnavá, pri potoku v doline Raj, cca. 1 km nad dedinou; leg. Šmídt I., 20.08.1990; (KO). — Belanské Tatry, chodník Kopské sedlo – Zadné Medodoly; leg. Najvarová Š., 30.08.1968; (TNP). — Belanské Tatry, Dolina Siedmich prameňov, Lavínový žľab; 1450 m a.s.l.; leg. Odložilíková L., 12.08.1955; (TNP). — Belanské Tatry, Dolina Siedmich prameňov, Lavínový žľab; 1500 m a.s.l.; leg. Odložilíková L., 01.09.1955; (TNP × 3 vouchers). — Západné Tatry: Tichá Dolina, pod Štrkami; leg. Šmarda J., 25.08.1955; (TNP). — Belanské Tatry, Zadné Medodoly, nad Bránkou; 1210 m a.s.l.; leg. Pačlová L., Šoltésová A., 31.08.1989; (TNP × 2 vouchers). — Belanské Tatry, nad chatou Plesnivec v Doline Siedmich prameňov; 1350 m a.s.l.; leg. Šoltés R., Šoltésová A., 11.09.1974; (TNP). — Západné Tatry: Tichá Dolina, pod Štrkami; leg. Pačlová L., 25.08.1957; (TNP). — Belanské Tatry, Dolina Čiernej vody (Rakúskej); 900 m a.s.l.; leg. Odložilíková L., 14.08.1955; (TNP). — Belanské Tatry, Červená hlina; 1370 m a.s.l.; leg. Odložilíková L., 21.08.1955; (TNP). — Belanské Tatry, Dolina Čiernej vody (Rakúskej); 980 m a.s.l.; leg. Odložilíková L., 09.09.1955; (TNP). — Bad Lucsivna [Lučivná]; leg. Scherfel A., 08.1891; (TNP). — Poprad; leg. Scherfel A.; (TNP).

Aconitum lasiocarpum* subsp. *lasiocarpum

SLOVAKIA: Užská hornatina: Rabia skala, pieskovec, skalky, južný svah; 1190 m a.s.l.; leg. Michalko J., 08.1956; (SAV × 4 vouchers). — Vihorlat, vrch Vihorlat, *Calamagrostidetum arundinaceae*; 1050 m a.s.l.; leg. Michalko J., 26.07.1967; (SAV). — pod vrcholom Stinskej; leg. Marhold K., 18.08.1988; (SAV). — vrch Vihorlat, tesne pod vrcholom; leg. Michalko J., 27.08.1963; (SAV). — Slovenské rudohorie: Slovenský raj. Stratená, Dobšiná. Andrejisko. Orientatio S(N), acclivitas 35°, substratum calcit, terra rendzina, vegetatio *Fagetum*; 1100 m a.s.l.; leg. Hajdúk J., 09.08.1956; (BRA). — Slovenský raj, Stratská hornatina. Stratená, Strat. pila. Hnilec, Vodná nádrž. Orientatio S2, NW, acclivitas 50°, substratum CaCO₃, terra litozem, rendzina, vegetatio *Piceetum calcicolum*; 840 m a.s.l.; leg. Hajdúk J., 31.07.1956; (BRA). — Slovenský raj, Stratské vrchy. Dobšiná. Andrejisko, hrebeň Andrejiská. Orientatio SN, acclivitas 15°, substratum calcit, terra rendzina, vegetatio *Abieto-Piceetum*; 1200 m a.s.l.; leg. Hajdúk J., 09.08.1956; (BRA). — Nízke Beskydy: Osadné, nad Δ Čereniny v bučine, pri hranici; leg. Májovský J., 29.09.1975; (SLO). — Vysoké Tatry: Kriváň; leg. Májovský J., 21.08.1957; (SLO). — Vihorlat [vrch], lúky (typu *Calamagrostion*) na najvyššom vrchole, J; 1060 m a.s.l.; leg. Májovský J., Michalko J., 10.09.1953; (SLO). — Slovakia; leg. ?; ?; (SLO × 3 vouchers). — Nízke Beskydy: Osadné, pri chodníku štátnej hranice na Δ Čereniny, nízky zárasť *Salix caprea*; 950 m a.s.l.; leg. Májovský J., 29.09.1975; (SLO). — Nízke Tatry, Slovenský raj: Javorina nad Vernárem; leg. Sillinger P., 06.08.1931; (NI).

Aconitum lasiocarpum* subsp. *kotulae

SLOVAKIA: Pusté Pole; leg. Futák J., 21.08.1973; (SAV). — Užská hornatina: Rabia skala, pieskovec, skalky, južný svah; 1190 m a.s.l.; leg. Michalko J., 08.1956; (SAV × 2 vouchers). — Vihorlat, pod vrcholom vrchu Vihorlat; leg. Michalko J., 27.08.1963; (SAV × 2 vouchers). — vrch Vihorlat, tesne pod vrcholom; leg. Michalko J., 27.08.1963; (SAV × 4 vouchers). — Šíp [vrch], obec Stankovany; 1100 m a.s.l.; leg. ?, 23.08.1975; (BRA). — Čierny Váh: na lúke Stará Poľana; leg. Ferjanec J., 26.07.1965; (SLO).

Aconitum* × *pawlowskii

SLOVAKIA: Čierny Váh: na lúke Stará Poľana; leg. Ferjanec J., 26.07.1965; (SLO). — Belianske Tatry: Ždiarska Vidla – Havran; leg. Májovský J., 23.08.1957; (SLO).

Aconitum degenii* subsp. *degenii

SLOVAKIA: Slovakia orientalis, Pri chyžke u potoku Stuzka; leg. Šmidt[?], 13.08.1964; (SAV × 2 vouchers).

Aconitum* × *gayeri

SLOVAKIA: medzi Čolom a Rabiou skalou, vo Fagete pod hranicou; leg. Šmidt[?], 15.08.1964; (SAV).

Aconitum* × *cammarum

SLOVAKIA: Bardejov; leg. Berganský J., 06.08.1928; (BRA × 7 vouchers). — Bardejov: Wachterhof; leg. Berganský J., 25.07.1924; (BRA). — Bardejov: Roch-Vorgelände; leg. Berganský J., 09.10.1929; (BRA). — Pri železničnej stanici Čirč; leg. ?, 30.07.1957; (BRA). — Sasinkovo, v zahrade; leg. ?, 30.06.1963; (BRA × 2 vouchers). — Vrátna [dolina]; leg. Brančík K., 08.1904; (SLO). — Felka [Veľká, Tatry]; leg. Scherfel A., 10.08.1924; (TNP × 2 vouchers). — Felka eig. Garten [Veľká, Tatry]; leg. Scherfel A., 14.08.1923; (TNP × 3 vouchers).

Aconitum firmum* subsp. *firmum

SLOVAKIA: Belianske Tatry; 1250 m a.s.l.; leg. Futák J., 14.07.1861; (SAV). — Slavkovský potok [Vysoké Tatry]; leg. Grebenščikov O., 07.08.1954; (SAV). — Vysoké Tatry, Zelené pleso hezin; 1550 m a.s.l.; leg. Hubová O., 23.06.1969; (SAV × 2 vouchers). — Vysoké Tatry, Veľická dolina, Veľické pleso; 1700 m a.s.l.; leg. Fabianková, 09.09.1974; (SAV). — TANAP, Biely Váh dolina; 1350 m a.s.l.; leg. Futák J., 27.08.1957; (SAV). — Lúč. Malá Fatra, vrch Reváň; 1100 m a.s.l.; leg. Schidlay E., 20.08.1949; (SAV). — Belianske Tatry, Javorina, Zadné Koperšady (Zadné Medodoly) v smrečine; 1400 m a.s.l.; leg. Schidlay E., 09.08.1946; (SAV). — Vihorlat: Popričný vrch, v sédole; leg. Šramko E., 08.1953; (SAV). — Pílsko, Babia hora; leg. Futák J., Magic D., 11.08.1964; (SAV). — Djumbier [Dumbier], svahy; leg. Ptý [Ptačovský], 09.1928; (SAV). — Vihorlat: V ridoli potoka; leg. Šramko E., 08.1953; (SAV). — Vys. Tatry, Veľická dolina; leg. Futák J., 08.08.1962; (SAV). — B.T., Široká dolina, dolná časť; 1500 m a.s.l.; leg. Flašíková, Hubová O., 02.08.1971; (SAV × 2 vouchers). — Bel. Tatry: Faixová; leg. Ptý, 22.07.1939; (SAV). — Bel. Tatry: pri Bielom plese (smerom na Belanskú kopy); 1600 m a.s.l.; leg. Zahradníková K., Šípošová H., 19.08.1980; (SAV × 3 vouchers). — Vysoké Tatry: N svahy

Kežmarského štítu nad Zeleným plesom; 1600 m a.s.l.; leg. Zahradníková K., 07.08.1969; (SAV). — Vysoké Tatry: cestou na Kriváň; leg. Zahradníková K., 15.08.1970; (SAV). — Vysoké Tatry: južný svah Jahňacieho štítu; 2000 m a.s.l.; leg. Zahradníková K., 09.08.1969; (SAV). — Vysoké Tatry: Kriváň; leg. Zahradníková K., Jasičová M., 11.08.1966; (SAV). — Votrubova chata; leg. Ptý, 07.1941; (SAV). — V kleči u Popradského plesa; leg. Ptý, 07.1939; (SAV). — Záp. Beskydy: Babia hora; leg. Zahradníková K., 21.07.1973; (SAV). — Vysoké Tatry: Furkotská dolina – Wahlenbergovo pleso – Bystré sedlo; leg. Zahradníková K., 13.08.1970; (SAV). — Tatry. Faixová a Votrubova chata; leg. Horák P., 25–26.08.1937; (SAV). — [Chočská] Fatra, Biela Skala; 1600 m a.s.l.; leg. Futák J., 24.08.1966; (SAV × 3 vouchers). — Slovakia borealis: montes Vysoké Tatry, ad ripas lacus “Popradské pleso” in *Pineto mughi*, solo granitico; 1640 m a.s.l.; leg. Hlavaček A., ?; (SAV). — Západné Tatry: Osobitá, Suchá dolina, pri potoku; 1000 m a.s.l.; leg. Futák J., 15.07.1965; (SAV × 2 vouchers). — Vys. Tatry; leg. Szép R., 20.08.1904; (BRA). — Vys. Tatry; 2000 m a.s.l.; leg. ?, 07.1936; (BRA). — Vys. Tatry: ad margines sylvae, Štrbské Pleso et Vyš. Hágy; 1200 m a.s.l.; leg. Valenta V. 01.08.1935; (BRA). — Nízke Tatry: pramenité svah pod vrchom Krupovy hole; 1550 m a.s.l.; leg. Dvořák J., 07.08.1973; (BRA). — Vysoké Tatry; leg. Berganský J., 28.08.1928; (BRA × 2 vouchers). — Vysoké Tatry; leg. Vaslora L. [?], 07.08.1952; (BRA). — Vysoké Tatry: Javorová dolina; 1900 m a.s.l.; leg. Drévlackanský Fr., 12.10.1962; (BRA). — Štrbské pleso; leg. Černý J., 08.07.1924; (BRA). — Slovenské rudohorie: Volovské vrchy, Slovenský raj. Dedinky, Mlynky, collis Kukla, flumen Hnilec. Orientatio N, acclivitas 35°, substratum silik.+carb., vegetatio *Mugedio–Aconitetea*; 980 m a.s.l.; leg. Hajdúk J., 18.07.1956; (BRA). — Vysoké Tatry: montis Satan; leg. Weber F., 08.1936; (BRA). — Vysoké Tatry: montis Končistá; 2450 m a.s.l.; leg. Weber F., 08.1925; (BRA). — Bielské Tatry: montis Tokárna; 1200 m a.s.l.; leg. Weber F., 08.1936; (BRA). — Vysoké Tatry: ad lac. Ladové pleso; leg. Weber F., 08.1925; (BRA). — Bielské Tatry: montis Havran; leg. Weber F., 08.1936; (BRA). — Vysoké Tatry: montis Vel. Vysoká; 2400 m a.s.l.; leg. Weber F., 08.1925; (BRA). — Tatra; leg. Scherfel A., ?; (BRA). — Belianské Tatry: Tatr. Kotlina – “Zadné Meďodoly”; 1400 m a.s.l.; leg. Z., 07.08.1969; (BRA). — Záp. Tatry: Bystrá dolina, ad ripam rivi “Bystrý”; 1300 m a.s.l.; leg. Dvořák J., 28.07.1976; (BRA). — Orava – Osobitá; leg. Magdolenová S., 08.1967; (BRA). — Bielske Tatry; leg. ?, 07.08.1970; (BRA). — Žiarska dolina; leg. ?, 31.07.1942; (BRA). — Vysoké Tatry: Česká dolina, kamenité svahy nad Českým plesom [now Ťažké pleso]; 1620 m a.s.l.; leg. Dvořák J., 07.09.1974; (BRA). — Záp. Tatry: Bystrá dolina, ad ripam rivi “Bystrý”; 1300 m a.s.l.; leg. Dvořák J., 28.07.1976; (BRA). — Vysoké Tatry; 950 m a.s.l.; leg. Kavka V., 15.07.1930; (BRA). — Vysoké Tatry: Kriváň; leg. Májovský J., 21.08.1957; (SLO). — Vysoké Tatry: cestou z Popradského na Žabie plesá; leg. Gálišová E., 23.08.1956; (SLO). — Nízke Tatry: Ohnište; leg. Májovský J., 20.07.1957; (SLO). — V. Tatry: Velická dolina; leg. Schidlay E., 10.10.1952; (SLO). — V. Tatry: Hincovo pleso; leg. Háberová I., 16.07.1968; (SLO). — Záp. Beskydy: Babia hora; leg. Murín A., 27.07.1983; (SLO × 2 vouchers). — Rašelinisko Mutné (okres Námestovo); leg. Májovský J., 30.07.1955; (SLO). — Kamenné vráta; leg. Futák J., 19.08.1946; (SLO × 3 vouchers). — Belianske Tatry: hrebeň; leg. Futák J., 09.07.1946; (SLO). — Tatranská Lomnica: Kežmarskej Źlaby, okolie; leg. Futák J., 08.07.1946; (SLO). — Popradské pleso; leg. Futák J., 23.08.1943; (SLO). — Zlomisková dolina, Ladové pleso, V. Tatry; leg. Futák J., 26.08.1943; (SLO). — Belianske Tatry: Javorina; leg. Futák J., 14.07.1946; (SLO). — Belianske Tatry: Tristárska dolina; leg. Futák J., 14.07.1946; (SLO × 3 vouchers). — Vysoké Tatry: Litvorová kotlina; leg. Vartíková E., 28.09.1971; (SLO × 2 vouchers). — Vysoké Tatry: circum l. d. “Zelené pleso”; leg. Nábělek V., 12.08.1940; (SLO × 2 vouchers). — Belské Tatry: sev. svah Hlúpeho; leg. Futák J., 21.08.1943; (SLO). — Jánska dolina, JZ svahy Ohništa, nad lesom na trávnatých svahoch a v suti; leg. Májovský J., 15.07.1967; (SLO × 2 vouchers). — Javorová dolina pri Javorine vo V. Tatrách; leg. Futák J., 17.08.1943; (SLO). — Dolina Mlynica vo V. Tatrách; leg. Futák J., 25.08.1943; (SLO × 4 vouchers). — Belianske Tatry: Zadné Koperšady; leg. Nábělek V., 12.08.1940; (SLO). — Kamenné vráta; leg. Futák J., 19.08.1946; (SLO). — Žiarska dolina: hojne na prameniskách a popri potokoch v pásme kosodreviny; leg. ?, 16.08.1992; (NI). — montes Vysoké Tatry: in valle Zlomisko, solo granitico; leg. Osvačilová V., 14.08.1951; (NI). — Belianske Tatry: Tristárska dolina; leg. Eliáš P. jun., 1998; (NI × 2 vouchers). — Račkova dolina; leg. Mikoláš V., 08.1956; (KO). — Sliezsky Dom vo V.T. [horský hotel na prahu Velickej doliny vo Vysokých Tatrách]; leg. Vojtún A., 03.08.1969; (KO × 2 vouchers). — ud. Bocianky, str.–sp. časť; leg. Mikoláš V., 15.08.1984; (KO). — Račkova dolina; leg. Mikoláš V., 08.1956; (KO). — Priehyba pod Krivánom [vo Vysokých Tatrách]; leg. Vojtún A., 16.07.1968; (KO × 2 vouchers). — Západné Tatry: v kosodrevine pod Tomanovským sedlom; 1650 m a.s.l.; leg. Šoltésová A., 18.09.1974; (TNP). — Bujačí Vrch – južný svah, v kosodrevine; 1800 m a.s.l.; leg. Šoltés R., Šoltésová A., 11.09.1974; (TNP). — Belianské Tatry. Bujačí Vrch, medzi Malým a Veľkým Košiarom; 1850 m a.s.l.; leg. Šoltésová A., 17.09.1974; (TNP). — Belianské Tatry. Bujačí Vrch, pod Veľkým Košiarom; 1850 m a.s.l.; leg. Šoltésová A., 17.09.1974; (TNP). — V. Tatry. V. Studená dol.; leg. Pačlová L., 29.08.1954; (TNP). — V. Tatry. Velická dol.; leg. Najvarová Š., 23.07.1968; (TNP). — Vysoké Tatry: pri značovanem chodníku zo Šalvejového prameňa na Zelené pleso; leg. Najvarová Š., 27.07.1967; (TNP). — Velická dolina, Kvetnica; 1820 m a.s.l.; leg. Odložilíková L., 06.07.1954; (TNP). — Vysoké Tatry. Veľká Studená dol.; 1610 m a.s.l.; leg. Odložilíková L., 15.08.1954; (TNP). — Belianské Tatry, pod Siedmimi prameňmi; 1230 m a.s.l.; leg. Odložilíková L., 08.08.1956; (TNP). — Mengersdorfer [Mengusovce]; leg. Scherfel A., 08.1923; (TNP × 2 vouchers). — Felka [Poprad-Veľká] eig. Garten [Veľká, Tatry]; leg. Scherfel A., 08.1923; (TNP). — Felka eig. Garten [Veľká, Tatry]; leg. Scherfel A., 08.1923; (TNP). — Mengersdorfer [Mengusovce]; leg. Scherfel A., 13.07.1924; (TNP). — Salbauffal; leg. Scherfel A.; (TNP). — Gwostar [?]; leg. Scherfel A., 07.1880; (TNP).

Aconitum firmum* subsp. *moravicum

SLOVAKIA: Malá Fatra, vrch Chleb; leg. Nábělek V., 29.08.1942; (SAV). — Malá Fatra, Šútovská dolina; leg. Zahradníková K., 22.07.1973; (SAV). — Vysoké Tatry, popri turistickom chodniku z Kriváňa na Tri studničky (po zelenej značke); 2300 m a.s.l.; leg. Peniašteková M., 20.08.1987; (SAV). — Manínska úžina (v jej prvej – dolnej polovici); leg. Schidlay E., 08.09.1945; (SAV). — Krivánska Malá Fatra: hrebeňovka V. Kriváň – Biele skaly – Suchý; 1500 m a.s.l.; leg. Futák J., Jasičová M., Zahradníková K., 11.09.1964; (SAV × 2 vouchers). — Krivánska Malá Fatra: Snilovská dolina – pri potoku; leg. Letz R., 1994; (SAV). — Malá Fatra: Malý Kriváň, v porastoch kosodreviny; 1600 m a.s.l.; leg. Grebensčíkov O., 22.09.1954; (SAV). — Vysoké Tatry: Bielovodská dolina; 1700 m a.s.l.; leg. Drévlackanský Fr., 07.10.1962; (BRA). — ad valle Studenec ad pedem m. Malý Kriváň; leg. Holuby J., 29.07.1896; (BRA). — [Krivánska] Malá Fatra. Veľký Rozsutec; leg. Drévlackanský Fr., 11.07.1961; (BRA). — ad valle Vrátna; leg. Brancsik C., 09.1898; (BRA). — Malá Fatra: in saxis montis Rásutec [Rozsutec] supra pag. Štefanová; leg. Weber F., 07.1935; (BRA). — Fatra Minor: in declivibus saxosis m. Kriváň; 1500 m a.s.l.; leg. Brižický J., 27.08.1940; (SLO). — Malá Fatra: Kriváň; leg. Činčura F., 19.07.1971; (SLO × 4 vouchers). — Rašelinisko Mutné (okres Námestovo); leg. Májovský J., 30.07.1955; (SLO × 2 vouchers). — Kamenné vráta; leg. Futák J., 19.08.1946; (SLO). — Javorová dolina pri Javorine vo V. Tatrách; leg. Futák J., 17.08.1943; (SLO). — Rozsutec – horná hranica lesa; leg. ?, 09.1981; (NI). — Horné diery; leg. Mikoláš V., 18.08.1984; (KO). — Horné diery (Kriv. MF); leg. Mikoláš V., 12.09.1984; (KO). — Vysoké Tatry: pri značkovanom chodníku zo Šalvejového prameňa na Zelené pleso; leg. Najvarová Š., 27.07.1967; (TNP).

Aconitum firmum* subsp. *maninense

SLOVAKIA: Kamenné vráta; leg. Futák J., 19.08.1946; (SLO). — Manín [vrch]; leg. Futák J., 25.08.1971; (SAV). — Súľov; leg. Futák J., 25.08.1971; (SAV). — Žilina. In saxosis calcareis in convalle Sedlovina et Kopaná ad Kunerad; 700 m a.s.l.; leg. Valenta V., 28.07.1937; (BRA).

Aconitum firmum* nothosubsp. *paxii

SLOVAKIA: Fatra Minor: in declivibus saxosis m. Kriváň; 1500 m a.s.l.; leg. Brižický J., 27.08.1940; (SLO). — Belianske Tatry: Tristárska dolina; leg. Futák J., 14.07.1946; (SLO).

Aconitum firmum* nothosubsp. *zapolowiczii

TANAP, dolina Bieleho Váhu, pri potoku; 1250 m a.s.l.; leg. Futák J., 27.08.1857; (SAV). — Vys. Tatry, Velická dolina, pri jazierku; 1825 m a.s.l.; leg. Futák J., Hubová O., 06.08.1962; (SAV). — V. Tatry: Hincovo pleso; leg. Háberová I., 16.07.1968; (SLO).

Appendix 2. Specimens cited in published sources***Aconitum moldavicum* subsp. *moldavicum***

SLOVAKIA: Spišská Nová Ves (ad stationem viae ferr. “Štiavnik”), 600 m; leg. J. Suza, 20.06.1931; (KRA 003671); source: Mitka (2008a). — Tomašovská Belá, nad potokiem w lesie bukowym; leg. K. Oklejewicz, 07.08.2004; (KRA); source: Mitka (2008a). — Stratená, Fagetum; leg. J. Mitka, 18.07.2006; (KRA); source: Mitka (2008a). — Liptovské Revúce, near torrent; leg. J. Mitka, 23.07.2006; (KRA); source: Mitka (2008a). — Liptovský Ján, Jánska dolina, ok. 800 m; leg. J. Mitka, 20.08.2007 (KRA); source: Mitka (2008a).

Aconitum variegatum* subsp. *variegatum

SLOVAKIA: Północne obrzeże wapienne, Kopieniec Wielki, góra część łąk Hali Koprowej; leg. H. & T. Tacik, 18.08.1966; (KRAM); source: Mitka (2003). — Comit. Szepes, in apertis piceetorum “Gähe Leit” supra Tátrabarlaglagent; leg. A. Boros, 24.8.1938; (BP); source: Mitka (2003). — Kiczora od strony Dol. Międzyzyciennej, przerzedzony, skalisty las, wapień, 1100-1200 m; leg. B. Pawłowski, 22.07.1939; (KRA); source: Mitka (2003). — Eisernes Thor, 1550 m; leg. F. Pax, 21.08.1905; (BP); source: Mitka (2003). — wycieczka w Spiskie Tatry w 1878, Dolina między Hawranem a Nową ku szczytowi Czamego Wierchu; leg. A. Rogalski, 28.07.1878; (KRAM); source: Mitka (2003).

Aconitum lasiocarpum* subsp. *lasiocarpum

SLOVAKIA: Bieszczady Zach.: Rabia skala, wśród zarośli z *Rubus idaeus*, 1150 m; leg. P. Bochenek, J. Mitka, 23.08.1995; (KRA); source: [Mitka \(2003\)](#). — Stinska, na skraju *Fagetum* wśród *Rubus idaeus*; P. Bochenek, leg. J. Mitka, 24.08.1995; (KRA); source: [Mitka \(2003\)](#). — Stinka, 1093 m; leg. Z. Dolezalova, ?; (PRC); source: [Mitka \(2003\)](#). — Wyhorlat; leg. C. Chyzer, 21.08.1879; (BP); source: [Mitka \(2003\)](#). — Comit. Gömör, in apertis silv. pedis montis Hanneshöhe prope Ladová jaskyňa, 900 m; leg. A. Boros, 03.09.1955; (BP); source: [Mitka \(2003\)](#).

Aconitum lasiocarpum* subsp. *kotulae

SLOVAKIA: Nizke Tatry, Tále; leg. J. Mitka, 19.08.2007; (KRA 0464740); source: [Mitka](#), personal communication. — Wyhorlat, ex herbario doctoris Cornelii Hyzer; leg. ?, 21.08.1879; (BP); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — In monte Vihorlat, 1074 m; leg. F. Belšán, 25.07.1936; (PRC); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Rabia skala, 1150; leg. J. Mitka, P. Bochenek, J. Terray, 23.08.1995; (KRA); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Rabia skala, cota 1168; leg. J. Soják, 23.08.1963; (KRAM); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Slov. orient, montes Poloninské Karpaty, in monte Rabia skala supra pagum Nová Sedlica, 1160 m; leg. J. Soják, 20.08.1963; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Slov. orient, montes Poloniny, ad cacumen montis Čereniny supra pagum Zvala; 933 m; leg. J. Soják, 21.08.1963; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Na skale Rabia skala, trig. 1168 m; leg. J. Šourek, 18.07.1957; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Výslunna škála nad Runinou; 1150 m; leg. J. Šourek, 7.08.1957; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — montes Poloniny, ad cacumen jugi inter pagos Zvala et Ruské, c. 1000 m; leg. J. Soják, 21.08.1963; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Nízke Poloniny, Runina, výslunné skály na Rabia skale, 1150 m; leg. J. Soják, 6.08.1957; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Dol. Koprowa [Kôprová dolina] – skraj lasu, ok. 1300 m, leg. B. Pawłowski, 19.8.1925; (KRAM-Pawłowski 320228, Paratypus); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#).

Aconitum* × *pawłowskii

SLOVAKIA: Dol. Koprowa (górna), zarośla mieszane; leg. J. Trela, 19.08.1825; (KRA); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Tatras, Kôprová dolina; leg. K. Zahradníková, M. Jasičová, 13.08.1966; (SAV); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Belianske Tatry, Lysá Polana; leg. K. Zahradníková, M. Jasičová, 14.08.1966; (SAV); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — apud rivulorum Javorový potok supra pag. Javorina (Tatranská Javorina), 1050 m; leg. J. Dostál, 15.08.1955; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Nízke Tatry, Dudlavá skala; leg. V. Kneblóvá, 08.09.1955; (PR); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Dobšiná jégbevlong szkecenei völgy [jégbarlang, Sztraczenai völgy?] leg. D. Filarszky, 23.08.1911; (BP); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Křižovatka; leg. A.G., 27.05.1970; (KO); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Hrad Mt.; leg. ?, 24.08.1959; (KO 13340); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#). — Údolie Dudlavky (ad Kamenná vrata); leg. V. Mikoláš, 16.08.1984; (KO); source: [Mitka & Starmühler \(2000\)](#) and [Mitka \(2003\)](#).

Aconitum* × *gayeri

SLOVAKIA: Rabia skala, 1150 m; leg. J. Mitka, P. Bochenek, J. Terray, 23.8.1995; (KRA); source: [Mitka \(2003\)](#).

Aconitum* × *cammarum

SLOVAKIA: Veľká Fatra, Donovaly; leg. J. Mitka, 27.07.2007; (KRA 0347248); source: [Mitka](#), personal communication.

Aconitum* × *berdau

SLOVAKIA: Tatras, Javorová dolina; leg. A. Sutkowska, 18.08.2009; (herb. J. Mitka); source: [Mitka](#), personal communication. — Malá Fatra, Terchová; leg. J. Mitka, 31.07.2009; (KRA 0464764); source: [Mitka](#), personal communication. — Wycieczka w Tatry Spiskie w r. 1878. *Aconitum napellus* L., Podspady nad Jaworzynką; leg. A. Rogalski, 25.07.1878; (KRAM 13348); source: [Mitka \(2003\)](#). — *Plantae exsiccatae regni Hungariae*, Comit. Szepes, in m. Hollókö pr. Sztraczena; leg. G. Lengyel, 01.08.1901; (BP); source: [Mitka \(2003\)](#).

Aconitum firmum* subsp. *firmum

SLOVAKIA: pod szczytem Łomnicy; leg. H. Zapalowicz, 12.08.1908; (KRAM); source: [Mitka \(2003\)](#). — in subalpinis

montium Magas-tatra supra Kešmark: Tokarna Zóltó; leg. L. Simonkai, 7-8.07.1890; (BP); source: [Mitka \(2003\)](#). — Hinszko [lake Hincó]; leg. D. Filarszky, Kümmerle, 07.08.1909; (BP); source: [Mitka \(2003\)](#). — [Vysoké and Západné Tatry Mts.] bélei Tiefergrund; leg. Magócsy, ?; (BP); source: [Mitka \(2003\)](#). — [Vysoké and Západné Tatry Mts.] ad lacum Buckholz [Buchholz ?], 1950 m; leg. G. Lengyel, 04.08.1931; (BP); source: [Mitka \(2003\)](#). — im Kleinkohlachtale, Hohe Tatra; leg. A. Margittai, 28.7.1930; (M); source: [Mitka \(2003\)](#). — Breznóbánya, Zadny Uplaz; leg. S. Kupcok, 02.08.1904; (PR); source: [Mitka \(2003\)](#). — ad Javorinam, in alt. c. 900 m; leg. G. Lengyel, 17.07.1931; (BP); source: [Mitka \(2003\)](#). — Alpenkessel zwischen Nový und Havran; leg. K. Ronniger, 09.07.1918; (W); source: [Mitka \(2003\)](#). — Fatra: Hungaria, comitatus ad confin. Árva et Trencsén, montes Kriván Fatrae, in declivibus montis Roszudec, alt. c. 1300 m, solo dolomitico; leg. E. Nyárady, 27.07.1907; (SIB); source: [Mitka \(2003\)](#). — Fatra, Terchová; leg. K. Domin, 01.08.1918; (PRC); source: [Mitka \(2003\)](#). — in rupestribus “Magas Fáttra” loco “Bartya” [*A. firmum* Rchb., rev. G. Gayer]; leg. L. Simonkai, ? 08.1904; (BP); source: [Mitka \(2003\)](#). — Comit. Árva, in pineti infra locus “Rohačsi savak” – alpinum liptoviensium, c. 1500 m [*A. tatrae* Borb., rev. G. Gayer 1913]; leg. A. Jávorka, 22.08.1911; (BP); source: [Mitka \(2003\)](#). — [Rohače Mts.], Liptauer Alpen, Rohacs [Roháč], 1600 m [*A. napellus* L. var. *tauricum* (Rchb.) Ser., rev. De Rapaics]; leg. F. Pax, 14.08.1905; (BP); source: [Mitka \(2003\)](#). — Comit. Liptó, Tatra Inf., in valle Lucsany ad Deményfalven; leg. G. Lengyel, 25.07.1928; (BP); source: [Mitka \(2003\)](#).

Aconitum firmum* subsp. *moravicum

SLOVAKIA: Montes Liptovské Tatry, in alpinis Osobitá, alt. c. 1580 m, solo calcareo; leg. M. Deyl, 07.07.1938; (PR); source: [Mitka \(2003\)](#). — Roháče, u Zverovky; leg. Kopecký, 06.08.1968; (PR); source: [Mitka \(2003\)](#). — Hungaria, comitatus Liptó, montes Tatrae Liptoviensae, in saxis “Javor” in parte superiore vallis Tycha, alt. cca. 1500 m, solo calc.; leg. E. Nyárady, 01.08.1907; (SIB); source: [Mitka \(2003\)](#). — Siná, 1500–1550 m; leg. P. Sillinger, 27.07.1930; (PRC); source: [Mitka \(2003\)](#). — in lapidosis in declivi merid. montis Chleb, cca. 1600 m, solo calcareo; leg. J. Soják, 04.08.1950 (PR); source: [Mitka \(2003\)](#). — Fatra – Kriván [*A. napellus* L. var. *multifidum* Koch. var. *canescens* (Schleich.) Rapaics, det. Rapaics; leg. ?, 13.08.1889; (BP); source: [Mitka \(2003\)](#). — [Malá] Fatra, Terchová; leg. K. Domin, 01.08.1918; (PRC); source: [Mitka \(2003\)](#). — Slovakia, Malá Fatra, Révalov, Révalovská dolina, along torrent 1150–1200 m; leg. J. Mitka, 02.09.2001; (KRA); source: [Mitka \(2003\)](#).

Aconitum firmum* subsp. *maninense

SLOVAKIA: Galicia, ad pedem montis Tatrae Magnae, in valle Poduplaski, alt. c. 1000 m, solo granitico; leg. E. Nyárady, 27.07.1908; (SIB); source: [Mitka \(2003\)](#). — [Súľovské vrchy] Manínská soutěska, Súľov; leg. K. Domin, 01.08.1920; (PRC); source: [Mitka \(2003\)](#). — Manín (SW od Súľovských vrchov), ok. 400 m; leg. Skalický, Skalická, 27.07.1973; (PRC); source: [Mitka \(2003\)](#). — Manín, Strážovské vrchy Mts., Manínska úžina, in oak-hornbeam forest *Tilio-Carpinetum* along a torrent; leg. J. Mitka, 03.09.2001; (KRA); source: [Mitka \(2003\)](#). — Slovakia, Manín, Strážovské vrchy Mts., Manínska úžina, on steep stone slope; leg. J. Mitka, 03.09.2001; (KRA); source: [Mitka \(2003\)](#).

Aconitum firmum* nothosubsp. *paxii

SLOVAKIA: Montes Liptovské hole, mons Červené vrchy, in locis lapidosis calcareis rupium Javorové skalky, alt. 1450 m [*A. firmum* (Rchb.) Neilr. mutatio – cum pilis adscendentibus copissimus, rev. V. Skalický]; leg. J. Dostál, 21.08.1930; (PRC); source: [Mitka \(2003\)](#). — Liptovské hole, Tichá dolina; leg. P. Svoboda, 07.08.1935; (PR); source: [Mitka \(2003\)](#).

Aconitum firmum* subsp. *firmum* × *A. firmum* subsp. *maninense

SLOVAKIA: Belianske Tatry Mts.: Comitatus Szepes, Contes Bélaenses, in valle Drechslerhäuschen sub monte Stirnberg, alt. c. 1400–1500 m; leg. E.G. Nyárady, 01.09.1907; (SIB); source: [Mitka \(2003\)](#).

RESEARCH ARTICLE

The influence of alcoholic extract from leaves of *Helianthus annuus* L. on germination and growth of *Sinapis alba* L.

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Abstract

The knowledge of plants interactions is used for their better protection and cultivation. The aim of this study was to determine the influence of alcoholic extracts from the leaves of the common sunflower (*Helianthus annuus*) on selected physiological parameters of plants of white mustard (*Sinapis alba* 'Barka'). The seeds germination, the growth, the fresh and dry masses of plants grown from seeds and germinated on the sunflower extracts and plants watered by *H. annuus* extracts during the growth phase were studied. In the growth phase, the fresh masses of all organs were increased on 5% extract, however significantly decreased on 10% extract. The dry mass of *S. alba* was not significantly changed. In the germination phase, much less changes of these parameters were revealed. In general, extracts from *H. annuus* leaves inhibited germination of *S. alba* seeds, but stimulated growth of plants in case of application of 5% concentrations during the germination phase and inhibited their growth in case of application of 10% concentrations during the growth phase.

Keywords: *Helianthus annuus*, *Sinapis alba* 'Barka', mustard, sunflower, allelopathy, growth, morphology, seed germination

Introduction

The progressive degradation of the environment and the need to reduce the use of chemical pesticides caused the interest in the allelopathy phenomenon (Kato-Noguchi *et al.* 2002).

The studies on allelopathic effects of different species constitutes the possibility of application of substances of plant origin, which are naturally biodegradable and therefore have a high economic significance (Oliwa *et al.* 2016). Investigations of the number of

allelopathic compounds show that mechanisms of interaction between plants are extremely complex, but nevertheless they are effectively applied in agricultural practice (Lipińska 2006; Afridi & Khan 2014, 2015). Allelopathy refers to secretion of chemical substances by plants, which modify properties of the environment in their immediate surroundings, depending from the intensity of abiotic factors as well as genetic determinants of organisms (Amini *et al.* 2014). The absorption mostly by roots and the transport of allelopathic substances to other plant organs are assisted by regulatory mechanisms at both cellular and tissue levels.

Allelopathic interaction between plants is a result of competition about the environmental resources, revealing by emission of chemical compounds by plants (Kasperczyk & Szewczyk 2007). Allelopathic substances can demonstrate both negative and positive influence on the growth of plants (Ohno & Doolan 2001; Puła *et al.* 2016; Barabasz-Krasny *et al.* 2017).

Helianthus annuus L. (Asteraceae) has a high allelopathic potential. It can actively influence on the growth of the surrounding plants (Leather 1983; Macias *et al.* 2002; Możdżeń *et al.* 2016). In particular, *H. annuus* inhibits germination of such weeds as *Phalaris minor* Retz., *Centaurea* spp., *Erigeron canadensis* L. and *Amaranthus retroflexus* L. (Khalid *et al.* 2002). It was shown that extracts of sunflower reduced seed germination of wild barley (Ashrafi *et al.* 2008). The allelochemicals substances of sunflower also inhibit the germination and growth of *Agropyron repens* (L.) P. Beauv., *Avena fatua* L., *Digitaria ciliaris* (Retz.) Koeler and *Sida spinosa* L. seedlings (Azania *et al.* 2003). The suppressing effect of aqueous extracts from leaves of *H. annuus* was also observed in the case of germination and growth of *Sinapis alba* (Bogatek *et al.* 2006).

Leaves of sunflower are rich source of phenolic compounds, chlorogenic and isochlorogenic acids, and terpenoids, characterised by a wide spectrum of biological activity (Wilson & Rice 1968; Batish *et al.* 2002; Gniazdowska *et al.* 2004). The biochemical analyses of extracts from *H. annuus* leaves also indicated the presence of sesquiterpene

lactones exceptionally well soluble in alcohols (Spring *et al.* 1992).

The aim of this study was to evaluate the influence of alcoholic extracts from dry leaves of *H. annuus* at 5% and 10% concentrations on plants of *Sinapis alba* L. cultivar 'Barka'. The mustard cultivar 'Barka' is widely grown in Poland and therefore it was selected for the studies. In this study, (1) seeds germination, (2) growth of underground and aboveground organs and (3) their fresh and dry masses were measured for *S. alba* plants watered by *H. annuus* extracts during the germination and growth phases.

Material and methods

The experiments were conducted at the Department of Plant Physiology of Pedagogical University in Kraków (Poland) during September 2016. In the same conditions, the experiments were replicated five times.

Extracts preparation

The leaves of *H. annuus* were collected from cultivated field in south of Poland in August 2016. Alcoholic extracts of allelopathic substances from dried leaves of *H. annuus* of 5% and 10% concentrations were prepared by weighing of plant material (5 g and 10 g were taken, respectively) and soaking by ethanol (95 ml and 90 ml were added, respectively) for 24 hours. The extracts were filtered on a Büchner funnel with Whatman No. 1 filter paper by vacuum pump and placed in a laboratory dryer at 70 °C for next 48 hours to evaporate. Then, the extracts were soaked in appropriate amount of distilled water (5% – in 95 ml and 10% – in 90 ml, respectively).

Seeds germination

One hundred seeds of *S. alba* 'Barka' were placed in sterilised 9 cm Petri dishes with 3 layers of Whatman No. 1 filter paper. The dishes were wetted with 6 ml of alcoholic extracts from *H. annuus* leaves and distilled

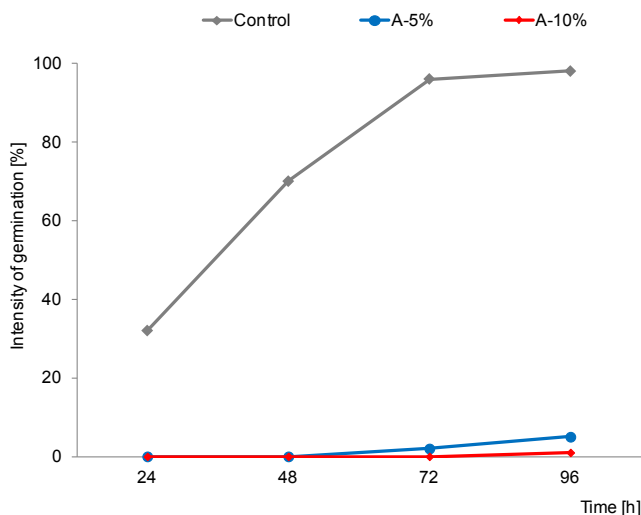


Fig. 1. The germination of *Sinapis alba* 'Barka' seeds on alcoholic extracts from the leaves of *Helianthus annuus* with concentrations of 5% and 10%.

water (control group). The seeds were placed in dark growth chamber at temperature 25 °C. After 24, 48, 72 and 96 hours, the number of germinated seeds was counted. As germinated seeds were considered that seeds, which had at least 2 mm of radical length. The percentage of the germinated seeds was calculated using the formula: germination [%] = (germinated seed × 100) / total seeds sown.

Plant growth conditions

After 72 h of germination, the seedlings of *S. alba* grown on the Petri dishes with *H. annuus* extract were rinsed with distilled water and planted in pots with sand. The first group of plants was watered every 48 h with distilled water and once a week – by Steiner culture medium (Steiner 1961). The second group included plants grown from seedlings germinated on distilled water and they were watered every second day with alcoholic extracts from the leaves of *H. annuus* and once a week during the growth – with culture medium. The control group of plants was watered with distilled water and the Steiner culture medium. Plants were grown in a growth chamber (Angelantoni Lifescience, Italy) with 12/12 h photoperiod, 300 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ light intensity, 25 °C/20 °C alternation, and 60–70 % of relative humidity.

Biometric analysis, fresh and dry mass

The lengths of the root, hypocotyl and epicotyl of *S. alba* plants were measured with caliper and average lengths were calculated. The fresh mass and the dry mass (fresh mass dried at 105 °C for 48 h) of plant material were determined.

Statistical analysis

The data were subjected to ANOVA with Statistica 10.0 for Windows. The significance of differences was determined using the Duncan test for homogeneous groups, the mean \pm SD of $n = 5$, $p < 0.05$.

Results and discussion

Allelopathic interactions usually result in delay or inhibition of the seeds germination and plants growth, which mostly depend from concentration of the active substances contained in substrate (Możdżeń & Oliwa 2015; Oliwa *et al.* 2016). In this study, with increasing of the concentrations of chemical substances in the alcoholic extracts from *H. annuus* leaves, the inhibition of seeds germination of *S. alba* was observed (Fig. 1). After 48 hours of germination on the alcoholic extract from leaves of

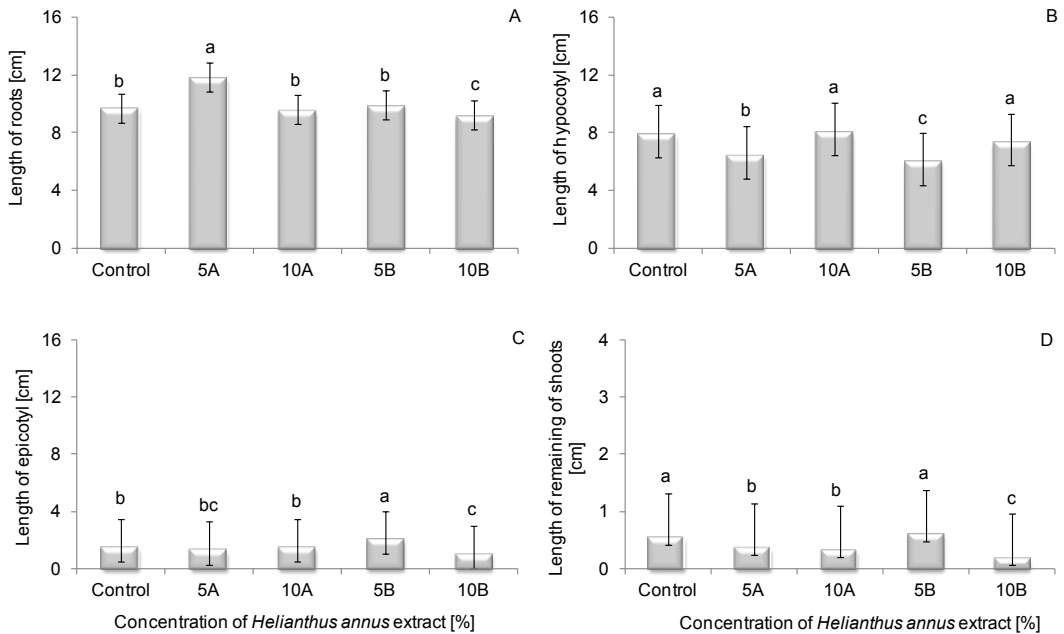


Fig. 2. The length of particular organs of *Sinapis alba* 'Barka' watered with alcoholic extracts from the leaves of *Helianthus annuus* with concentrations of 5% and 10% in germination (A) and growth (B) phases. Mean values \pm SD (n=5); different letters are significantly different according to Duncan's test at $p < 0.05$.

H. annuus no germinated seeds of *S. alba* were observed, while in control group 70% of seeds were germinated. The first seeds germinated on 5% extracts were observed after 72 h. Higher concentration of allelopathic extracts (10%) resulted in complete inhibition of seeds germination up to 96 hours. In such case, after 96 h, only 1% of *S. alba* seeds were germinated. The destabilisation of metabolic pathways in different conditions has both primary and secondary origin, and it has a reflection in the physiological processes associated with growth and development of plants (Możdżeń & Repka 2014). The plant growth inhibition can be a competition between the plants for water, minerals substances, oxygen, and carbon dioxide. Among other factors reducing the plant growth may be the changes of pH and osmotic potential of the soil, and disorder of nitrogen uptake mechanisms (Inderjit & Duke 2003).

In this study, the morphometric analysis revealed that in the germination phase the 5% extract of *H. annuus* increased the root

length and decreased the hypocotyl length in *S. alba* plants. Comparing with a control group, the remaining of shoots was inhibited on the 10% extracts. In the growth phase, 10% extracts inhibited the growth of mustard plants (Fig. 2).

The changes in the values of fresh and dry masses of *S. alba* grown on the alcoholic extracts of *H. annuus* were observed (Tab. 1). The differences of roots mass in plants grown on 5% extracts both during the germination and growth phases were revealed. In growth phase the roots mass was higher than in the germination phase (Tab. 1). This was related also with small decrease in the value of fresh and dry mass of the hypocotyl of *S. alba* watered with *H. annuus* extracts during the growth phase. However in growth phase, higher losses of the fresh and dry masses of hypocotyl were caused by 10% extracts. The fresh mass of epicotyl was decreased in higher concentrations of allelopathic substances both in germination and growth phases, and was

Table 1. The fresh and dry masses of organs of *Sinapis alba* 'Barka' watered with alcoholic extracts from the leaves of *Helianthus annuus* with concentrations of 5% and 10% in germination (A) and growth (B) phases. Mean values \pm SD (n=5); different letters differ significantly within a row by Duncan's test at $p < 0.05$.

Organ	Control	Alcoholic extracts of <i>H. annuus</i>			
		5%		10%	
		A	B	A	B
Fresh mass, g					
Root	0.68 ^c	0.87 ^b	1.12 ^a	0.74 ^c	0.63 ^c
Hypocotyl	0.89 ^a	0.93 ^a	0.75 ^b	0.84 ^a	0.57 ^c
Epicotyl	0.14 ^b	0.13 ^b	0.21 ^a	0.08 ^c	0.04 ^c
Blade leaf	0.49 ^b	0.49 ^b	0.58 ^a	0.31 ^c	0.12 ^d
Whole plant	2.51 ^b	2.93 ^{ab}	3.30 ^a	2.35 ^b	1.59 ^c
Dry mass, g					
Root	0.02 ^b	0.02 ^b	0.03 ^a	0.03 ^{ab}	0.02 ^{ab}
Hypocotyl	0.04 ^c	0.07 ^a	0.05 ^b	0.04 ^c	0.04 ^d
Epicotyl	0.01 ^a	0.01 ^a	0.01 ^a	0.01 ^a	0.01 ^a
Blade leaf	0.05 ^b	0.05 ^b	0.06 ^a	0.04 ^c	0.02 ^d
Whole plant	0.35 ^b	0.38 ^b	0.47 ^a	0.37 ^b	0.34 ^b

increased only on the lower concentration of allelopathic substances in the growth phase. Similar trend was observed during analysis of these parameters for leaf blades too. The dry mass of *S. alba* epicotyl was not significantly changed in the germination and growth phases, comparing to the control group. The lowest values of leaves mass in the growth phase were in the plants watered by 10% extracts. In the growth phase, the fresh masses of all organs increased on 5% extract, however on 10% extract they were significantly decreased. In the germination phase, much less changes of these parameters were revealed (Fig. 2; Tab. 1).

In many cases *H. annuus* extracts inhibit or suppress germination and growth of other plants (Leather 1983; Khalid *et al.* 2002; Macias *et al.* 2002; Azania *et al.* 2003; Bogatek *et al.* 2006; Ashrafi *et al.* 2008; Możdżeń *et al.* 2016). But numerous studies also showed that extracts of leaves, stems, roots, flowers and fruits performing toxic effects on many other plant species, although in low concentrations can stimulate selected physiological processes in such plants (Batish *et al.* 2002; Gniazdowska *et al.* 2007; Siegień *et al.* 2008).

There is a positive correlation between the effects of allelopathic substances and plants morphology (Skrzypek *et al.* 2016). The allelopathic substances modify the permeability of cell membranes and disturb the transportation of the mineral substances. They have negative effect on the protein synthesis, inhibit the efficiency of oxidative phosphorylation and photosynthesis, and reduce chlorophyll content (Skrzypek *et al.* 2015, 2016). Many allelopathic substances inhibit the cell division and elongation, and stimulate the oxidation of endogenous and biologically active compounds (Burgos *et al.* 2004).

Conclusions

1. The inhibition of germination of *S. alba* seeds on the 10% alcoholic extracts from the leaves of *H. annuus* was observed;
2. Extracts applied during the germination phase significantly influenced on the plants growth. The growth and the mass of mustard plants were stimulated by 5% concentration of alcoholic extracts of common sunflower;

3. The growth of *S. alba* plants during the growth phase was significantly inhibited by 10 % concentration of alcoholic extracts of common sunflower.

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CORRESPONDENCE

Influence of different concentrations of jasmonic acid on *in vitro* development of *Catasetum fimbriatum* Lindl. (Orchidaceae)

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Abstract

In vitro seeding constitutes an indispensable tool for propagation of the main commercial species of orchids. This study aimed to analyze the *in vitro* development of *Catasetum fimbriatum* under different concentrations of jasmonic acid in Murashige & Skoog culture medium compound of ½ macronutrients. After 180 days of cultivation, concentration of 0.25 and 0.50 $\mu\text{L} \cdot \text{L}^{-1}$ caused a significant increase in number of roots and leaves. The concentration of 1.00 $\mu\text{L} \cdot \text{L}^{-1}$ showed the best result for the length of longest root and largest leaf, the total length of seedling, and the total fresh and dry masses. Nevertheless, the use of this plant regulator on *in vitro* culture media still requires further investigations to determine the optimal concentration in order to obtain desirable phytotechnical characteristics in different orchid species.

Keywords: *Catasetum fimbriatum*, jasmonic acid, plant regulator, *in vitro* propagation

Introduction

Including 7 genera that inhabit tropical regions of the Americas, *Catasetinae* Lindl. (Orchidaceae) spreads from sea level to locations with more than 1,000 m of altitude (Hoehne 1949; Romero 1990; Pridgeon *et al.* 2009; Pedroso-de-Moraes

et al. 2012). These deciduous, sympodially growing orchids have well developed pseudo-bulbs, related to the storage of water during periods of drought (Dodson 1975; Moraes & Almeida 2004). The representatives of the subtribe occur in treetops or on the litter, from where their fleshy roots absorb nutrients (Hoehne 1938).

The main Brazilian genus, under intense extractivism, for having species of high commercial value, is *Catasetum* Rich. ex Kunth (Joly 1998; Pedroso-de-Moraes *et al.* 2007). It presents plants with extensive ecomorpho-physiological specializations related to cross fertilization (Zimmerman 1991; Moraes & Almeida 2004). In particular, sexual trimorphism described for flowers of subgenus *Orthocatasetum* Mansf. (Hoehne 1938) drew attention of many orchidologists (Moraes & Almeida 2004).

The genus *Catasetum* is composed of about 300 species, which can present diclinic masculine, feminine and monoclinic flowers; such floral typologies can coexist even on the same floral rachis (Hoehne 1938; Zimmerman 1991; Bicalho & Barros 1998; Pridgeon *et al.* 2009). This fact gave rise to controversies regarding its adaptive derivational status (Zimmerman 1991; Moraes & Almeida 2004).

Catasetum fimbriatum Lindl. is a monoic species that show male and female diclinic flowers quite different morphologically from each other, as well as incomplete monoclones (Pedroso-de-Moraes 2002) are frequently visited by bumble bees (*Euglossa* sp.), which pollinate them (Cardoso 2014). The male inflorescences are more frequent, longer, with 10–15 flowers of 7–9 cm diameter, with durability between 15–30 days after anthesis, colored from green to yellow (Cardoso 2014). It has fleshy roots related to the fixation and absorption of decaying organic matter, as well as thin secondary roots related to aeration of the root system (Pedroso-de-Moraes 2002). Pseudobulbs have an average length of 12–27 cm and a diameter of 6–13 cm. Leaves usually light green and pleated, 30–36 cm of length in average (Pedroso-de-Moraes 2002; Cardoso 2014).

Catasetum orchids are considered ornamental plants of great prominence in orchid culture, mainly due to the exoticism of their flowers, being used as potted plants. In addition, the high valuation on the market is due to slow metabolism of the majority of orchids, resulting the lower rates of conventional vegetative propagation and higher expenses for

cultural treatment (Pedroso-de-Moraes 2000). Therefore *in vitro* propagation, whether by sowing or regenerating tissues, is actively applied to increase the production of seedlings of high genetic quality and reduction of production costs (Stancato *et al.* 2001).

There are no specific culture media suitable for particular orchid's taxa. It is difficult to explain why in certain combinations of components of environment and culture conditions one results are successful, whereas others – not (Ventura *et al.* 2002). This question is even more ambiguous when analyzing data on the use of different concentrations of recently discovered phytohormones and plant regulators, such as jasmonic acid (JA).

For jasmonates, in relation to morphophysiology, both promoter and inhibitory effects are reported on representatives of different plant groups. A number of studies were carried out to investigate its role on the regulation of several physiological processes, such as: senescence (Parthier 1990), accumulation of storage proteins (Staswick 1992), development of embryos (Wilen *et al.* 1991) and biosynthesis of secondary metabolites (Facchini *et al.* 1996). Such mechanisms of action are result of alterations in gene expression (Reinboth *et al.* 1994).

Exogenous application of JA promotes senescence, petiole abscission, root formation, coiled tendrils, ethylene and β -carotene synthesis (Staswick 1992). In addition to the promoter effects, this plant growth regulator can inhibit seed germination, and inhibit or stimulate callus growth, root growth, chlorophyll production and pollen grain germination relating from applied concentrations (Parthier 1990; Vick & Zimmerman 1986).

In relation to the *in vitro* culture, the influence of different concentrations of JA on the development of post-germination plantlets of *C. fimbriatum* is unknown. Since, the aim of this work was to evaluate the seedling development of *C. fimbriatum* in MS medium (Murashige & Skoog 1962) influenced by different concentrations of JA after 180 days of *in vitro* culture.

Material and methods

Seeds were obtained from mature plants (9 months of development) after artificial cross fertilization and were provided by the Pedroso-de-Moraes Orchidarium (Santa Cruz das Palmeiras, SP, Brazil).

For *in vitro* seeding, MS media (Murashige & Skoog 1962) were composed of half of the macronutrient concentration, supplemented with 0, 0.25, 0.5 and 1.0 $\mu\text{L} \cdot \text{L}^{-1}$ of JA, 1 gL^{-1} of activated carbon and 30 gL^{-1} of sucrose, with 5.8 pH adjusted before the addition of 7 gL^{-1} of agar. Then, 50 ml of each culture medium containing the different JA concentrations were poured into four 250 ml sterilized flasks and autoclaved at 121 °C and 1 atm pressure for 20 minutes (Arditti & Ernst 1992).

For disinfection, seeds were agitated in the solution of 5% sodium hypochlorite for five minutes in centrifuge tubes. Tubes later were immersed in 70% alcohol and taken to the laminar flow chamber, where the seeds were washed four times with distilled water and deposited in the flasks containing the culture media (Pedroso-de-Moraes *et al.* 2009).

Four flasks were seeded by treatments with 1 g of inoculated seeds per container. The seeded flasks were sealed with a transparent or metallic plastic cap and maintained for 180 days in a climatic chamber (BOD MA 403) at constant temperature of 25 °C, under a photoperiod of 12 hours and light intensity of $cca. 116 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Dezan *et al.* 2012).

The following biometric phytotechnical characters were evaluated: number of roots (NR), number of leaves (NL), total length of seedling (LS), length of the largest root (LR), length of the largest leaf (LL), total fresh mass (FM) and total dry mass (DM). Total dry mass was calculated after drying of the material at 65 °C until reaching the constant dry mass (Dezan *et al.* 2012).

The results were processed through polynomial regression analysis using BioEstat 5.3 (Ayres *et al.* 2007). In order to select the regression model that best fits the obtained data, we considered the non-significance of regression deviation, the degree

of significance present for the highest order model, and finally the value of the coefficient of determination (R^2) (Fernandes *et al.* 2012).

Results and discussion

Number of roots under all applied concentrations was higher than in control group of *C. fimbriatum* (Fig. 1 A). In general, it confirms assertion that the exogenous application of JA increases the rhizogenesis (Staswick 1992). In case of *C. fimbriatum*, the addition of 0.25 $\mu\text{L} \cdot \text{L}^{-1}$ of JA to the culture medium showed the best result for NR in comparison to other concentrations. However, it also was shown that increasing concentration of JA determines the decrease of the number of roots for *Cattlianthe* Jewel Box (Borin *et al.* 2015).

Application of 0.25 and 0.50 $\mu\text{L} \cdot \text{L}^{-1}$ concentrations of JA resulted in increasing number of leaves (Fig. 1 A). Application of 0.50 $\mu\text{L} \cdot \text{L}^{-1}$ of JA showed the highest influence on foliar genesis. However, decrease in leaf formation occurred with the use of 1.00 $\mu\text{L} \cdot \text{L}^{-1}$ of the plant regulator, indicating a phytotoxic effect. For *Cattlianthe* Jewel Box, the addition of 0.25 $\mu\text{L} \cdot \text{L}^{-1}$ of JA to the culture medium had no statistically significant influence on the number of leaves. For this hybrid, the increase in concentrations in the culture media (from 0.50 $\mu\text{L} \cdot \text{L}^{-1}$ to 1.00 $\mu\text{L} \cdot \text{L}^{-1}$) resulted in decrease of NL (Borin *et al.* 2015) and foliar senescence. In case of *Phalaenopsis* Blume, addition of 0.25 $\mu\text{L} \cdot \text{L}^{-1}$ of JA promoted the number of leaves, while further increase of concentrations decreased it (Hsu 2003).

Our findings regarding NR and NL stimulation by JA are in agreement with those obtained for *Zea mays* L. (Vick & Zimmerman 1986). However in other plant groups, the application of JA caused inhibition of rhizogenesis and decreased number of leaves (Staswick 1992).

Application of 0.25 $\mu\text{L} \cdot \text{L}^{-1}$ concentration of JA showed the weakest result in LR and LS in comparison to control (Fig. 1 B). However, there was an increase in LR and, consequently,

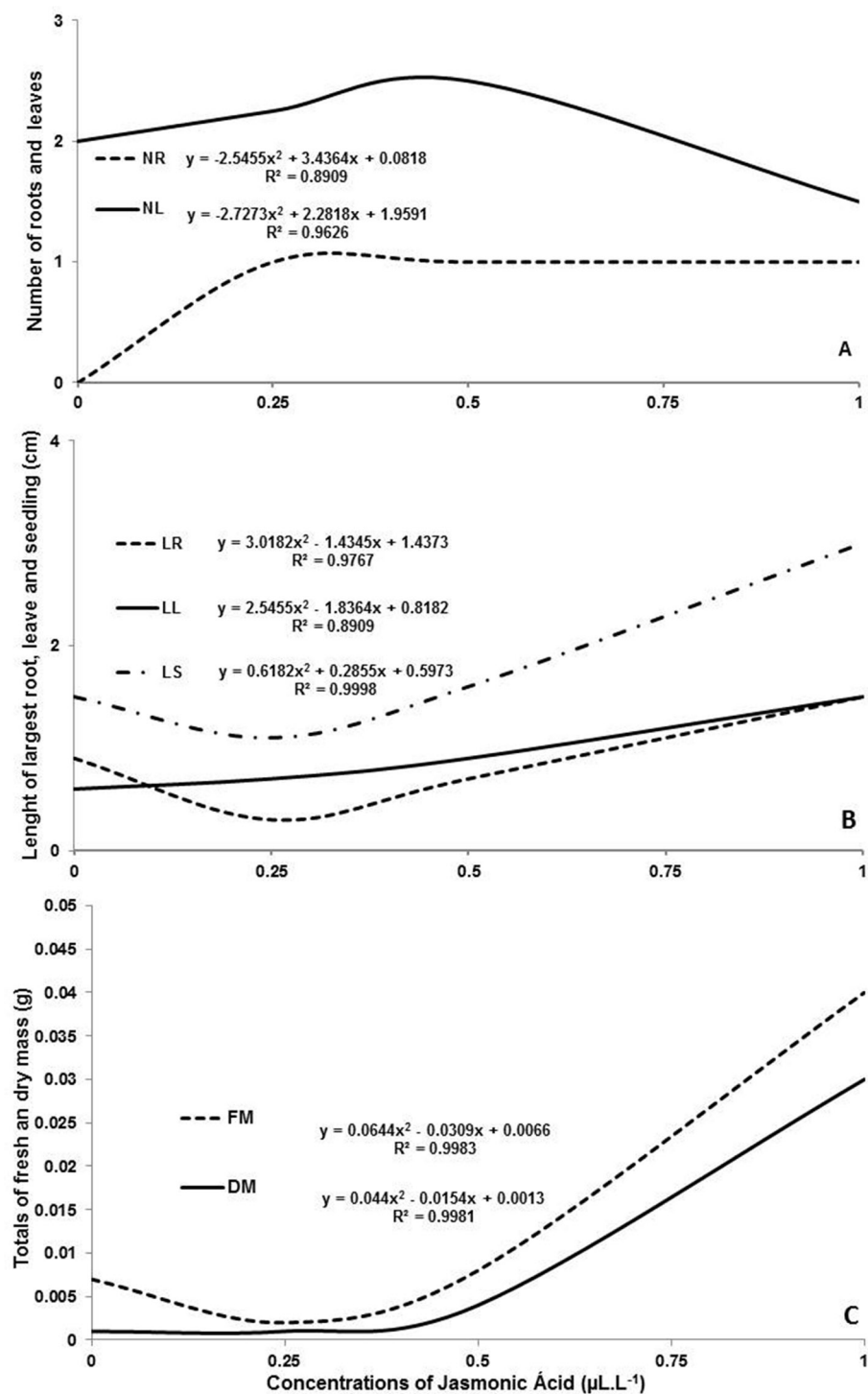


Fig. 1. Polynomial correlation of biometric variables of *Catasetum fimbriatum* seedlings: **NR** – number of roots; **NL** – number of leaves, **LR** – length of largest root; **LL** – length of largest leaf; **LS** – total length of seedlings; **FM** – fresh mass; **DM** – dry mass.

of LS with the increasing concentrations of JA; application of $1.00 \mu\text{L} \cdot \text{L}^{-1}$ of JA demonstrated the highest results of LR and LS. In case of LL, all applied concentrations were effective, with the concentration of $1.00 \mu\text{L} \cdot \text{L}^{-1}$ presenting the best result (Fig. 1 B). Such outcomes contradict to findings reported for *Cattlianthe* Jewel Box, for which the concentration of $0.25 \mu\text{L} \cdot \text{L}^{-1}$ was the most effective for LR, LL and LS, with increasing concentration generating the worse results (Borin *et al.* 2015). Regarding LL, our results corroborate the observations recorded for *Allium cepa* L., *Phaseolus coccineus* L. ($50 \mu\text{M}$) and *Zea mays* (0.1 , 10 and $100 \mu\text{M}$), with highest concentrations increasing the length of leaves (Parthier 1990; Maksymiec & Krupa 2007). From other side, for the *Phalaenopsis* the lowest concentration of JA ($25 \mu\text{M}$) promoted the highest increase in leaf length (Hsu 2003).

Regarding the FM variable, application of $0.25 \mu\text{L} \cdot \text{L}^{-1}$ concentration of JA added demonstrated the inhibitory effect. However, increasing concentration of the plant regulator promoted FM, where application of $1.00 \mu\text{L} \cdot \text{L}^{-1}$ of JA was the most effective (Fig. 1 C). This confirms results obtained for soybean, when the increase of JA concentrations resulted in increased fresh mass of seedlings (Koda 1992). These findings also confirms that JA has a positive effect on the cellular water balance, promoting smaller reductions in contained water contents, and promoting greater tissue turgidity (Koda 1992; Maksymiec & Krupa 2007; Kerbauy 2008).

Similarly, our studies showed that the increase in JA concentration (mainly $1.00 \mu\text{L} \cdot \text{L}^{-1}$) in the culture media promoted an increase in the dry mass of seedlings, (Fig. 1 C). It was also shown that application of JA, pure or fermented by *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx, promotes significant increases in the dry mass of floral buds and fruits of *Capsicum frutescens* L. and *Physalis angulata* L., up to the highest ($1.00 \mu\text{M}$) treated concentration (Linares *et al.* 2010). The increase in dry mass induced by JA can be explained by increasing gene expression related to photosynthetic processes and carbohydrate assimilation (Parthier 1990; Koda 1992).

Conclusions

Based on the results of this study it is evident that: a) the concentration of JA of $0.25 \mu\text{L} \cdot \text{L}^{-1}$ and $0.50 \mu\text{L} \cdot \text{L}^{-1}$ showed a significant increase for number of roots and number of leaves respectively; b) application of $1.00 \mu\text{L} \cdot \text{L}^{-1}$ of JA was the most effective for increasing in length of the largest root, length of the largest leaf, length of seedlings, as well as for fresh and dry mass; c) the use of JA as a plant growth regulator for *in vitro* cultivation still requires further investigation to determine the concentration to be applied by producers, with the aim of obtaining a higher number of desired phytotechnical characteristics in different species of orchids.

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CORRESPONDENCE

A new record for alien flora of Turkey: *Symphyotrichum pilosum* (Willd.) G.L. Nesom var. *pilosum*

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Abstract

Symphyotrichum pilosum var. *pilosum* from Asteraceae family was collected from Ereğli region (Zonguldak province, NW Turkey) in 2016 and is reported here for Turkey for the first time. It is naturalized alien plant for Turkey. The description and photos of collected plants, as well as distribution map and new key for the genus *Symphyotrichum* in flora of Turkey are provided here.

Keywords: *Symphyotrichum pilosum*, Asteraceae, new record, alien plant, Turkey

Introduction

The genus *Symphyotrichum* that belongs to Asteraceae family is mostly composed of perennial and herbaceous plants. There are about 100 species belonging to this genus in the world, most of which are distributed in the North, Central and South Americas. Some *Symphyotrichum* species occur in Europe as invasive plants (Tutin *et al.* 1976; Kubitzki 2007; Mohlenbrock 2015). In Turkey, there are known two species of *Symphyotrichum* – *S. laeve* (L.)

Á. Löve & D. Löve and *S. squamatum* (Spreng.) G.L. Nesom (Davis 1965–1985).

Material and methods

During the floristic investigations in and around Ereğli district of Zonguldak province in Turkey, plant samples belonging to *Symphyotrichum pilosum* var. *pilosum* (Willd.) G.L. Nesom were collected in August – September 2016. Herbarium specimens of *S. pilosum* var.

pilosum, as well as specimens of *S. laeve* and *S. squamatum* (E00531253!, E00531250!, E00249092! and E00563223!) were used for comparative investigation and then they were deposited in the Herbarium of the Faculty of Forestry at the University of Düzce (DUOF).

The collected samples were checked according to the “Flora of Turkey” and its supplements as well as according to “A checklist of the Flora of Turkey (Vascular plants)” but they were not determined (Davis 1965–1985; Güner et al. 2000, 2012). After it was recognized that the collected samples belong to the *Symphyotrichum* genus, with the detailed review of literature it was determined that this is a new taxon for flora of Turkey *S. pilosum* var. *pilosum* originated from North America (Mohlenbrock 2015).

Morphological descriptions of the species in this paper are based on literature (Tutin et al. 1976; Davis 1988; Kubitzki 2007; Mohlenbrock 2015) and supplemented with observations of the collected plants. The nomenclature has been checked with Euro+Med Plant Base (Greuter 2006–2017). Distribution of the studied taxon within the territory of Turkey was mapped in a Davis grid system accepted in “Flora of Turkey”. Data on the abundance of species, condition of the populations and characteristics of *S. pilosum* var. *pilosum* habitats in Turkey are provided on the base of field observations.

Results and discussion

The new taxon of *Symphyotrichum* is recorded for Turkey and considering that fact, it is necessary to present a new key for identification.

1. Annual or biennial plants ***S. squamatum***

1*. Perennial plants **2**

2. Lots of cauline leaves more than 1 cm wide, leaves glaucous above ***S. laeve***

2*. Lots of cauline leaves not more than 1 cm wide, stem and leaves pubescent ***S. pilosum* var. *pilosum***

Symphyotrichum pilosum (Willd.) G.L. Nesom var. *pilosum*, *Phytologia* 77: 289. 1995. (Fig. 1).

Basionym: *Aster pilosus* Willd.

Description: Hemicryptophyte, characterized by rapid growth and formation of a strong taproot. Stem from 1 to several, erect or oblique; branches near to base, sparsely to densely hirsute; 100–180 cm tall. Leaves with ciliate margins, pilose, from linear to linear-lanceolate, 6–9 cm long, 2.8 cm wide; basal leaves absent in flowering time. Main inflorescence is open panicle with ascending to spreading branches gathering 40 or more heads; involucre bracts linear to subulate, involucre 3–5 mm long and 3–4 mm wide. Phyllaries in 4 or 5 series unequal, green apex innolled at the margin. Ligules white, often becoming purplish, 1.4 mm wide. Pappus white 3–4 mm (Mohlenbrock 2015).

Distribution in Turkey (Figs 2 & 3): Turkey (Western Black Sea Region): A3-Zonguldak, Ereğli district at elevation of 10–150 (200) m, at a roadside, field side and abandoned fields, 29.VII.2016, B. Tunçkol 4400 & H. Yaşayacak (DUOF 7023).

Symphyotrichum pilosum var. *pilosum* has been recorded in ruderal habitats, in out parts of main road. It is quite widespread on southern slopes in Ereğli in lengthwise areas of about 10–200 m elevation. The species is a constituent of the ruderal vegetation of *Rubus sanctus* Schreb., where it is an almost dominant. There are three populations of *S. pilosum* var. *pilosum* in Ereğli district (Fig. 3) separated by short interruptions with about 30–40 individuals in each. Individuals of all stages of development, from early vegetative, flowering, and up to late stage of fructification were recorded. Besides *S. pilosum* var. *pilosum*, following taxa were recorded in that habitats: *Rubus sanctus*, *Artemisia vulgaris* L., *Solanum nigrum* L., *Clinopodium nepeta* Kuntze subsp. *glandulosum* (Req.) Govaerts and *Lactuca serriola* L.

Symphyotrichum pilosum var. *pilosum* is native to the eastern part of North America and several southern provinces of Canada. However, it is anthropogenically spread to almost all parts of North America (Chmielewski & Semple 2001). As a naturalized species, it was recorded in India, where it inhabits ruderal



Fig. 1. General appearance of *Symphotrichum pilosum* var. *pilosum*: **A** – habitus; **B** – inflorescence; **C** – capitula, **D** – stem, **E** – basal leaves (DUOF 7023).

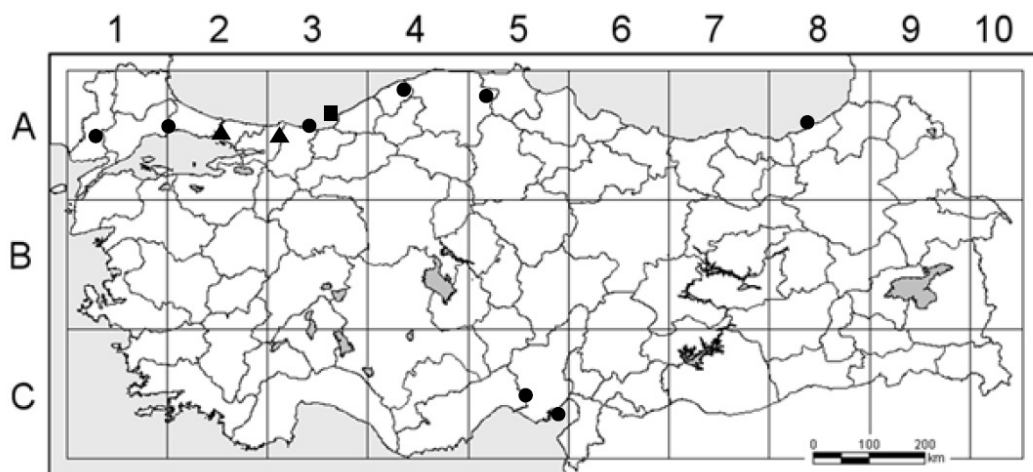


Fig. 2. Distribution of *Symphyotrichum* in Turkey: ■ – *S. pilosum* var. *pilosum*; ● – *S. laeve* (Davis 1988; Güner et al. 2012); ▲ – *S. squamatum* (Davis 1988; Güner et al. 2012).

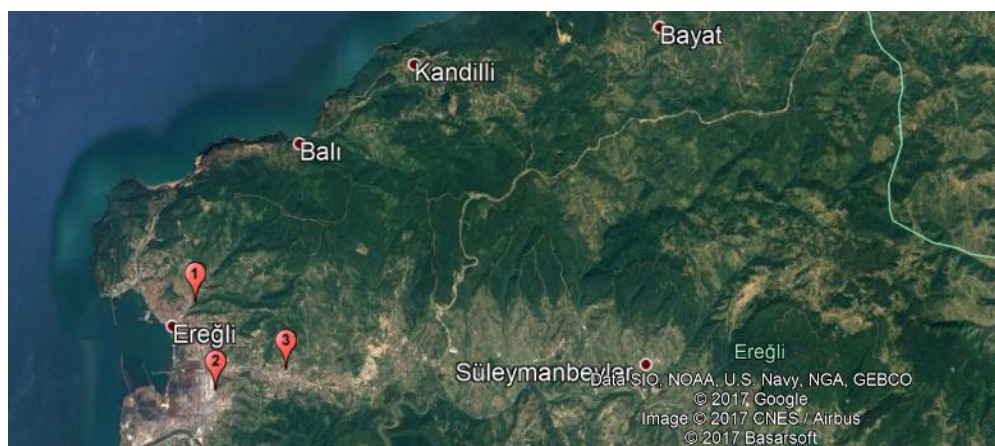


Fig. 3. Distribution of *Symphyotrichum* in Ereğli district.

areas along the roadsides (Kaul 1975). It was also recently reported as naturalized neophyte for flora of Piemonte in Italy (Conti et al. 2005; Celesti-Grapow et al. 2010). In Europe, this species occurs in different habitats, mostly in agricultural and ruderal, along the coastline, at roadsides, in vine yards, gardens, city parks, on meadows, orchards, trash dumps and roads embankments.

In the study on the weed distribution in Zonguldak conducted by Cimalová (2012),

S. pilosum var. *pilosum* is not mentioned. However Cimalová (2012) noticed that there are 69 other taxa belonging to 24 families, and Asteraceae is one of the most represented families (10.14%).

Interruption of nature, urbanization and climate changes cause the deterioration of the natural vegetation and spread of alien plant species. In the last checklist of alien flora of Turkey with a taxonomic composition and ecological attributes, two other *Symphyotrichum*

species (*S. laeve* and *S. squamatum*) are mentioned as naturalized (Uludağ *et al.* 2017). Hence, *S. pilosum* var. *pilosum* is a new alien taxon reported here, which also seems to be naturalized in Turkey. This new finding seems not to be the last, and a lot of new observations can be made there. According to Aksoy (2014), up to 242 alien taxa are represented in the flora of Turkey. However, in later investigation of Uludağ *et al.* (2017) already 340 alien taxa were mentioned for Turkey. This shows the importance of local and regional investigations of flora for the new records of alien taxa and further monitoring of their populations.

There are many studies on flora and vegetation conducted in Turkey, but the number of alien plants in Turkey is constantly increasing. These species start their spreading mostly in disturbed and unnatural areas, and then they invade the natural sites. Thus, there should be detailed studies about how these alien species come, which areas they occupy, how they compete and how interact with a native flora.

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RESEARCH ARTICLE

The pea development after UV-B irradiation

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Abstract

The effect of UV-B radiation on the growth and development of pea plants was studied. Pea is a self-pollinating plant with unlimited branching and short vegetative period. The number of flowers and pods per plant is limited by the conditions of vegetation. It is established that after irradiation of seedlings in doses from 2 to 15 kJ/m² shoot growth decreased. Dose of 2 kJ/m² inhibited growth, dry mass accumulation of shoots, formation of flowers and pods. The dose of 4 kJ/m² activated increasing quantity of flowers and pods. Further increasing the dose of UV-B irradiation suppressed growth and development of pea plants. UV-B irradiation in a wide range of doses caused growth inhibition of pea plants. Pea plants are convenient object for investigation of UV-B radiation effects.

Keywords: *Pisum sativum*, ultraviolet stress, growth, flower, pods

Вступ

Ультрафіолетова радіація як частина спектру сонячного опромінення досягає поверхні землі, тому належить до важливих чинників навколишнього середовища, що впливають на розвиток та продуктивність продовольчих культур. Рівень ультрафіолету в природних умовах коливається в межах

від 0 до 12 кДж/м² (Kakani *et al.* 2003). У помірних широтах рівень УФ-В досягає 4 кДж/м² біля поверхні ґрунту. Зростання рівня ультрафіолету обумовлене зменшенням вмісту озону в атмосфері, яке спричинене антропогенною активністю, зокрема збільшенням концентрації сполук хлору, метилброміду, хлорофлюорокарбону, оксиду азоту у повітрі. Денний пік ультрафіолету

співпадає з максимумом температури повітря у період вегетації та формуванням репродуктивних органів більшості рослин. Вивчення впливу хронічного опромінення УФ-В на рослини гороху дозволило встановити, що воно спричиняло зменшення довжини та кількості стебел, поверхні листка (Mepsted et al. 1996; Gonzalez et al. 1998). Показано, що дія УФ-В здатна викликати зміни у морфології всів рослин гороху, що пропонують використовувати як критерій для діагностики (Brosche & Strid 2000). Актуальність досліджень дії ультрафіолету на продовольчі культури обумовлена його здатністю інгібувати ріст, наростання біомаси, зменшувати врожай (Choudhary & Agrawal 2014).

Нами показано, що високотемпературний стрес спричиняв гасіння природної флуоресценції нативних хлоропластів (Musienko et al. 2014). Іншими дослідниками виявлено, що вирощування гороху за дії високої температури та УФ-В спричиняло зменшення накопичення біомаси, газообміну, флуоресценції хлорофілу, ушкодження компонентів фотосистем, зокрема пластохінону, зростання генерації активних форм кисню (Martel & Quaderi 2016; Hayes et al. 2017). Встановлено, що в природних умовах вирощування культурних рослин за дії абіотичних чинників навколишнього середовища відбувалась деструкція пігментного комплексу та хлоропластів у листків, що було ідентифіковано також і за інтенсивністю флуоресценції інтактних клітин мезофілу пшениці (Singh et al. 2011; Zhuk & Musyenko 2012, 2013).

Проведені нами раніше дослідження дозволили встановити, що опромінення рослин гороху незначними дозами УФ-С спричиняло гормезисний ефект, який проявлявся у стимуляції захисних систем, підвищенні активності антиоксидантних ферментів, стабілізації пігментного комплексу клітин листкового мезофілу, що підвищувало стійкість до повторної дії ультрафіолету у інгібуючих дозах (Mikheyev et al. 2016). Відкриття у рослинних клітинах акцептора сигналу для УФ-В – специфічного

фоторецептора UV Resistance Locus 8 (UVR8) – стимулювало дослідження формування відповіді рослинної клітини на цей стресовий чинник середовища (Jenkins 2009; Vanhaelewyn et al. 2016; Ferreyra et al. 2016). Однак вивчення впливу УФ-В на рослини гороху відбувалось в умовах його хронічної дії з використанням не більше однієї-двох його доз та переважно завершувались задовго до закінчення вегетаційного періоду (Kakani et al. 2004). Останнім часом інтерес до дослідження дії гострого УФ-В опромінення на рослини посилюється у зв'язку з підвищенням інтенсивності сонячної радіації у полуденні години (Yoon et al. 2017). Горох належить до зручних об'єктів для дослідження дії УФ-В завдяки його нетривалому вегетаційному періоду, високій чутливості до абіотичних стресів, самозапиленню квіток. Метою нашої роботи було вивчення дії гострого УФ-В опромінення у широкому діапазоні доз на ріст, розвиток та продуктивність рослин гороху протягом онтогенезу.

Матеріали і методи досліджень

Рослини гороху (*Pisum sativum* L.) сорту Ароніс вирощували в умовах вегетаційного досліду у посудинах місткістю 3 кг ґрунтової суміші, яка містила дві частини дерново-підзолистого ґрунту і одну частину перліту. В період росту рослин від сходів до дозрівання підтримували температуру повітря +20–22 °С. У кожній посудині знаходилось по 12 рослин. Режим освітлення складав 8 год. темноти і 16 год. світла інтенсивністю 2,2 кЛк. Опромінення рослин проводили через 10 діб після посіву і 8 діб від появи сходів. У цей період рослини мали три сформовані яруси листків і знаходились у вегетативній фазі росту та розвитку. Джерелом УФ-В-випромінювання були лампи Philips Special fluorescent lamp 35 W. Потужність опромінення УФ-В складала 6,0 Вт/м² на рівні верхніх листків, дози – 2, 4, 8 і 15 кДж/м². Обрані дози опромінення проявляють дію від слабкої до стабільно інгібуючої, яка, однак, не спричиняє загибелі

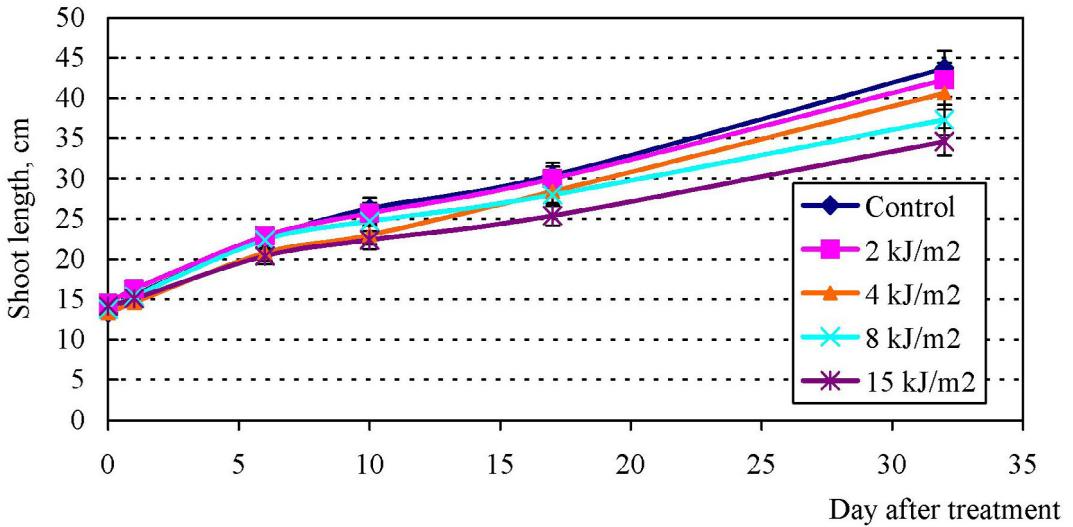


Рис. 1. Вплив УФ-В на ріст пагонів гороху.

Fig. 1. Effect of UV-B on growth of pea shoots.

рослин. Повторність досліджу 5-разова. Кожні п'ять діб після опромінення вимірювали довжину усіх пагонів у кожній посудині, після дозрівання визначали масу сухої речовини однієї рослини у кожній посудині. У період цвітіння підраховували кількість квіток у кожній рослині та у період дозрівання – кількість стручків. Статистичний аналіз результатів досліджень проводили у програмі Microsoft Excel. На графіках наведені середні значення і середньоквадратичні відхилення від них. На рисунках позначено статистично значима різниця за рівня значимості $P < 0,05$.

Результати та їх обговорення

Встановлено, що опромінення рослин гороху УФ-В у широкому діапазоні доз спричиняло інгібування росту пагонів у довжину (Рис. 1). Ефект проявлявся уже через п'ять діб після дії УФ-В у всіх дозах і у подальшому посилювався. Ріст пагонів, які опромінювали УФ-В, інгібувався порівняно з контрольними рослинами протягом 30 діб досліджу.

Вимірювання висоти рослин наприкінці їх вегетації виявило її прогресивне зменшення

зі збільшенням дози УФ-В-променів (Рис. 2). Однак найзначніше достовірне її пригнічення відзначено після опромінення у дозах 8 і 15 кДж/м².

Інгібування росту гороху за дії усіх доз УФ-В променів свідчить про його високу чутливість до даного чинника навколишнього середовища.

Визначення маси сухої речовини рослин гороху після завершення їх вегетації виявило, що вона зменшувалась після опромінення УФ-В у дозі 8 кДж/м², яка відповідає підвищеному природному фону (Рис. 3). Після дії УФ-В у дозах 2 та 4 кДж/м² суха маса рослин була близька до тієї, яка відзначена у контрольних рослин.

Зростання дози УФ-В променів до 15 кДж/м² порівняно з попередньою дозою 8 кДж/м² мало не настільки інгібуючий вплив на накопичення сухої маси пагонів.

Опромінення рослин гороху низькою дозою УФ-В 2 кДж/м² достовірно зменшувало кількість квіток на рослинах (Рис. 4). Зростання дози УФ-В до 4 кДж/м² спричиняло збільшення числа квіток більш ніж вдвічі, що могло бути обумовлено активацією відновних процесів після гострого опромінення.

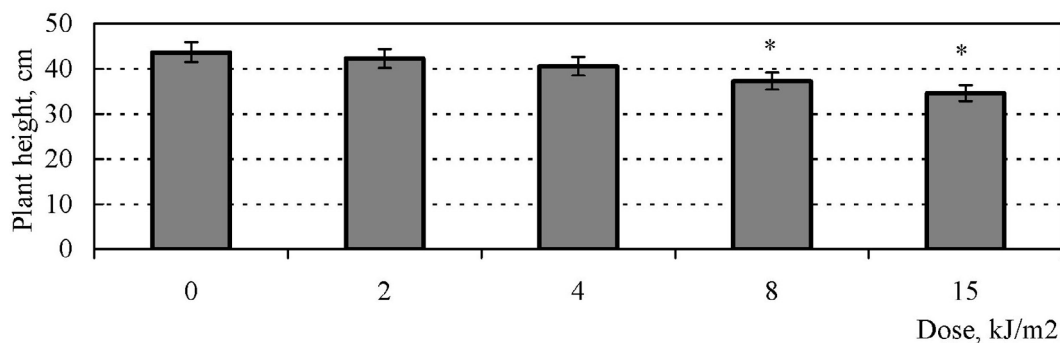


Рис. 2. Вплив УФ-В на висоту рослин гороху після закінчення росту. * вказує значиму різницю в порівнянні з контролем за $P < 0,05$.

Fig. 2. Effect of UV-B on the height of pea plants at the end of growth. * indicates the level of significance between control and UV-B treated plants at $P < 0.05$.

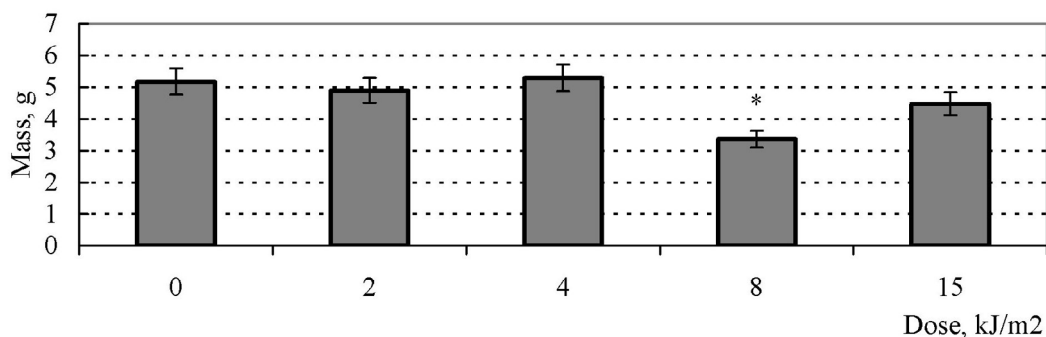


Рис. 3. Вплив УФ-В на масу сухої речовини пагонів гороху після дозрівання. * вказує значиму різницю в порівнянні з контролем за $P < 0,05$.

Fig. 3. Effect of UV-B on dry mass of pea shoots after maturation. * indicates the level of significance between control and UV-B treated plants at $P < 0.05$.

Подальше збільшення дози УФ-В променів викликало зменшення утворення квіток. Найменша кількість квіток відмічена після дії дози 15 кДж/м², і була вчетверо меншою порівняно з тією, що виявлена після опромінення рослин дозою 4 кДж/м². Число зрілих стручків було меншим, порівняно до квіток у більшості варіантів дослід. Редукція частини квіток могла бути обумовлена різними причинами, у тому числі і дефіцитом асимілятів, які надходять з листків.

Найзначніше зменшення кількості стручків порівняно до кількості квіток було

відзначено після опромінення рослин гороху дозою 4 кДж/м², яка нерідко спостерігається в умовах помірної зони. Незначна доза УФ-В 2 кДж/м², яка відповідає природному фону УФ-В променів, спричиняла пригнічення росту та формування генеративних органів у рослин гороху, що свідчить про їх високу чутливість до даного чинника навколишнього середовища. Подальше збільшення дози УФ-В променів пригнічувало ріст пагонів, утворення квіток і стручків, але рослини залишались здатними до росту і плодоношення.

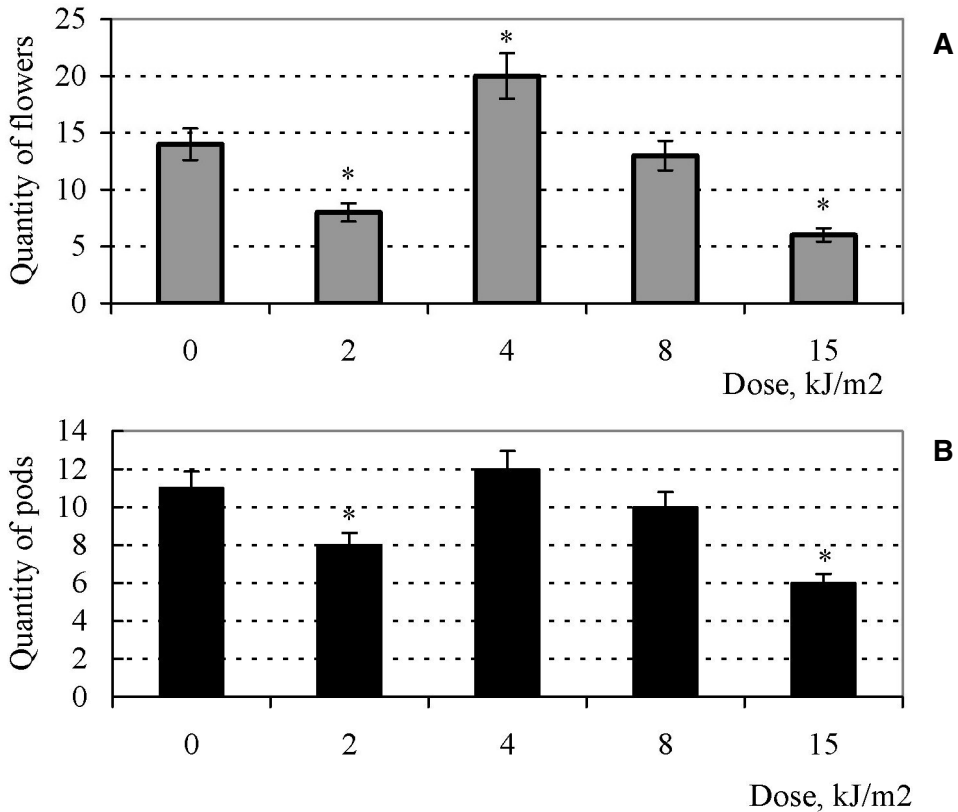


Рис. 4. Вплив УФ-В опромінення на кількість квіток (А) та стручків (В) у рослин гороху протягом вегетації. * вказує значиму різницю в порівнянні з контролем за $P < 0,05$.

Fig. 4. Effect of UV-B on quantity of flowers (A) and pods (B) of pea plants during vegetation. * indicates the level of significance between control and UV-B treated plants at $P < 0.05$.

Отже, інгібування росту та розвитку рослин гороху відбувалось після опромінення низькими і високими дозами УФ-В променів. Подібний ефект було отримано за дії УФ-В на рослини квасолі у польових умовах, у якій відзначено пригнічення росту, зміни складу та вмісту пігментів, антиоксидантів (Singh *et al.* 2011). Таким чином, окремі представники бобових виявили високу чутливість до УФ-В у широкому діапазоні доз, що робить можливим їх використання у якості чутливих індикаторів дії ультрафіолету на рослини. Критеріями дії УФ-В опромінення можуть бути процеси морфогенезу, що дозволяє отримати кількісні характеристики відповіді рослин на абіотичний стресовий чинник

навколишнього середовища. Виявлено, що зміни у рості та морфогенезі рослин після дії УФ-В обумовлені зменшенням вмісту гормонів, які активують ріст – ауксинів і гіберелінів і збільшенням абсцизової та саліцилової кислоти, оксиду азоту, етилену (Vanhaelewyn *et al.* 2016). Критичною дозою УФ-В для рослин гороху в наших дослідженнях була 8 кДж/м^2 , що виявила інгібуючу дію на ріст, біомасу, врожай в інших дослідженнях для цієї культури (Choudhary & Agrawal 2014). Попередньо також відзначалася одночасна генерація оксидного стресу, накопичення флавоноїдів, саліцилової кислоти, збільшення транспірації та продишового опору, зменшення вмісту хлорофілу

(Martel et al. 2016). Гостре опромінення УФ-В у високих дозах здатне спричинити запрограмовану загибель клітин, що може бути однією з причин затримки росту та розвитку у дводольних (Ferreyra et al. 2016). Подальше дослідження дії УФ-В на рослини гороху дозволить доповнити знання про цей важливий стресовий чинник навколишнього середовища.

Висновки

Встановлено, що дія гострого опромінення УФ-В на рослини гороху в дозах від 2 до 15 кДж/м² інгібувала ріст рослин, формування квіток і стручків, приріст сухої маси. Виявлено, що наслідком дії дози УФ-В 2 кДж/м², яка відповідає природному фоновому рівню ультрафіолету у помірних широтах, було зменшення висоти рослин, утворення квіток і стручків. Опромінення рослин гороху УФ-В дозою 4 кДж/м² викликало подальше пригнічення росту у висоту, але стимулювало розвиток квіток і стручків. Доза 8 кДж/м² була пороговою для дії УФ-В на горох. Подальше збільшення дози УФ-В посилювало пригнічення ростових процесів у пагонах гороху, формування квіток і стручків. Однак дія УФ-В променів у найбільшій дозі, яка перевищувала природний фон майже у 10 разів, не спричиняла загибелі рослин, що свідчить про невисоку руйнівну здатність даного компоненту сонячного світла. Рослини гороху виявили високу чутливість до УФ-В-опромінення у широкому діапазоні доз і можуть бути використані для тестування відповіді на даний компонент сонячного світла.

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RESEARCH ARTICLE

Vegetative anatomical adaptations of *Epidendrum radicans* (Epidendroideae, Orchidaceae) to epiphytic conditions of growth

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Abstract

The anatomical properties of leaf, stem, and root of *Epidendrum radicans* Pav. ex Lindl., belonging to the subfamily Epidendroideae (Orchidaceae) were investigated for adaptations to stressed habitats. The anatomical investigation revealed that leaves of *E. radicans* have a thick cuticle (3–4 μm) and paracytic type of stomata. Foliar epidermal cells are conical on the adaxial surface and rectangular in the abaxial surface, distinct hypodermis absent, and uniseriate fiber bundles are arranged in both sides of the leaves. The foliar mesophyll is homogenous and starch grains and raphides present. The leaf sheath covering the stem have cuticle restricted to the outer surface and air spaces are present. The stem has a cuticularized uniseriate epidermis and a uniseriate hypodermis. The cortex and a parenchymatous ground tissue of the stem are separated by a layer of sclerenchymatous band. Vascular bundles are collateral and their size generally increases from the periphery towards the center. A sclerenchymatous patch covers the phloem pole, whereas the xylem is covered by thin-walled parenchymatous cells. The roots possess *Epidendrum*-type velamen. Cover cells present. Uniseriate dimorphic exodermis consists of U-thickened long cells and thin-walled passage cells. The endodermal cells O-thickened, pericycle sclerenchymatous, xylem 10–14 arched. The pith is sclerenchymatous, but parenchymatous at the center. The anatomical examination of *E. radicans* revealed adaptations to moisture stress conditions like thick cuticle covering the leaves and stem, water storage cells, multilayered velamen and dimorphic exodermis.

Keywords: *Epidendrum radicans*, cover cells, cuticle, idioblasts, raphides, starch grains, stegmata, velamen, water stress

Introduction

Epidendrum L. is the largest genus in the neotropical subtribe Laeliinae Benth. with around 1500 species and is native to the American continent (van den Berg *et al.* 2005; Pinheiro & Cozzolino 2013). The high diversity in *Epidendrum* is due to the different habitats they occupy ranging from dry tropical jungles to humid forests. In nature, most of these orchids grow as epiphytes, while some exist as lithophytes or terrestrial forms (Hágsater & Soto-Arenas 2005). Further, species of *Epidendrum* are also aggressive colonizers of the disturbed and denuded areas. *Epidendrum* taxa are proposed as a potential model for investigation on many evolutionary hypothesis including habitat selection and reproductive biology due to their wide variation in morphology, genetic diversity, ecological functionality and easy propagation (Pinheiro & Cozzolino 2013). The circumscription of *Epidendrum* is of great debate and several species that were once in this genus are now transferred to other genera (Chase *et al.* 2015). Economic importance of *Epidendrum* is limited when compared to other orchid genera. Stems of *E. mosenii* Rchb. f. are used as analgesic and *E. rigidum* Jacq. is reported to possess herbicidal activity. Infusion prepared from leaves of *E. chlorocorymbos* Schltr. is known to reduce blood cholesterol levels, cure spots on the skin, treat ear ache and stimulate dreaming (Asseleih *et al.* 2015). Nevertheless, species of *Epidendrum* are highly popular and most sought after by gardeners and hobbyists for their beautiful flowers and are popularly known as Poor man's orchid or Crucifix orchid (Stern & Carlsward 2009).

Anatomy helps in understanding the trends in plant evolution and adaptations although most of the recent concepts in these areas are based on molecular studies (Seago & Fernando 2013). Despite their huge species diversity and widespread popularity in horticulture, anatomical studies on species of *Epidendrum* are very limited. Baker (1972) noted that the lengths of guard cells in *E. parkinsonianum* Hook., ranged between 60 and 63 μm . Khasim & Mohana-Rao (1990) in their study on the

anatomy of some Epidendroideae reported the presence of fiber and compound midrib vascular bundles and the absence of trichomes, hypodermis and motor cells in leaves of *E. radicans* Pav. ex Lindl. In their comparative anatomy of the vegetative parts of Laeliinae, Stern & Carlsward (2009) examined the leaf anatomy of *E. anceps* Jacq. and *E. nocturnum* Jacq., among other members of the subtribe. The main foliar anatomical features noted were the absence of hairs, adaxial and abaxial (*E. anceps*) or abaxial (*E. nocturnum*) distribution of the stomata, reniform guard cells, lack of fibre bundles and hypodermis, undifferentiated mesophyll, and stigmata restricted to fibre bundles and vascular bundles. More recently, Moreira *et al.* (2013) compared the anatomical adaptations of the epiphytic *E. secundum* Jacq. and *Dichaea cogniauxiana* Schltr., growing in a nebular forest located at an amporupestre area at Serra da Piedade of Brazil to stresses of their microhabitats. In *E. secundum* the leaves growing in open light had more stomata and a thicker cuticle than *D. cogniauxiana* growing in the shade (Moreira *et al.* 2013).

Stem anatomy of *Epidendrum* is not well studied when compared to leaves and roots. Weltz (1897) examined the anatomy of stems in eight genera of Laeliinae and noted that the hypodermis in *Epidendrum* spp. was homogeneous, consisting of similar appearing cells. Investigations on the stem anatomy of *E. anceps* and *E. nocturnum* revealed the absence of hairs and stomata (Stern & Carlsward 2009). The cauline epidermis consisted of cells that was either thick- (*E. nocturnum*) or thin-walled (*E. anceps*), and the hypodermis contained cells with thickened walls. Vascular bundles were distributed in the ground tissue and the phloem and xylem were covered by crescent shaped sclerenchymatous caps. Stigmata often associated with the phloem sclerenchyma (Stern & Carlsward 2009).

Dycus & Knudson (1957) while examining the role of velamen in aerial roots of orchids observed that the number of velamen layers in *E. xanthium* Lindl., *E. boothianum* Lindl., *E. ibaguense* Lindl. and *E. radicans* ranged from 3 to 5. Pridgeon *et al.* (1982) examined

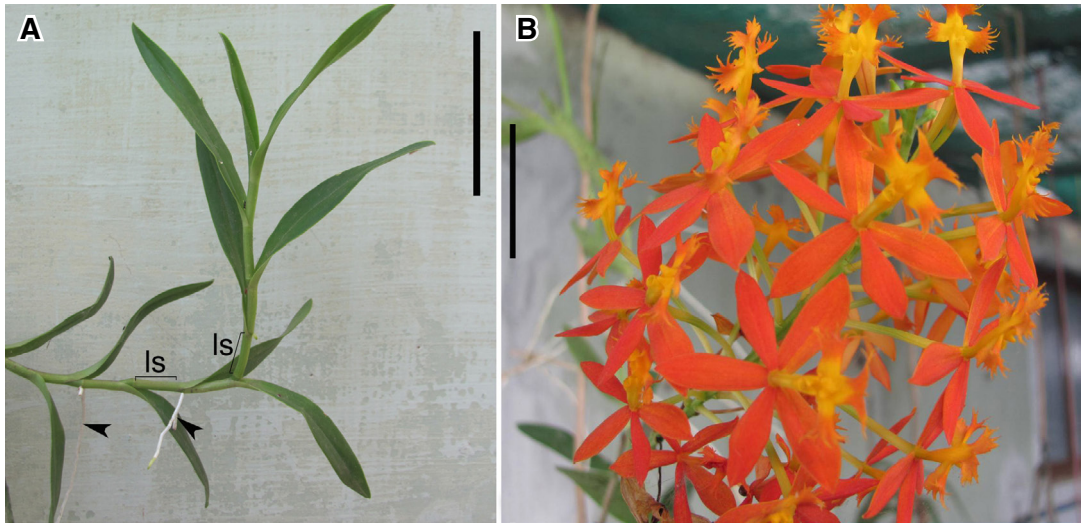


Fig. 1. Habit of *Epidendrum radicans*. **A** – vegetative shoot with roots (**arrow heads**) and the stem covered by leaf sheath (**ls**); **B** – flowering shoot. Scale bars = 5 cm (**A**) and 2 cm (**B**).

13 species of *Epidendrum* for the presence of tilosomes in roots. Of these, elaborate tilosomes were present only in *E. coriifolium* Lindl., and tilosomes of limited nature were observed in *E. ensatum* A. Rich. & Gal., *E. pseudepidendrum* (Rchb. f.) Rchb. f. and *E. secundum* Jacq. Moller & Rasmussen (1984) indicated the occurrence of conical silica structures in *Epidendrum*. Porembski & Barthlott (1988) in their classification of velamen radicum grouped orchids with velamen resembling *E. bifidum* Abul. as *Epidendrum*-type. Epivelamen cells in this velamen type are smaller than the radially elongated endovelamen cells. Further, endovelamen cells have thickenings that are fused into composed ledges and large pores. Tilosomes are infrequent and walls of the endodermis lightly thickened. Cortex may possess tracheoidal idioblasts (Porembski & Barthlott 1988). Zankowski *et al.* (1987) investigated the developmental anatomy of velamen and exodermis in aerial roots of *E. ibaguense* and concluded that casparian strips in the exodermis are obscure and are not a functional equivalent of endodermis. Oliveira & Sajo (1999) studied the root anatomy of *E. campestre* Lindl. and *E. secundum* growing at Instituto de Botânica de São Paulo. Root hairs were present in *E. campestre*. The velamen

in *E. campestre* was heterogeneous with periclinally flattened exovelamen and an endovelamen with finely reticulate thickened walls. Cover cells were present in both the species. The velamen consisted of 5–6 cell layers in both species, exodermal cells were O-thickened (*E. campestre*) or U-thickened (*E. secundum*), cortex was 9–12-layered, endodermis was O-thickened and the xylem arches ranged from 11 to 17. The root cortex of *E. campestre* had fungal hyphae and water storing idioblasts. Nevertheless, velamen cell layers, as well as the water storing idioblasts, in roots were observed in the both species (Oliveira & Sajo 1999). These anatomical modifications indicate adaptation of the two orchids to their microhabitats (Moreira *et al.* 2013). The anatomy of *E. anceps* and *E. nocturnum* roots was characterized by the lack of tilosomes and cortical cell wall banding, and exodermal cells with U-thickened walls. Endodermal and pericycle cells in these taxa were isodiametric with O-thickened walls (Stern & Carlsward 2009).

Among the different species of *Epidendrum*, the ground dwelling *E. radicans* (Fig. 1), commonly known as fire star orchid or ground root orchid, is indigenous to Central America (Devadas *et al.* 2010). In addition to terrestrial

habitats, *E. radicans* also thrives as epiphyte in the Asian tropics (Khasim & Mohana-Rao 1990). Unlike other orchids where flowering is usually seasonal, this orchid produces flowers year round (Janzen 1987; Suttleworth *et al.* 1994). *E. radicans* often occurs in anthropogenically disturbed areas like the roadsides at an altitude of 1,000–2,000 m a.s.l. (Bierzuchudek 1981). Human activities are shown to increase the abundance of *E. radicans* (Dressler 1981). Epiphytic habitats are always stressful due to the lack of water and nutrient holding medium. Plants inhabiting these habitats have evolved several structural and physiological adaptations that enable these plants to successfully thrive in these circumstances. In spite of its weedy nature, *E. radicans* is susceptible to stresses and requires optimum moisture, light and nutrients for normal growth (Dressler 1981). Therefore, the aim of the present study was to examine the vegetative anatomy of *E. radicans* and to record the adaptations that help this species to survive in epiphytic and other stressful habitats.

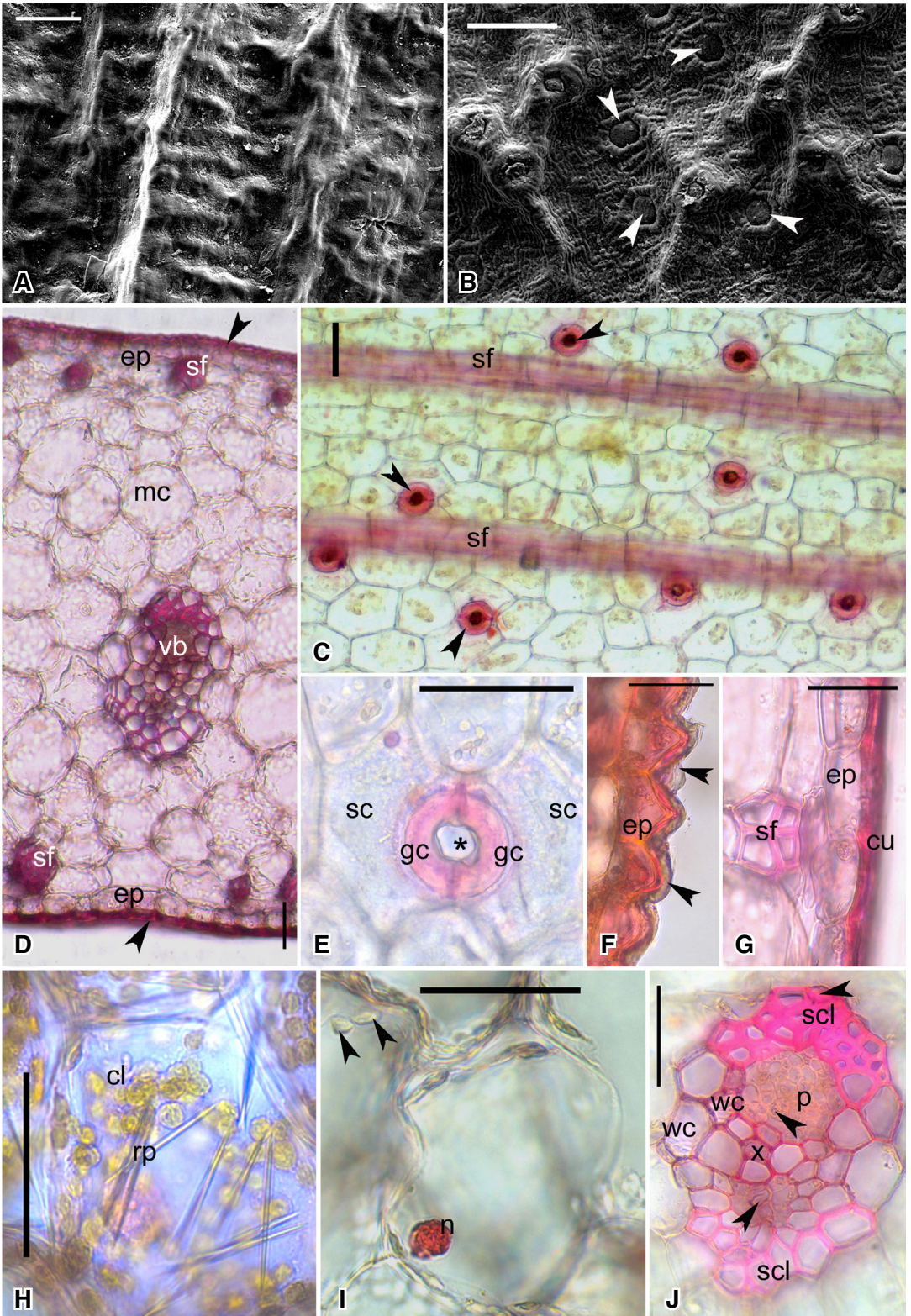
Material and methods

The vegetative material of *E. radicans* for anatomical studies was obtained from a home garden (10°59'54.2" N, 76°59'22.9" E, 411 m a.s.l.) in Coimbatore, Tamilnadu, India. The average maximum and minimum temperature of Coimbatore are 32.5°C and 21.3°C. The relative humidity ranges from 49–88%. The plants were growing in a light intensity of 27.87 candelas and in 18 cm clay orchid pots filled with charcoal, broken bricks and coconut husk in the ratio of 1 : 1 : 2. The plants were watered once the potting

medium dried and standard fertilizer was applied as foliar spray once every fifteen days. Plant samples from three potted plants were collected during January of 2017. Fresh stems, roots and leaves were collected, washed and fixed in FAA (formalin – acetic acid – alcohol) mixture until processing (Johansen 1940). For uniformity, fifth leaf from the tip was selected for examination and the sections were made midway between the tip and the base. Similarly, stem and root were sectioned 7.5 cm and 5 cm respectively from the tips. Free hand sections 30–40 µm thick were taken using a razor blade and stained with safranin. Lamellar suberin was tested by staining with sudan IV, toluidine blue / HCl-phloroglucinol was used to locate lignin and tannin, and iodine was used to detect the presence of starch (Johansen 1940).

The sections either stained or treated with various histochemical reagents were mounted in glycerine and observed under an Olympus BX51 light microscope. A calibrated ocular scale was used to measure the dimensions of the cells and the size of the different regions in the sections. The values are presented either as range or length [mean ± standard error (SE)] × width (mean ± SE) or mean ± SE accordingly. Microphotographs of the sections observed were captured with a ProgRes3 camera attached to the Olympus BX 51 microscope. Stomatal index (%) was calculated (n=10) from the number of epidermal cells and stomata in ten randomly selected microscopic fields (×200) according to Salisbury (1927). For scanning electron microscopy (SEM), 5 mm² of leaf bit or thin transverse section of stem and root were fixed with double-sided adhesive tape onto labelled stubs. The specimens were coated with gold and processed in Philips SEM 515.

Fig. 2. Leaf anatomy of *Epidendrum radicans*. **A–B** – scanning electron microscope images of the adaxial leaf surface showing ridges and groves (**A**) and abaxial surface with stomata (**white arrow heads**) (**B**); **C** – epidermal peeling of leaf showing stomata (**black arrow heads**) and sclerenchymatous fiber bundles (**sf**); **D** – transverse section (**t.s.**) of leaf showing cuticle (**black arrow heads**), epidermis (**ep**), sclerenchymatous fiber bundle (**sf**), homogenous mesophyll (**mc**) and collateral vascular bundle (**vb**); **E** – paracytic stomata (**asterisk**) on the abaxial leaf surface surrounded by guard cells (**gc**) and subsidiary cells (**sc**); **F** – t.s. of leaf showing adaxial epidermis (**ep**) with triangular cells and cuticle (**black arrow heads**); **G** – t.s. of leaf showing abaxial epidermis (**ep**) covered by a cuticle (**cu**) and the presence of sclerenchymatous fiber bundle (**sf**); **H** – t.s. of leaf showing mesophyll cells containing chloroplasts (**cl**) and raphides (**rp**); **I** – t.s. of leaf showing mesophyll cell containing intact nucleus (**n**) and starch grains (**black arrow heads**); **J** – t.s. of leaf showing vascular bundle with xylem (**x**), phloem (**p**), sclerenchymatous cap (**scl**), water cells (**wc**) and stegmata (**arrow heads**). Scale bars = 50 µm (**A–J**).



Results

Leaf

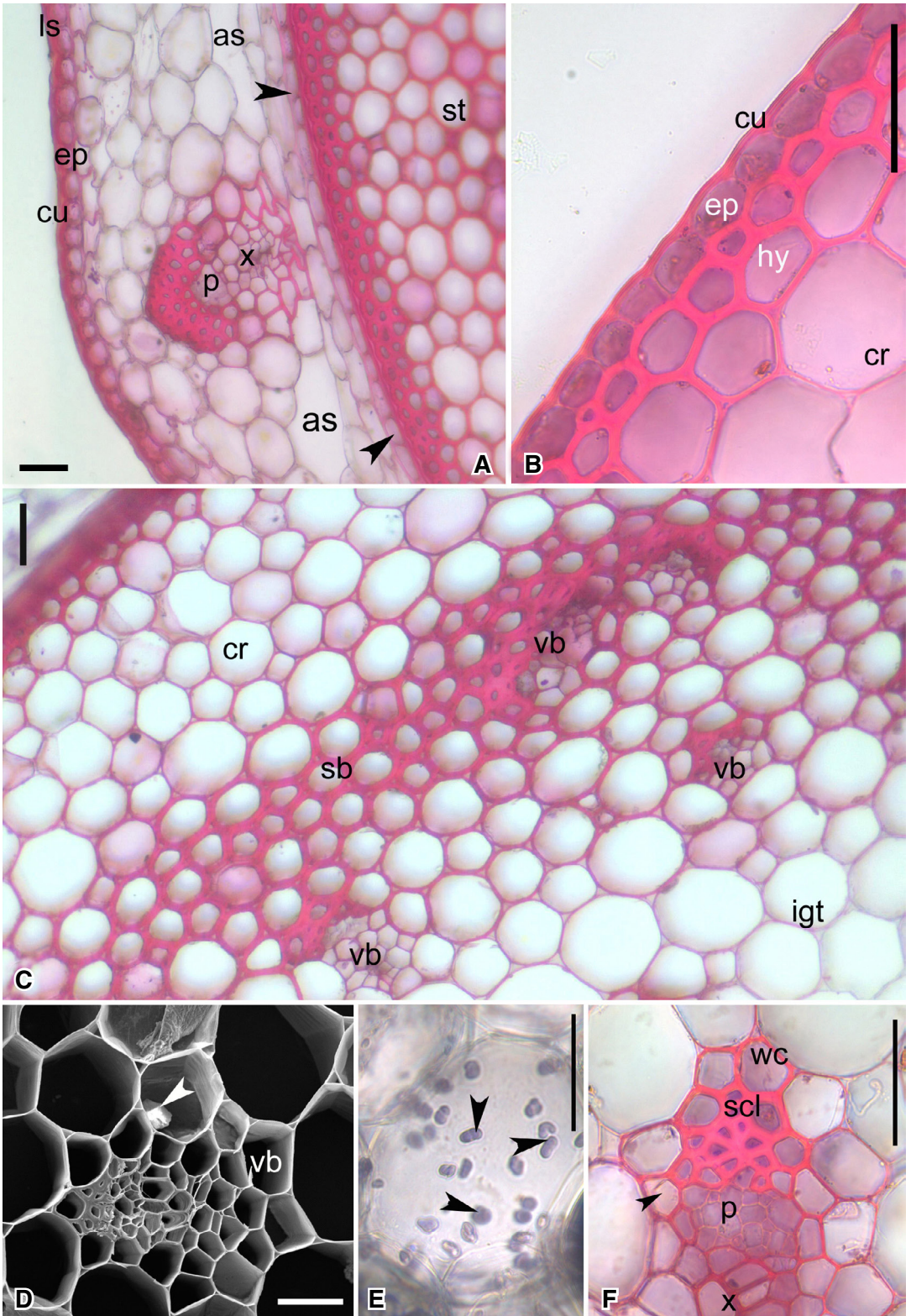
Cuticle 3–4 μm thick and smooth, present on both abaxial and adaxial surfaces of the leaf (Fig. 2 A, D, F, G). Paracytic stomata restricted to the adaxial surface, with two subsidiary cells having longitudinal axes parallel to the guard cells of the aperture (Fig. 2 B, C, E). Substomatal chambers small and irregularly shaped. The subsidiary cells fail to meet over the poles. The pore measures $14.05 \pm 0.18 \times 13.65 \pm 0.18 \mu\text{m}$ (length \times breadth). The guard cells measures $31.9 \pm 0.33 \times 12.6 \pm 0.28 \mu\text{m}$ and subsidiary cells measure $33.15 \pm 0.55 \times 54.25 \pm 0.69 \mu\text{m}$. The calculated stomatal index is $9.77 \pm 0.34\%$. Upper and lower epidermis uniseriate, compactly arranged, thick walled, nucleate and parenchymatous. Cells of the upper epidermis are conical and measures $25.57 \pm 0.55 \times 38.65 \pm 0.41 \mu\text{m}$, and those of the lower epidermis are rectangular and measures $33.00 \pm 0.54 \times 23.50 \pm 0.41 \mu\text{m}$. Hypodermis consists of thin walled parenchymatous cells. Fiber bundles present in the hypodermal region of both the upper and lower surfaces. However, the number of fiber bundles in the adaxial region is higher compared to that of the abaxial region. Mesophyll cells 23–25 μm (23.5 ± 0.17) wide, homogenous and not differentiated into palisade and spongy layers. Starch grains that stains brown with iodine are present in most of the mesophyll cells. Raphides present. Vascular bundles arranged in a single row in the median. Xylem and phloem are covered by a sclerenchymatous cap (Fig. 2 D, G, J). The sclerenchymatous cap covering the phloem is much bolder than those covering the xylem. Small water storing idioblasts present next to the phloem. Largest vascular bundle is present

in the midrib region. In the lamina, a large vascular bundle alternates 2–4 smaller vascular bundles. Stegmata present in xylem, phloem and sclerenchymatous cap surrounding the phloem (Fig. 2 J).

Stem

Stems circular, green, smooth, hairs absent, and the leaf sheath cover 50 % of the internode. Leaf sheath consist of circular to oval thick-walled epidermal cells covered by a 2–5 μm thick cuticle (Fig. 3 A–C). Epidermis and cuticle restricted to the adaxial surface of the leaf sheath. The epidermis is followed by a 7–8 layers of parenchymatous cells that are circular to irregular enclosing small triangular intercellular spaces. The innermost layer of the leaf sheath consists of 1–2 rows of rectangular cells with slightly thickened walls. Large airspaces present in the leaf sheaths. Leaf traces collateral with the phloem pole covered by a thick sclerenchymatous cap and the xylem pole covered by cells that are lightly thickened compared to the phloem pole. Cuticle covering the stem is 2–6 μm thick. Cuticle in the stem in regions covered by leaf sheath is thinner (2–4 μm) than those regions not covered by the leaf sheath (3–6 μm) (Fig. 3 A, B). Epidermis is uniseriate, with compactly arranged rectangular parenchymatous cells and measures $29.03 \pm 0.67 \times 20.07 \pm 0.45 \mu\text{m}$. Hypodermis consists of 1–3 layers of compactly arranged thick walled sclerenchymatous cells. Tissue inner to the hypodermis consists of 49–55 layers of cells and differentiated into an outer cortex and inner ground tissue regions. The cortex and the ground tissue are separated by 4–5 layered sclerenchymatous bands. Outer cortical region is 4–6 layered parenchymatous or sometimes chlorenchymatous enclosing triangular

Fig. 3. Leaf sheath and stem anatomy of *Epidendrum radicans*. **A** – transverse section of leaf sheath (**ls**) covering the stem (**st**), showing cuticle (**cu**), epidermis (**ep**), air spaces (**as**) and the inner layer of the leaf sheath (**black arrow heads**); **B** – stem transverse section showing cuticle (**cu**), epidermis (**ep**), hypodermis (**hy**) and cortex (**cr**); **C** – outer cortex (**cr**) and inner ground tissue (**igt**) separated by a sclerenchymatous band (**sb**) and vascular bundles (**vb**); **D** – SEM image of the inner ground tissue containing stegmata (**white arrow head**) and vascular bundle (**vb**); **E** – starch grains (**black arrow heads**) in the ground tissue cell; **F** – vascular bundle with xylem (**x**), phloem (**p**), sclerenchymatous cells (**scl**), water cell (**wc**) and stegmata (**black arrow head**). Scale bars = 50 μm (**A–F**).



intercellular spaces. Vascular bundles absent in the cortex. The inner ground tissue contains scattered vascular bundles with the outermost bundles immersed in the sclerenchymatous band. The size of the vascular bundles generally increases towards the centre of the stem. Starch grains are present in most of the ground tissue cells (Fig. 3 E). The vascular bundles are collateral and the phloem pole is covered by sclerenchymatous cells. Stegmata present both in the cortical and phloem regions (Fig. 3 D, F).

Root

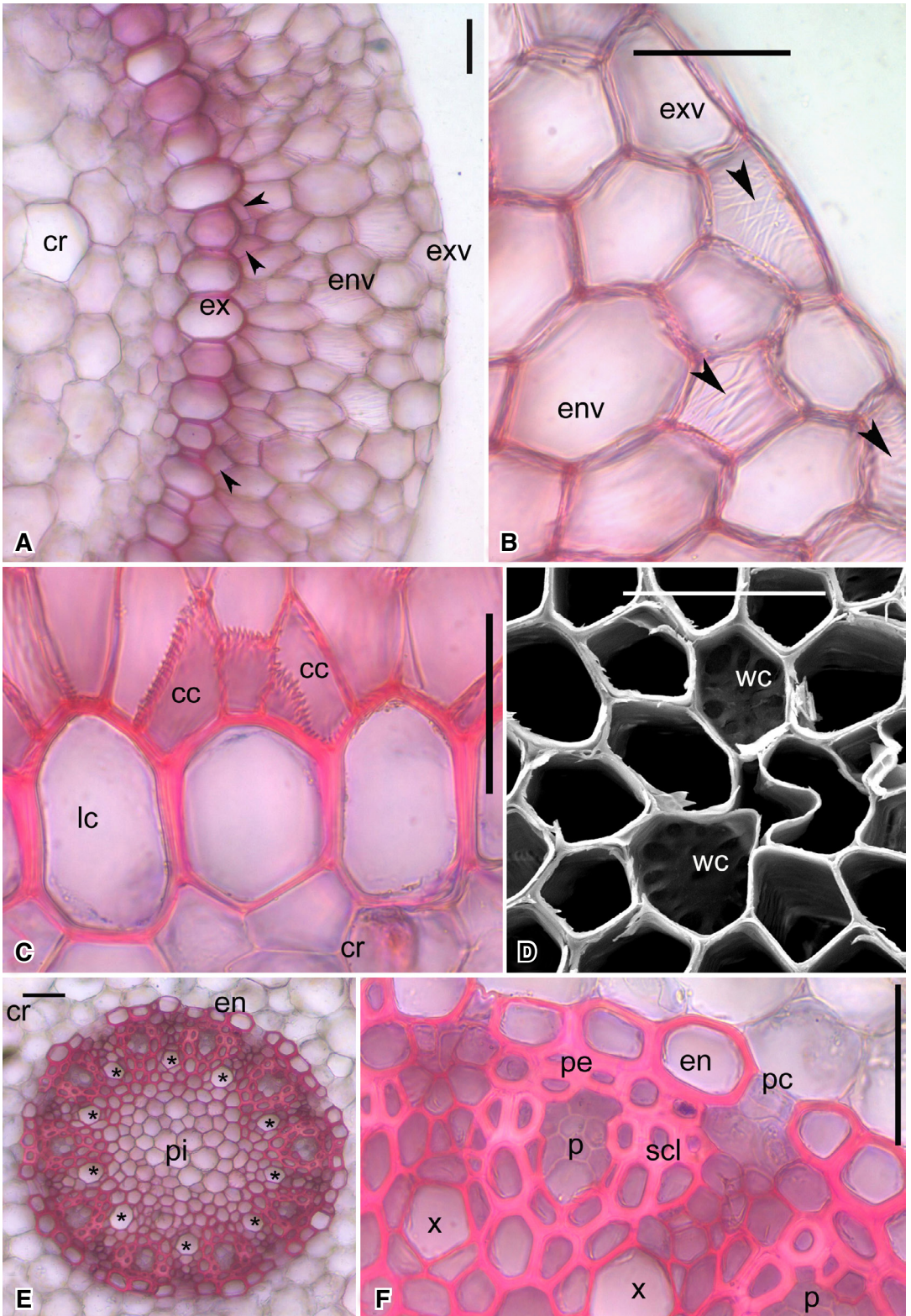
The aerial roots of *E. radicans* are circular, whereas those attached to the substratum have a flattened region at the point of contact with the substratum. The root is covered by a velamen that is 4–6-layered (Fig. 4 A, B). Velamen is differentiated into an outer exovelamen and an inner endovelamen. The exovelamen is uniseriate with isodiametric cells. The endovelamen is 3–5-layered consisting of isodiametric to radially elongated cells. Cells of the endovelamen are comparatively larger. Wall striations are present in the cells of both the exovelamen and endovelamen. The average velamen cell measures $63.35 \pm 1.01 \times 35.25 \pm 0.64 \mu\text{m}$. The velamen is followed by the exodermis, which is the outermost cortical layer. The exodermis consists of long and short cells. The long cells of the exodermis are U-thickened and the short passage cells are comparatively thin-walled. The wall of the long cells reacts positive to suberin. The exodermis cell measures $60.75 \pm 0.54 \times 35.45 \pm 0.84 \mu\text{m}$. Small cover cells are present above the exodermal layer. Below the exodermis is an 8–9-layered thin-walled parenchymatous cortex (Fig. 4 C–E). The cortical cells of the substrate roots contain fungal pelotons in root portions attached to the substrate. The ultimate cortical layer is

differentiated into uniseriate endodermis. The endodermal layer consists of cells with O-thickened walls that reacts positive to suberin, and is interspersed with 1–2 thin-walled passage cells (Fig. 4 F). Pericycle uniseriate, cells facing the xylem are thin-walled and those opposite to the phloem are thick-walled. Vascular cylinder 10–14-arched. Vascular tissue is surrounded by sclerenchymatous tissue. The pith is sclerenchymatous with thick-walled cells. But pith cells at the centre may be thin-walled enclosing small intercellular spaces. Water cells present in the cortical and pith region (Fig. 4 D).

Discussion

Generally, plants that thrive in extreme environmental conditions have adaptations that help them to overcome the different stresses. *E. radicans* is able to exist in open and rocky areas, which are strictly terrestrial, and sometimes grows as an epiphyte. Though *E. radicans* shows a tendency of weedy growth, the plant cannot tolerate stress and it needs adequate light, water and nutrients supplement (Dressler 1981). Anatomical adaptations are evident in all the vegetative parts of *E. radicans*. The leaves and stems lack hairs as reported for other *Epidendrum* species. This contradicts the studies where frequent occurrence of trichomes was reported in Laeliinae including *Epidendrum* species (Solereeder & Meyer 1930; Baker 1972). Stern & Carlsward (2009) also failed to detect trichomes on leaves of Laeliinae members including *E. anceps* and *E. nocturnum*. These authors suggested that the lack of trichomes in Laeliinae could be due to the fragile thin-walled nature of the terminal cells of the trichomes that detach off very easily. Nevertheless, examination of young unopened leaves of *E. radicans* also revealed the absence of trichomes suggesting

Fig. 4. Root anatomy of *Epidendrum radicans*. **A** – transverse section of root showing exovelamen (**exv**), endovelamen (**env**), cover cells (**black arrow heads**), exodermis (**ex**) and cortex (**cr**); **B** – striations (**black arrow heads**) in cells of exovelamen (**exv**) and endovelamen (**env**); **C** – exodermis with long cells (**lc**) and cover cells (**cc**); **D** – scanning electron microscopic image of water cells (**wc**) in the cortex; **E** – cortex (**cr**) and stele showing endodermis (**en**), xylem arches (**asterisks**) and pith (**pi**); **F** – endodermis (**en**) with passage cells (**pc**) and pericycle (**pe**) covering the phloem (**p**) and xylem (**x**) embedded in sclerenchymatous tissue (**scl**). Scale bars = 30 μm (**D**) and 50 μm (**A–C, E, F**).



that foliar trichomes were absent in this orchid. The leaves of *E. radicans* are hypostomatic like in other *Epidendrum* species. But stomata of *E. radicans* are not tetracytic as in other Laeliinae members (Khasim & Mohana-Rao 1990; Stern & Carlsward 2009), they are paracytic as reported by Khasim & Mohana-Rao (1990). The size of guard cells is well within the range reported for other members of Laeliinae (Stern & Carlsward 2009).

In this study, leaf sheath and stems of *E. radicans* were covered by a cuticle that varied in thickness. The thick cuticle is suggested as an adaptation to minimize water loss in epiphytic orchids (Moreira *et al.* 2013; Yang *et al.* 2016). The cuticle is also known to play a key role in the exchange of gases, it protects plants against environmental stresses and generates a suitable microenvironment for phyllosphere organisms (Yeats & Rose 2013; Fernández *et al.* 2016). Epidermal cells of *E. radicans* have an outer undulating anticlinal walls resulting in conical shaped cells similar to those reported in several desert plants (Gibson 1996). Nevertheless, the foliar epidermal cells on the abaxial side are periclinal. Though foliar hypodermis has been reported in several members of Laeliinae, it appear to be absent in *Epidendrum* species as observed in the current and other studies (Stern & Carlsward 2009). Foliar fibre bundles in *E. radicans* are distributed on both sides of the leaves resembling those of *E. nocturnum*. Nevertheless, the distribution of foliar bundles was mostly abaxial in most members of Laeliinae including *E. anceps* and appears to be the usual anatomical feature in this group (Stern & Carlsward 2009). Foliar fiber bundles are mechanical in function supporting the leaves and have no systematic value as they occur in many epiphytic orchid taxa across various groups (Stern & Carlsward 2009). The mesophyll of *E. radicans* is homogenous as in other species of *Epidendrum* (Khasim & Mohana-Rao 1990; Stern & Carlsward 2009). Unlike in *E. anceps* and *E. nocturnum*, where the vascular bundles occur in two rows, the vascular bundles in leaves of *E. radicans* are arranged in a single median row. The distribution of stegmata in vascular bundles is

similar to those of *E. anceps* and *E. nocturnum* (Stern & Carlsward 2009).

In *E. radicans*, leaf sheaths cover almost half of the internode of the stem and may provide mechanical support to the stem. To our knowledge anatomical features of the leaf sheath in *Epidendrum* or any other orchid species has never been examined before. The anatomy of leaf sheath is different from those of the leaves. The leaf sheaths are characterized by distinctive anatomical characters like the presence of large air spaces, the absence of cuticle on the abaxial surface, stegmata and idioblasts. Vascular bundles occupy nearly two third width of the leaf sheath. The sclerenchymatous patch covering the phloem is more prominent than those covering the xylem.

Idioblasts with different types of thickenings were found in all vegetative organs of *E. radicans*. It is believed that idioblasts can assist in the storage of water (Pridgeon 1982) and are termed as water cells by Stern & Carlsward (2009). However, these specialized cells can also afford mechanical support by preventing cell collapse during water stress and appears to be an adaptation to xeromorphic conditions of the epiphytic habitats (Olatunji & Nengim 1980; Holtzmeier *et al.* 1998). According to Pridgeon (1982), during the shortage of water, these cells either get filled with air or may become involved in water storage.

The stem anatomy of *E. radicans* resembles in general other members of Laeliinae (Stern & Carlsward 2009). The stem of *E. radicans* is devoid of hairs and stomata, and the thickness of the cuticle covering the stem is well within the range reported for other members of Laeliinae (Stern & Carlsward 2009). The variation in the thickness of the stem cuticle in the leaf sheath covered and uncovered portion clearly suggests the role of leaf sheath in minimizing water loss in additions to aiding rigidity to the stem. The epidermal cells in *E. radicans* are conical similarly to those of *E. nocturnum* and thin-walled (Stern & Carlsward 2009). Though most members of Laeliinae lack a cortex, *E. radicans* has a cortex that is 4–6 cells wide. A fibre band in *E. radicans* covers the ground tissue, as in *Broughtonia* R. Br. (Stern & Carlsward 2009).

The ground tissue in *E. radicans* consists of both large and small roundish cells unlike in *Orleanesia* Barb. Rodr., where the cells of the ground tissue are more or less evenly sized (Stern & Carlsward 2009). Though cauline stigmata were reported in stems of *E. anceps* and *E. nocturnum* by Stern & Carlsward (2009), Khasim & Mohana-Rao (1990) did not observe any stigmata in stems of *E. radicans*. In contrast, stigmata were observed in cells adjoining the vascular bundles in *E. radicans* in the present study. Moller & Rasmussen (1984) suggested that silicon enters epiphytic plants mainly due to the settling of the air-borne dust or through the water run-offs containing silica from the phorophytes. Further, the widespread presence of stigmata in xerophytes and their absence in mesophytes suggests the possibility of the relation of stigmata to arid conditions (Moller & Rasmussen 1984).

The aerial roots of *E. radicans* are cylindrical whereas the roots attached to the substratum are flattened at the point of contact and the former lacked root hairs. This is similar to the observations of Stern & Carlsward (2009) and Moreira *et al.* (2013) where root hairs were absent in aerial roots of studied *Epidendrum* species. In contrast, root hairs were present in root regions that were in contact with the substratum as in *E. secundum* (Moreira *et al.* 2013). The velamen in *E. radicans* resembles the velamen in other members of Laeliinae and is of typical *Epidendrum*-type. The velamen is an important structure in orchid roots adapted for the uptake of water and dissolved nutrients. It helps in the quick absorption of water and prevents moisture loss from the roots, apart from providing mechanical protection, reflecting infra-red radiation, screening of roots against ultraviolet radiation and absorbing immobilized nutrients from rain water (Thangavelu & Ayyasamy 2017). Apart from this, the other functions that can be attributed to this tissue include amplifying access to mineral-rich solutions (Benzing *et al.* 1982), and exchange of carbon dioxide and oxygen between the root and atmosphere (Moreira & Isaias 2008).

The occurrence of velamen is generally associated with an epiphytic habit (Zotz

& Winkler 2013), though it occurs also in terrestrial orchids (Porembski & Barthlott 1988; Uma *et al.* 2015). The size of the velamen can be related to specific environmental factors like temperature and water. Hence, orchids like *E. radicans* occurring in dry or exposed habitats have a multilayered velamen, while those occurring in humid environments are characterized by few-layered velamen (Sanford & Adanlawo 1973). Moreover, the velamen in *E. radicans* is differentiated in two layers, the exovelamen being uniseriate and the endovelamen – multiseriate. On the other hand, wall striations were observed in the cells of both exovelamen and endovelamen in *E. radicans*. The striations in the exovelamen of *E. radicans* falls into the type-IIIa and those of the endovelamen falls into the type-IIb according to Sanford & Adanlawo (1973) classification. The wall thickenings in the velamen provide mechanical support to the cells avoiding their collapse during the dehydration (Noel 1974; Oliveira & Sajo 1999).

Exodermis, the outer layer of the cortex (Engard 1944) in *E. radicans* is heterogeneous consisting of long and short cells. The long cells of the exodermis develop secondary thickenings in their walls during maturity and die (Pridgeon 1986). The high lignin and suberin content in the walls of the exodermal long cells provide mechanical strength and maintains high humidity around the cortex (Sanford & Adanlawo 1973; Benzing *et al.* 1983; Moreira & Isaias 2008). The shorter passage cells are thin-walled and living, they play a significant role in the nutrition and hydration as in other epiphytic orchids. The 2–4 wedge-shaped cover cells above the short cells of the exodermis as seen in *E. radicans* are formed from the innermost layer of the velamen (Carlsward *et al.* 2006). Though cover cells are associated with tilosomes, in some orchids we did not observe any tilosomes originating from the outer walls of the exodermal passage cells (Pridgeon *et al.* 1983). Tilosomes were also absent in *E. anceps*, *E. nocturnum* and *E. secundum* (Moreira *et al.* 2009; Oliveira & Sajo 1999). The cortex in *E. radicans* is parenchymatous, and the presence of pelotons

of the mycorrhizal fungi was restricted to the cells of cortex that were in contact with the substrate (Thangavelu & Ayyasamy 2017). Similar observations were made by Moreira & Isaias (2008) in *Sophronitis pumila* (Hook) Van den Berg & M.W. Chase, *Prescottia montana* Barb. Rodr., *Habenaria petalodes* Lindl., and *Polystachya estrellensis* Rchb. f.

The endodermis in *E. radicans* is uniseriate and its cells are thickly-walled. The thick-walled endodermal cells are interrupted by the presence of thin-walled passage cells. The endodermal thickenings form an efficient apoplastic barrier for the transfer of water and nutrients (Ma & Peterson 2003; Moriera & Isaias 2008). Hence the presence of passage cells is as essential in the endodermis as in the exodermis.

The vascular tissues in *E. radicans* are embedded in sclerenchymatous tissues, as it has been observed in many epiphytic orchids. This anatomical feature is considered to be a highly significant character that is related to the endurance of the plant during drought conditions (Nawaz *et al.* 2013; Thangavelu & Ayyasamy 2017). The pith is sclerenchymatous but the cells in the center are thinly-walled with small intercellular spaces.

Terrestrial orchids usually develop less anatomical and morphological adaptations for water stress. Nevertheless, an analysis of the vegetative structures discussed above clearly shows that *E. radicans* possess several anatomical adaptations that could aid in the survival of this orchid in stressful habitats. The presence of thick cuticle on adaxial and abaxial surfaces of the leaf and the presence of adaxial stomata substantiate the xeromorphic conditions tolerated by the plant. Velamen tissue in roots is basically important for the absorption of water and nutrients. Velamen in *E. radicans* resembles those of epiphytic than terrestrial orchids. Similarly, the nature of exodermis and endodermis is significant to provide mechanical protection and prevents evaporation of water. The absence of special storage organs in *E. radicans* is compensated by presence of the storage idioblasts in almost all the vegetative parts of this orchid. However, further experimental studies examining the

growth of this orchid in different environment and on different substrates would reveal the morphological plasticity of this orchid.

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CORRESPONDENCE

Morphological characteristics of a rare endemic species, *Erysimum croceum* M. Pop. (Brassicaceae) from Trans-Ili Alatau, Kazakhstan

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Abstract

Erysimum croceum is a rare endemic species listed in the Red Book of Kazakhstan. In 2015–2017 we studied three populations of this species on the territory of the Ile-Alatau National Park (Trans-Ili Alatau, Northern Tian-Shan). As a result of the inventory of sampling plots, we estimated the elevation range occupied by the species and identified age structure and population density. Our results confirmed earlier reports of low population counts of *E. croceum*. We also studied biometric characteristics of virginal and generative individuals of *E. croceum* from different populations, and measured parameters of their seeds. The morphometric parameters were highly variable across the studied populations. The only exception was the morphometric parameters of the seeds, which had low or very low variability. We conclude that these parameters are the most stable characteristics of the species. The results of the study can contribute to our understanding of population structure and dynamics of *E. croceum* and assist in developing effective conservation strategies for this species.

Keywords: *Erysimum croceum*, population, age structure, virginal individuals, generative individuals, density, biometrics

Introduction

Wallflower (*Erysimum* L.) is one of the largest genera of the family Brassicaceae Burnett

representing a monotype tribe Erysimeae Dumort (Al-Shehbaz 2012). Globally, the number of *Erysimum* species ranges from 200 to 350 (Polatschek & Snogerup 2002;

Al-Shehbaz 2012). Wallflowers are perennial or biennial plants distributed throughout Europe, in the Mediterranean, the Middle East and East Asia, and also in North and Central America (Polatschek & Snogerup 2002). South-East Asia and the Mediterranean region are the main centers of species diversity of the genus; recently, several new species have been described in these regions (Mozaffarian 2008; Polatschek 2008, 2010, 2011; Abdelaziz et al. 2011; Moazzeni et al. 2014).

Some species of *Erysimum* are used in the official and traditional medicine (Varlakov & Masina 1943; Maslennikova et al. 1961; Makarevich et al. 1974; Lei et al. 2000; Grudzinskaya et al. 2014).

The majority of publications on *Erysimum* addressed systematic questions (German 2005, 2014; Abdelaziz et al. 2011, 2014; Ouarmim et al. 2013; Lorite et al. 2015; Czarna et al. 2016; Moazzeni et al. 2016; Mahmoodi et al. 2017), and only a few, the phylogenetics and morphology of certain species (Mutlu 2010; Abdelaziz et al. 2014).

The Flora of Kazakhstan (Vasiliyeva 1961) listed 15 species of *Erysimum*, while a new taxonomic treatment by Abdulina (1999) mentioned 16 species. However, only one of them, *Erysimum croceum* M. Pop. has been listed in the Red Data Book of the Republic of Kazakhstan (Roldugin 2014) as a rare endemic species. For the first time *E. croceum* was collected and described by M.G. Popov in the gorge of the river Malaya Almatinka. The description first published by Popov (1935) was later cited in the Flora of the USSR and the Flora of Kazakhstan (Bush 1939; Vasiliyeva 1961). Among all Middle Asian representatives of the genus, *E. croceum* is the only species that has bright orange petals; hence the specific epithet “croceum”. Some taxonomic treatments reduced *E. croceum* under the synonymy of *E. virgatum* (Polatschek 2010; The Plant List 2013). However, many Kazakh and Russian botanists recognize *E. croceum* as an independent species (Vasiliyeva 1961; Adylov 1974; Baitenov 1985; Czerepanov 1995; Abdulina 1999; Roldugin 2014). Following years of extensive field research,

the authors of the present study agree with the latter authors.

Despite a relatively large distribution area which includes Trans-Ily Alatau, Ketmentau, Kungey and Kyrgyz Alatau the species remains poorly studied due to low population numbers and sporadic distribution. Plants occur individually or in small groups up to ten individuals in number. Only on two occasions we found populations consisting of several hundred individuals; even those groups did not exceed 500 plants in number. Population numbers widely fluctuate as a result of the biennial life cycle and preference for frequently disturbed habitats such as rocky cliffs, mudflows, and cascade pebbles. The number of *E. croceum* populations is declining due to habitat destruction and uncontrolled harvesting of flowering plants for ornamental purposes. To protect the species in the wild, all existing populations have to be examined and monitored, and conservation measures developed. This, in turn, requires detailed knowledge of morphology and biology of the species, as well as an assessment of the state of its populations. We have been studying the species since 2015; we have also collected material allowing us to study interpopulation genetic variability.

The novelty of our research lies in the discovery and subsequent study of the three new populations of *E. croceum*, one of which was found at an extremely low elevation of 1728 m a.s.l. Our results allow to estimate more precisely than before the elevational preferences of the species, which was earlier found only between 2000–2600 m a.s.l. For the first time, we collected data on the age structure, population density, morphological characteristics of vegetative and generative plants, and their variability.

Material and methods

The present study was carried out in the framework of the project 0497/GF4 “Assessment of the state of cenopopulations of some rare medicinal plants of the Trans-Ili



Fig. 1. *Erysimum croceum* inflorescence.

Alatau using botanical and molecular genetic methods” funded by the Committee on Science of the Ministry of Education and Science of the Republic of Kazakhstan.

The object of the study was *E. croceum* (Fig. 1). The most detailed description of the plant has been provided by Vasiliyeva (1961: 101). “Plants biennial, 9–65 cm tall. Stems simple or occasionally branched, straight, pubescent with bifid hairs, edges sharp. Leaves 2–5 (8) cm long, 0.5–1.5 (2) mm wide, lanceolate or oblong-lanceolate; margins oblong-crenate, finely toothed or almost entire; basal leaves petiolate, upper sessile, with an indumentum of stellate hairs (mixed with bifid hairs along the veins). Flowers on pedicels up to 6 mm long. Sepals 6–7.5 mm long, on the dorsum pubescent with mixed hairs. Petals reddish orange, yellowish when dry, 9–13.5 mm long, egg-oblong or nearly round, 8–9 mm wide. Fruit-stalks up to 9 mm long. Siliques straight, tetrahedral, 3.5–8.5 cm long, 1.25 mm wide, with bilobate beaks 2 mm

long, gray with stellate hairs. Seeds oblong, ca. 2 mm long”.

In the central part of the Trans-Ili Alatau on the territory of the Ile-Alatau State National Park, we found and examined three new populations of *E. croceum* (Fig. 2). The first population (further in the text referred to as Population 1) was found on the south-east and north-west facing slopes of the Small Almaty Gorge at an altitude of 2413–2597 m a.s.l., on both banks of the river Malaya Almatinka (N 43° 06.316', E 077° 04.184'). Because the first description of *E. croceum* was made by M.G. Popov in this gorge, it is recognized as a *locus classicus* for the species. The second population (Population 2) was found on the north-facing slope of the Great Almaty Gorge at an altitude of 2236 m a.s.l., on the left bank of the river Kumbelsu (N 43° 04.790', E 076° 59.512'). The third population (Population 3) was found on the south-west facing slope of the Issyk Gorge at an altitude of 1728 m a.s.l., on the right bank of the river Issyk (N 43° 15.731', E 077° 29.522').



Fig. 2. The map of the study area with the locations of the three studied populations of *Erysimum croceum* marked by the red circles.

Wherever possible, at the sites of the populations of *E. croceum*, we established 20 sampling plots with a size 1×1 m along each transect. In each sampling plot, we counted the number of individuals of the studied species of the same age group and measured biometric characteristics. When the number of *E. croceum* plants was low, we counted and measured all individuals of the species found on the site. The mean weight of 1000 seeds was obtained by averaging the results of the three measurements. All statistical analyses were carried out in Microsoft Excel 2007.

Results

The analysis of the age structure revealed that the ratio of the virginal and generative individuals varied from one population to another (Fig. 3).

Population 1 had the largest number of generative individuals, while Population 2 had the largest number of virginal individuals; in Population 3, the numbers of generative and virginal individuals were nearly the same. This could be due to the fact that *E. croceum* is a biennial plant; according to [Kokoreva et al. \(2010\)](#), the numbers of individuals of different age can fluctuate markedly between years.

It was difficult to calculate population density, because in some places we were able to establish sampling plots of a size 1 m^2 , and in other places, we had to count all available individuals. The average population density across sampling plots was 7.3 ± 0.6 and 8.2 ± 1.2 individuals per m^2 in Populations 1 and 3 respectively.

In all populations, we measured morphological parameters of *E. croceum*. The biometric characteristics of the virginal and generative individuals are provided in [Tab. 1](#)

and Tab. 2. We found that the morphometric parameters of plants from different populations fluctuated considerably (Tab. 1).

In virginal individuals, the maximum height was recorded in Population 3, and the minimum – in Population 2. The number of leaves in the rosette, the length and width of the leaf were almost the same in individuals from Populations 1 and 3; the values of the same parameters were the lowest in Population 2.

In generative individuals, the maximum height was recorded in Population 2, and the minimum, in Population 3. The largest number of stem leaves was recorded in Population 3, and the smallest, in Population 2. The largest leaf size (length and width) was recorded in Population 2, while in the other two populations the average leaf size was nearly the same (Tab. 2).

When checked against the scale of variation in characteristics developed by Mamaev (1975), the level of variability of morphometric parameters observed in our study could be described as high or very high in both virginal and generative individuals.

In all three populations we studied morphometric characteristics of the generative organs; we calculated the number of flowers and

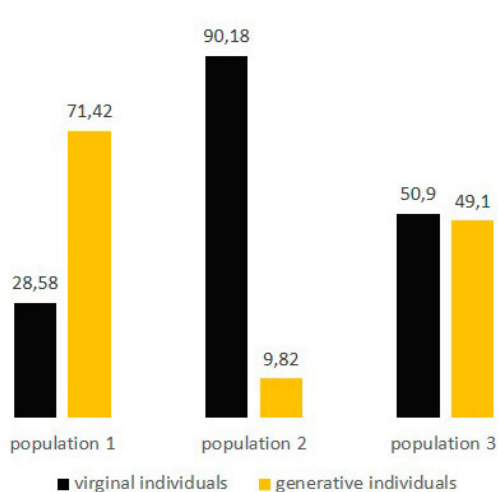


Fig. 3. The ratio of generative and virginal individuals in the three studied populations, %.

seeds per individual, and measured the length of seed pods (Tab. 3). Unfortunately, we failed to collect seeds in Population 2.

The maximum number of flowers per individual was recorded in Population 3; this can be due to a frequently occurring branching inflorescence. The individuals from Population 2

Table 1. Morphometric parameters of virginal individuals. **M** – mean value; **m** – standard error of the mean; **C_v** – coefficient of variation.

Population	Height, cm		Number of leaves		Leaf size, cm			
					Length		Width	
	M±m	C _v , %	M±m	C _v , %	M±m	C _v , %	M±m	C _v , %
1	3.6±0.4	66.6	13.6±0.9	41.8	3.0±0.1	63.7	0.6±0.02	41.6
2	1.9±0.3	143.9	6.3±0.3	52.6	1.8±0.1	104.8	0.5±0.01	51.5
3	5.8±0.4	51.7	13.9±1.2	59.9	3.0±0.1	56.1	0.6±0.01	53.7

Table 2. Morphometric parameters of generative individuals. **M** – mean value; **m** – standard error of the mean; **C_v** – coefficient of variation.

Population	Height, cm		Number of stem leaves		Leaf size, cm			
					Length		Width	
	M±m	C _v , %	M±m	C _v , %	M±m	C _v , %	M±m	C _v , %
1	45.9±1.8	26.2	22.0±1.4	42.6	3.8±0.1	28.6	0.9±0.02	32.8
2	49.8±3.6	24.02	15.3±1.0	21.5	6.2±0.2	19.2	1.4±0.05	25.9
3	36.5±2.6	46.1	22.8±1.8	49.8	4.3±0.1	44.5	0.8±0.02	51.0

Table 3. Morphometric characteristics of reproductive organs. **M** – mean value; **m** – standard error of the mean; **C_v** – coefficient of variation.

Population	Number of				Length of a seed pod, cm	
	flowers per individual		seeds per individual		M±m	C _v , %
	M±m	C _v , %	M±m	C _v , %		
1	13.8±1.1	51.6	610.8±97.3	90.1	7.5±0.1	11.4
2	11.5±1.5	43.9	na	na	na	na
3	37.4±7.1	119.3	664.7±83.0	48.4	6.2±0.2	22.8

Table 4. Morphometric parameters of seeds from Population 1 and 3. **M** – mean value; **m** – standard error of the mean; **C_v** – coefficient of variation.

Population	Size, mm				Weight of 1000 seeds, g	
	Length		Width		M±m	C _v , %
	M±m	C _v , %	M±m	C _v , %		
1	1.96±0.02	8.0	0.96±0.01	8.4	0.58±0.01	1.0
3	1.95±0.02	5.1	0.86±0.02	11.1	0.59±0.01	4.9

had the fewest flowers. The maximum seed pod length was recorded in Population 1. The level of variability in the number of flowers and seeds was very high in both populations, and in the fruit length, medium in Population 1 and high in Population 3. The size and weight of 1000 seeds were nearly the same in Populations 1 and 3 (Tab. 4). The level of variability in the size and weight of seeds was low or very low. Consequently, these characteristics were the least labile.

Conclusions

The results of our study support the earlier evidence of a low population size in *E. croceum* (Kokoreva et al. 2010; Ivashchenko 2012). We were able to calculate the average population density only in Populations 1 and 3; the difference between these populations was very small. The difference between the ratios of virginal and generative plants in each studied population was very large; this can be explained by a biennial life cycle. The morphometric parameters of virginal and generative individuals were very labile and

had a high or very high level of variability. The large number of flowers in Population 3 was due to a high number of the branching inflorescence; this characteristic also had a high level of variability. The length of seed pods in Population 1 was only slightly different from that in Population 3. The level of variability of this characteristic ranged from low (Population 1) to medium (Population 3). Among all studied characteristics, the seed size and weight of 1000 seeds were the least variable; i.e. these characteristics of *E. croceum* were the most stable.

Our study has contributed to earlier morphological descriptions of the species (Bush 1939; Vasiliyeva 1961). According to the results of our study, the stem length is 85–90 cm (against 65 cm stated by the earlier studies); the leaf length is 9–11 cm (against 5–8 cm); the leaf width is 2.1–2.5 cm (against 1.5–2.0 cm); and the length of seed pods is 1.5–9.0 cm (against 3.5–8.5 cm). We have also verified several characteristics of seeds: the seed length is 1.5–2.2 mm; the seed width is 0.7–1.1 mm; and the weight of 1000 seeds is 0.57–0.62 g.

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RESEARCH ARTICLE

Morphological and anatomical characteristics of *Phragmites australis* from Dnipro channel

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Abstract

Field and experimental studies of morphological and anatomical leaf parameters of *Phragmites australis* showed the structural adaptation of these plants to growth under different water supply regimes. The anatomical structure of the leaves of common reed, which grew in shallow water and dry land in the Venice channel of the Dnipro river in Kyiv, was studied with the light microscopy. The obtained results suggest that the anatomical structure and type of mesophyll cells are stable parameters for this species, while the size both of the leaf blade and its cells can be considered as adaptive and labile features varying depending on the conditions of water supply.

Keywords: *Phragmites australis*, leaf, morphology, anatomy

Вступ

Phragmites australis (Cav.) Trin. ex Steud. – геліофіт, багаторічна рослина-космополіт, яка зростає у різних екологічних умовах (Brix 1999; Clevering & Lissner 1999; Packer *et al.* 2017). Відомо, що зміна морфо-фізіологічних параметрів, включаючи розміри органів, елементів флоєми і ксилеми, швидкість асиміляції CO₂, активність RuBisCO, продиховий індекс тощо, часто залежать від умов зростання (Kühl *et al.* 1999; Lessmann

et al. 2001; Hansen *et al.* 2007). Так китайські дослідники (Chen *et al.* 2013), вивчаючи два екотипи *Ph. australis*, один з яких зростає на березі озера Qinshan, а другий – у горах Zaigong на суходолі, показали відмінності у деяких фізіологічних та анатомічних ознаках. Зокрема, вони виявили, що в рослин, які зростали у горах, був знижений майже удвічі вміст розчинних цукрів і у 1,3 рази – вміст проліну; а також зменшена у 1,2 рази щільність продихів, у 1,35 рази – товщина листків і в 1,8 рази – діаметр ксилеми у

порівнянні з рослинами, що зростали в озері. Автори вважають, що відповідні зміни у вмісті цукрів та проліну опосередковані оптимізацією метаболічних компонентів і енергетичними запасами за умов різного водопостачання, а зниження щільності продихів автори пов'язують із мінімізацією втрат води; зниження діаметра судин та збільшення щільності судин – із максимальним поглинанням та оптимізацією провідності води. Подібні думки були висловлені й у більш ранніх роботах інших дослідників (Lu *et al.* 1994; Sobrado 2007). Хоча є дані, що мінеральний склад ґрунту, особливо за умов посухи та його засолення, суттєво можуть впливати на ріст і структурно-функціональні ознаки рослин (Packer *et al.* 2017). Ми також дотримуємося гіпотези (Chen *et al.* 2013), що вода є одним з основних чинників, що забезпечує ріст, розвиток та продуктивність *Ph. australis*. Тому метою нашого дослідження було проаналізувати морфологію та анатомію листків очерету, що зростає за різних умов водозабезпечення в зоні м. Києва.

Матеріали і методи досліджень

Об'єктом дослідження були листки *Ph. australis*, який зростає на глибині від 10 до 50 см у Венеціанській протоці лівого берегу Дніпра у зоні м. Києва та на суходолі (піщаному ґрунті) на віддалі 7–10 метрів від берега (Рис. 1 А). Матеріал збирали на початку червня у фазі вегетативного росту. Фіксацію матеріалу для цитологічних досліджень проводили в польових умовах. Вирізки з найширшої частини листових пластинок, між другою та сьомою жилками від краю листка, відбирали для експериментів. Для дослідження брали кожний третій листок, що закінчив ріст розтягом із чотирьох водних і чотирьох суходільних рослин. Зразки фіксували для світлової мікроскопії сумішшю 3% глютарового альдегіду й 3% параформальдегіду (1:1 за об'ємом) на 1М фосфатному буфері з рН 7,2 протягом 24 годин, після чого промивали буфером, зневоднювали в розчинах спиртів зростаючої

концентрації й заливали в суміш епоксидних смол епону і аралдиту за загальноприйнятою методикою. Зрізи товщиною 1–2 мкм робили на ультрамікромомі RMC MT-XL (США), фарбували водним розчином 0,1% толуїдинового синього і вивчали під мікроскопом NF (Carl Zeiss, Germany). Для визначення лінійних розмірів листків використовували по три листки з восьми рослин. Лінійні розміри клітин визначали у 30–40 клітинах епідермісу та у 50–60 клітинах мезофілу з кожного зразка. Для визначення відносного вмісту води в листках використовували стандартний біохімічний метод (Yermakov 1982) з висушуванням зразків у термостаті при температурі 95°C до незмінної ваги. Отримані цитологічні та біохімічні дані обробляли статистично, використовуючи програму Microsoft Excel 2007.

Результати та їх обговорення

Водні рослини *Phragmites australis*

Висота водних рослин очерету у фазі вегетативного росту коливалася від 90 до 130 см. Стебло пряме, гладке, тригранне. Листки сидячі, листовка пластинка шиловидної форми, тонка та цупка. Адаксіальна поверхня листка плоска, в той час як абаксіальна, у зоні жилок – випукло-хвиляста. Листки очерету, що зростає на мілководді, були коротші та вужчі в порівнянні з листками рослин, що зростає на суходолі (Рис. 1 Б, В). Середній розмір листків очерету, який ріс у воді, становив $51 \pm 7 \times 1,3 \pm 0,7$ см. Вміст води у листках становив $62,8 \pm 0,5$ %. Дослідження структури поперечних зрізів листових пластинок показало, що верхня поверхня майже рівна, нижня – хвиляста (Рис. 2 А–В); на нижній поверхні виділяються впадини і виступи. Структура листка ізолатеральна. Товщина листової пластинки в зоні впадини коливається від 200 до 380 мкм, у зоні гребенів, де розміщувалися провідні пучки – від 300 до 600 мкм. На обох поверхнях листка виявлені продихи. Основні клітини

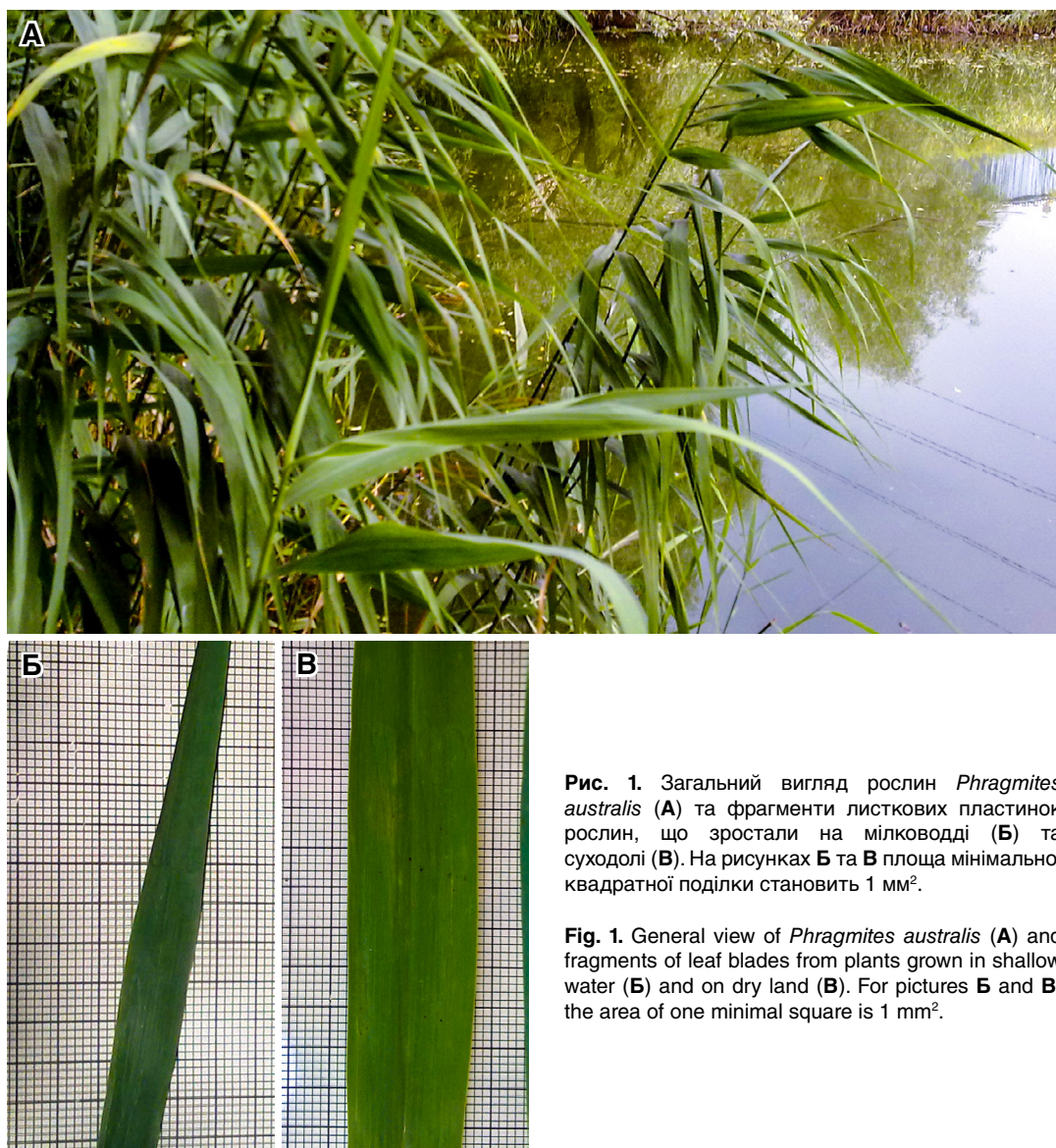


Рис. 1. Загальний вигляд рослин *Phragmites australis* (А) та фрагменти листових пластинок рослин, що зростали на мілководді (Б) та суходолі (В). На рисунках Б та В площа мінімальної квадратної поділки становить 1 мм².

Fig. 1. General view of *Phragmites australis* (A) and fragments of leaf blades from plants grown in shallow water (B) and on dry land (B). For pictures B and B, the area of one minimal square is 1 mm².

адаксіального й абаксіального епідермісу характеризуються товстими клітинними оболонками. Для абаксіального епідермісу характерна присутність у зоні впадни 6–7 великих пухирцевоподібних клітин; це моторні клітини, що допомагають скручуватися листку за несприятливих умов. Моторні клітини на зрізі майже прозорі, звужені зі сторони периклінальної стінки й розширені – у зоні контакту з мезофілом; ширина таких клітин коливається від 50 до

150 мкм, висота – від 100 до 150 мкм. Кількість шарів мезофілу в зоні впадни коливалась від чотирьох до шести, тоді як у зоні провідних пучків – 11–12 шарів. На поперечних зрізах форма епідермальних клітин овальна, клітин мезофілу – майже округла, зрідка видовжена в зоні провідних пучків. Середній розмір клітин верхнього епідермісу становив (висота × ширина) – $33 \pm 1,4 \times 63 \pm 2,1$ мкм, нижнього епідермісу – $38 \pm 2,1 \times 52 \pm 1,7$ мкм, клітин мезофілу – $56 \pm 4,3 \times 59 \pm 2,7$ мкм.

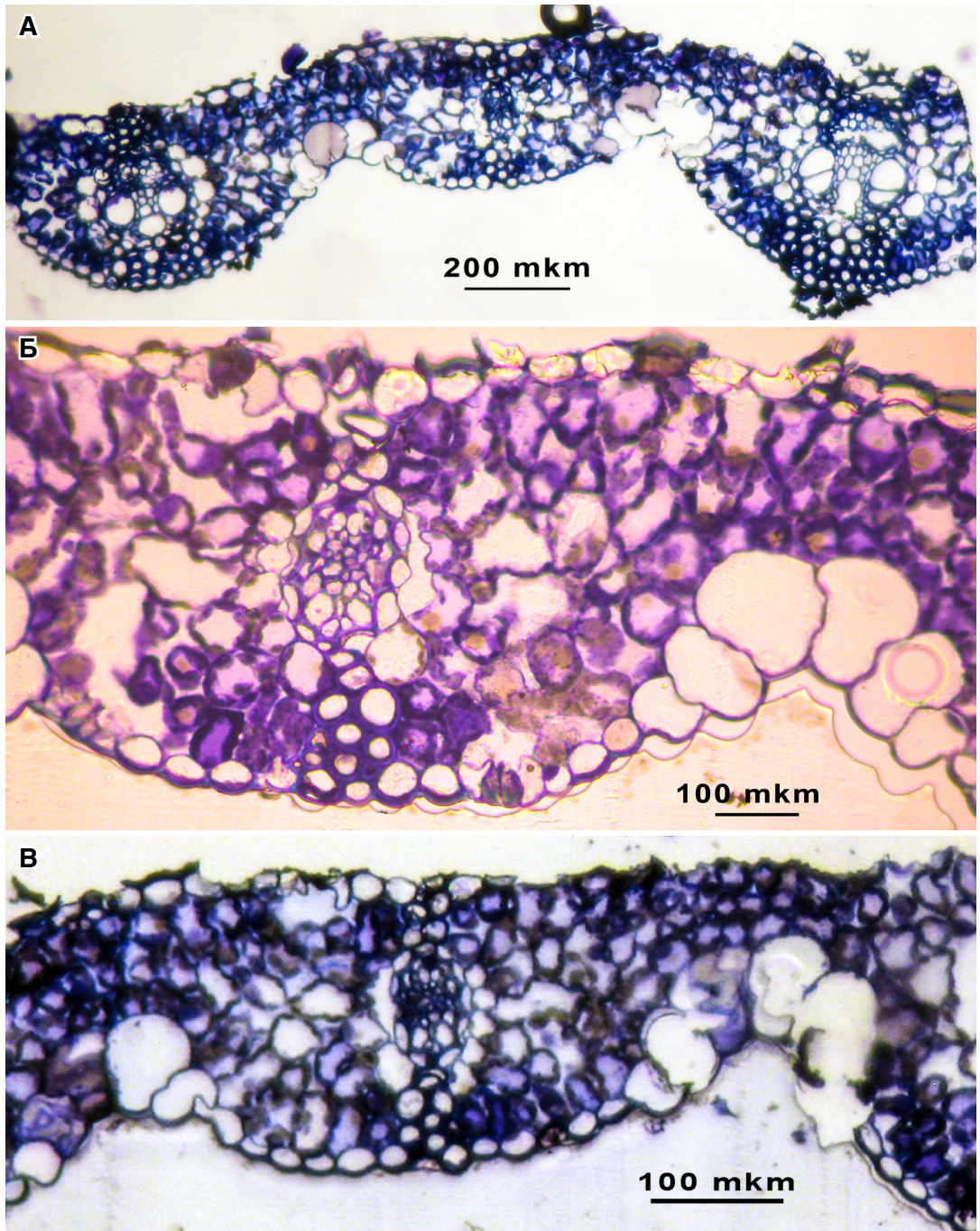


Рис. 2. Поперечні зрізи листових пластинок *Phragmites australis*: А–В – листок рослини, що зростала на мілководді; Г–Е – листок рослини, що зростала на суходолі.

Fig. 2. Cross sections of *Phragmites australis* leaf blades: А–В – leaf of a plant grown in shallow water; Г–Е – leaf of a plant grown on dry land.

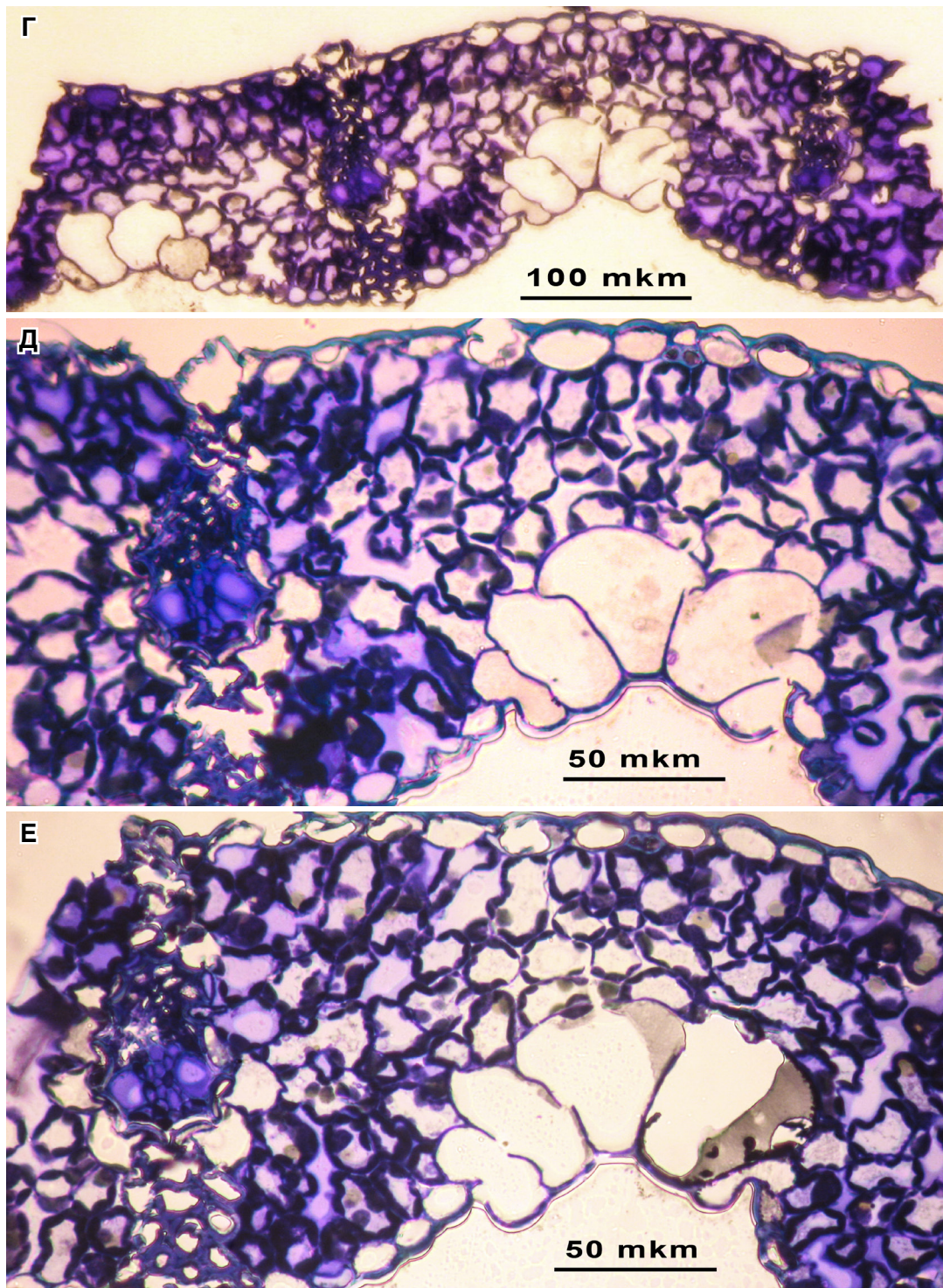


Рис. 2. Продовження.

Fig. 2. Continued.

Середня кількість хлоропластів на зрізі однієї клітини мезофілу становила $5,82 \pm 0,40$.

Суходільні рослини *Phragmites australis*

Висота суходільних рослин очерету коливалася від 120 до 165 см. Анатомо-морфологічні ознаки суходільних рослин очерету подібні до таких, що зростали у воді; будова пластинки – ізолатеральна. Відмінності проявлялися у збільшенні довжини та ширини листкової пластинки (Рис. 1 В), зменшенні товщини пластинки та розмірів клітин, зниженні кількості моторних клітин. Середній розмір листкової пластинки становив $71 \pm 8,9 \times 3 \pm 0,2$ см. Вміст води у листках суходільного очерету становив $57,2 \pm 0,7\%$. На поперечних зрізах листків (Рис. 2 Г–Е) верхня поверхня пластинки майже рівна, нижня – хвиляста з формуванням гребенів у зонах провідних пучків. У зоні впадіння міститься чотири шари мезофілу, у зоні гребенів, біля провідних пучків – від восьми до десяти шарів, товщина листків у зоні ложбинки становила 110–120 мкм, а в зоні гребенів коливалася від 150 до 170 мкм. Ширина гребенів в основі коливається від 200 до 230 мкм, висота становить близько 100 мкм. Продихи присутні на обох поверхнях листкової пластинки. Форма клітин епідермісу та мезофілу подібна до такої у водних рослин. Середній розмір клітин (висота \times ширина) верхнього епідермісу становив $11 \pm 1,7 \times 19 \pm 1,3$ мкм, нижнього епідермісу – $11 \pm 0,7 \times 14 \pm 1,1$ мкм, клітин мезофілу – $20 \pm 1,7 \times 17 \pm 0,9$ мкм. Середня кількість хлоропластів на зрізі клітини мезофілу становила $6,3 \pm 0,28$.

Таким чином, показано, що у фазі вегетативного росту листки рослин *Ph. australis*, які зростали у водно-береговій зоні протоки р. Дніпра в м. Києві за розмірами відрізнялися від листків суходільних рослин цього виду. Більші розміри листкових пластинок рослин, що зростали на певній віддалі від берега, можуть бути зумовлені збільшенням швидкості поділу, більш раннім їхнім розвитком, або ж більш оптимальними

умовами росту. Ми припускаємо наявність суттєвих відмінностей у швидкості проходження клітинного циклу в листках досліджуваних нами рослин, оскільки саме цей показник є досить чутливим до вмісту води в рослині та у ґрунті. Відомо, що за умов незначної ґрунтової посухи ($-2,2$ МПа) у клітинах *Zea mays* L. спотерігали інгібування S-фази клітинного циклу (Setter & Flannigan 2001), тоді як за умов тривалого водного дефіциту відносна швидкість поділу клітин листків *Helianthus annuus* L. зменшувалася на 39% за рахунок блокування проходження клітинного циклу у фазах G_0 та G_1 (Granier & Tardie 1999). Деякі автори вважають, що інгібування поділу клітин у рослинних тканинах є наслідком пригнічення синтезу певних циклінів, які задіяні у регуляції фаз клітинного циклу, та інгібування експресії відповідних генів (Matthias & Herskowitz 1994).

Оскільки досліджені нами рослини водного очерету нормально росли й розвивалися протягом усього вегетативного періоду аж до формування колосків, ми можемо припустити, що ці рослини є стійкими до затоплення кореневої системи. З іншого боку, менші розміри клітин епідермісу та мезофілу листків суходільного очерету можуть бути наслідком зниження швидкості та/або ж часу росту клітин розтягом, яке обумовлене як дією фітогормонів, так і фізіологічною відповіддю на зниження водного балансу коренів, аналогічно, як це описано для інших видів (Jackson & Drew 1984; Voesenek et al. 2004). Очевидно, що коренева система водного очерету, характеризується частковою гіпоксією, яка властива деяким трав'янистим рослинам, зокрема *Paspalum dilatatum* Poir., що також може зростати у воді (Insausti et al. 2001).

Ми також припускаємо, що рослини водного й суходільного очерету, які ми досліджували, характеризуються різною плоідністю, яка, як відомо, впливає на морфологічні ознаки, зокрема, на розмір стебел і листків, на висоту міжвузлів та розмір клітин листків очерету.

За літературними даними, октоплоїдні, гексаплоїдні і декаплоїдні рослини очерету (*Pauca-Comanescu et al.* 1999) відрізняються саме за морфологічними показниками. Показано, що чим меша плоїдність, тим менший розмір клітин (*Hansen et al.* 2007). Ряд авторів вважає, що морфологічні зміни є незалежними від умов місцевості, а обумовлені лише відмінностями числа хромосом (*Björk* 1967; *Clevering & Lissner* 1999; *Rolletschek et al.* 1999; *Pauca-Comanescu et al.* 1999). Ці дослідники описують морфологічні відмінності, як між різними популяціями *Ph. australis*, так і між різними клонами в межах однієї й тієї ж популяції, незалежно від умов місцевості. Цьому виду властивий досить широкий діапазон плоїдності, проте найчастіше трапляються тетраплоїди ($2n=48$) та октоплоїди ($2n=96$) (*Clevering & Lissner* 1999). Пагони октоплоїдів, як правило, довші й товщі, а також мають більші листки, ніж у тетраплоїдів. Октоплоїдний очерет іноді називають “гігантським”, а тетраплоїдний – “звичайним” (*Hanganu et al.* 1999; *Pauca-Comanescu et al.* 1999). Зазвичай проявом поліплоїдії є збільшення розміру клітин, однак інколи результатом поліплоїдії може бути також і зменшення кількості поділу клітин під час розвитку (*Stebbins* 1971). Таким чином, питання щодо клітинних механізмів мінливості морфо-анатомічних показників очерету, що зростає в зоні м. Києва потребує подальшого дослідження.

Висновки

Дослідження анатомо-морфологічних ознак листків очерету, що зростає у воді та на суходолі у фазі вегетативного росту, показало, що анатомічна будова і тип мезофілу є стабільними ознаками для цього виду, тоді як лінійні розміри листкових пластинок та розміри клітин – пластичними ознаками, які змінюються в залежності від локального водозабезпечення.

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