

# First record of *Legousia hybrida* (Campanulaceae) in the Bulgarian flora

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**Abstract.** The genus *Legousia* (Campanulaceae) has been represented so far by two species in the Bulgarian flora: *L. speculum-veneris*, a relatively widespread taxon in the lowlands across the country, and *L. pentagonia*, reported only from the Eastern Rhodopi Mts floristic region. During the recent field surveys in the coastal area of NE Bulgaria, a third species from the genus has been found: *L. hybrida*. The latter is reported here for the first time for the Bulgarian flora. Herbaceous annual, it is well distinguished from the other two congeneric species by its smaller flowers and a corolla about half as long as the calyx lobes. *Legousia hybrida* has been recorded in the Black Sea Coast (Northern) floristic region. It grows in relatively open grassland communities and can be abundant locally. A genome size of  $1C = 1.47\text{--}1.49$  pg has been estimated for the species. The distribution, phenology and habitat preferences of the species are presented.

**Key words:** Bulgarian flora, C-value, genome size, *Legousia*, new records

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## Introduction

*Legousia* Durande is a small genus of herbaceous annuals represented by six native and one alien species in Europe (cf. Castroviejo & al. 2010; Tan & al. 2015). In Bulgaria, only two species of the genus have been reported so far: *L. speculum-veneris* (L.) Chaix, a relatively widespread taxon in the lowlands across the country, and *L. pentagonia* (L.) Druce, reported

only from the Eastern Rhodopi Mts floristic region (Ančev 2012). In late April 2015, during a field survey in the coastal area of NE Bulgaria, a third species from the genus has been found. It impressed with its strongly undulate leaves, small flowers and a corolla about half as long as the calyx lobes, and was identified as *L. hybrida* (L.) Delarbre.

The aim of the present article is to report *L. hybrida* for the first time for the Bulgarian flora.

## Material and methods

Plant material and photographs have been taken from the Black Sea Coast (*Northern*) floristic region. Several field trips were organized in 2015–2021, in order to study the distribution of the species and collect herbarium material and material for laboratory investigation. Herbarium specimens were deposited in the Herbarium (SOM) of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences in Sofia. Determination of specimens was based on the *Legousia* account in *Flora Europaea* (Tutin 1976). Morphological description rested on the collected herbarium specimens and was compared with relevant taxonomic sources (e.g., Tutin 1976; Damboldt 1978; Sales & Hedge 2001). Data about abundance of the subpopulations and accompanying species has been noted from the field.

Genome size was estimated by a flow cytometer CyFlow SL Green (PARTEC, Germany) equipped with a green (532 nm) solid-state laser. Plant material was treated by CyStain PI Absolute P extraction and staining kit (SYSMEX & PARTEC), following the protocol provided with the kit. *Pisum sativum* 'Kleine Rheinländerin' (1C = 1Cx = 4.38 pg, Greilhuber & al. 2007) was used as an internal standard. The samples were measured at a rate of 10–20 nuclei per second, with 5000 nuclei for each measurement; five replicates were made for each sample, and only runs with CV < 5% were taken into account.

## Results and discussion

*Legousia hybrida* (L.) Delarbre, Fl. Auvergne, ed. 2 (1800) 47 (Figs. 1, 2)

Herbaceous, shortly hispid annual, 5–20 cm. Stem simple or branched at base, erect or ascending. Leaves 10–15 × 4–6 mm, alternate, oblong to oblong-ovate, strongly undulate, the lowest shortly petiolate, the middle and upper sessile to somewhat subamplexicaule. Flowers few, sessile, gathered in small terminal clusters. Calyx-lobes clearly shorter than ovary at anthesis, lanceolate to lanceolate-ovate, acute, erect to suberect. Corolla about ½ as long as calyx-lobes, lobes



Fig. 1. *Legousia hybrida*, entire plant.



Fig. 2. *Legousia hybrida*, flower (photo V. Vladimirov).

2–3 mm, ovate-lanceolate, acute at apex, lilac. Ovary 10–12 mm, much longer than wide.

Flowering April – May, fruiting May – June.

### Key to *Legousia* species in Bulgaria

*Legousia hybrida* is easily distinguished from the other two congeneric Bulgarian species by smaller flowers and a corolla about half as long as the calyx lobes. The key below is based on relevant taxonomic sources (Tutin 1976; Ančev 2012; Wahlsteen & Tyler 2019), as well as on the comparison of the available herbarium material.

1. Calyx lobes clearly longer than corolla,  $\pm$  erect in fruit; corolla-lobes 2–3 mm ..... *L. hybrida*
- 1\*. Calyx lobes shorter or as long as corolla, erect-to-patent in fruit; corolla lobes at least 5 mm ..... 2
2. Corolla lobes 10–12 mm; capsule 10–15 mm, narrowed at apex; calyx lobes as long as or slightly shorter than ovary at anthesis .....  
.....*L. speculum-veneris*
- 2\*. Corolla lobes 15–18 mm; capsule 20–30 mm, not narrowed at apex; calyx lobes about 1/3 to 1/2 as long as ovary at anthesis .....*L. pentagonia*

**Genome size:** Two plants of *L. hybrida*, each from a different locality, have been studied. The estimated genome size is within the range  $1C = 1.47$  to  $1.49$

pg (Table 1). Apparently, this is the first published data about the genome size of *L. hybrida*. For comparison, the genome size of three specimens from *L. speculum-veneris* has been measured too. The obtained mean value was  $1C = 0.85$  pg (Table 1). Only one published record of the genome size of this species has been found, for the flora of Netherlands,  $1C = 1.05$  pg (Zonneveld 2019). The chromosome number of *L. speculum-veneris* has been reported as  $2n = 20$  both for the Bulgarian (Ančev 1975, 1976; Petrova & Vladimirov 2020: 298) and for the Netherlands floras (Zonneveld 2019). Taking this into consideration, the observed difference in the genome size within the species was significant. Apparently, a larger number of populations of the species should be examined simultaneously for chromosome number and genome size.

The chromosome number of *L. hybrida* has not been studied from a Bulgarian accession. Nevertheless, data in the literature from other countries indicated that the species has  $2n = 20$  (e.g. Tutin 1976; Zonneveld 2019). Considering this, if the two *Legousia* species have been compared, it can be seen that *L. hybrida* has a significantly higher genome size. This also suggests that it is desirable to study a larger number of populations of both species, both for genome size and chromosome number.

**Table 1.** Genome size of the studied Bulgarian accessions of *Legousia*

No.	Species/locality	Number of plants measured	1C min [pg]	1C max [pg]	1C mean [pg]
<b><i>Legousia hybrida</i></b>					
1.	Bulgaria, Black Sea Coast ( <i>Northern</i> ): NE of Tyulenovo village, Shabla Municipality, ca. 15 m, 43.50426°N, 28.58777°E, 01.05.2021, coll. V. Vladimirov	1	-	-	1.49
2.	Bulgaria, Black Sea Coast ( <i>Northern</i> ): CapeKaliakra, SE of Balgarevo village, Kavarna Municipality, ca. 70 m, 43.38437°N, 28.44906°E, 01.05.2021, coll. V. Vladimirov	1	-	-	1.47
<b><i>Legousia speculum-veneris</i></b>					
1.	Bulgaria, Tundzha Hilly Country: along the road to Melnitsa village, Elhovo Municipality, ca. 115 m, 42.05286°N, 26.57024°E, coll. V. Vladimirov	3	0.84	0.87	0.85

**Distribution in Bulgaria:** Black Sea Coast (*Northern*): **1)** steppe communities E of Kamen Bryag village, Kavarna Municipality, 20–25 m a.s.l., ca. 43.45396°N, 28.55758°E, 29.04.2015, coll. V. Vladimirov (SOM); **2)** steppe grasslands S of Tyulenovo village, Shabla Municipality, ca. 20 m, 43.48382°N, 28.57856°E, 29.04.2016, coll. V. Vladimirov (SOM); **3)** steppe communities NE of Tyulenovo village, Shabla Municipality, ca. 12 m, 43.50359°N, 28.58817°E, 29.04.2016, coll. V. Vladimirov (SOM); **4)** steppe grasslands S-SW of Tyulenovo village, Shabla Municipality, between the road from Kamen Bryag to Tyulenovo village and the Black Sea shore, ca. 25 m, 43.48442°N, 28.57409°E, 01.05.2021, V. Vladimirov obs.; **5)** steppe grasslands NE of Tyulenovo village, Shabla Municipality, ca. 15 m, 43.50426°N, 28.58777°E, 01.05.2021, several hundred flowering specimens, V. Vladimirov obs. (Fig. 3); **6)** Cape Kaliakra, SE of Balgarevo village, Kavarna Municipality, Ponto-Sarmatic steppes in close proximity to a wind-farm, ca. 70 m, 43.38437°N, 28.44906°E, 01.05.2021, V. Vladimirov obs. (Fig. 4).

**General distribution:** *Legousia hybrida* is distributed in NW and C Europe, the Mediterranean area, including N Africa, SW Asia and eastwards up to C Asia (Wahlsteen & Tyler 2019). The discovery of the species in Bulgaria is not unexpected, since it has been distributed in all countries adjacent to Bulgaria, except for the Republic of North Macedonia (cf. Castroviejo & al. 2010).

**Habitats and populations:** In Bulgaria, *L. hybrida* has been so far recorded in relatively open grassland communities and may be locally abundant. In most localities, the habitats belong to the Ponto-Sarmatic steppes, situated on flat terrains with more or less developed soil cover on limestone rock and occupied by natural grasslands or secondary pastures.

In locality 2 (see above under ‘Distribution in Bulgaria’), several dozen scattered flowering *Legousia* specimens were recorded on ca. 100–150 m<sup>2</sup>. The total vegetation cover was about 60%. The accompanying species were: *Alyssum* sp., *Artemisia pedemontana* (ca. 15% cover), *Anthemis* sp. (10%), *Scandix australis* (10%), *Achillea setacea*, *Adonis flammea*, *Ajuga chia*, *Carduus nutans*, *Carthamus lanatus*,



Fig. 3. *Legousia hybrida*, general habit (photo V. Vladimirov).

*Convolvulus cantabrica*, *Crepis sancta*, *Eryngium campestre*, *Erodium cicutarium*, *Euphorbia helioscopia*, *Linaria genistifolia*, *Muscari neglectum*, *Plantago lanceolata*, *Scherardia arvensis*, *Senecio vernalis*, and *Viola kitaibeliana*.

In locality 3, several hundreds of scattered flowering *Legousia* specimens were recorded. The total vegetation cover was about 60%. The accompanying species were: *Scandix australis* (25% cover), *Convolvulus cantabrica* (10%), *Crepis sancta* (5%), *Adonis flammea*, *Alyssum* sp., *Anthemis* sp., *Cerastium* sp., *Crepis foetida*, *Erodium cicutarium*, *Eryngium campestre*, *Lamium amplexicaule*, *Medicago minima*, *Scherardia arvensis*, and *Senecio vernalis*.



Fig. 4. Habitat of *Legousia hybrida*: Ponto-Sarmatic steppes on Cape Kaliakra near a wind farm (photo V. Vladimirov).



Fig. 5. In most Bulgarian localities, individuals of *L. hybrida* have small size, unbranched stems and few flowers (photo V. Vladimirov).

In locality 4, scores of hundreds of scattered flowering *Legousia* specimens were recorded. The accompanying species in close proximity were: *Adonis flammea*, *Buglossoides arvensis*, *Cerastium* sp., *Convolvulus cantabrica*, *Crepis sancta*, *Euphorbia helioscopia*, *Eryngium campestre*, *Medicago minima*, *Muscari neglectum*, *Poa bulbosa*, *Scandix australis*, *Scherardia arvensis*, *Taraxacum serotinum*, etc.

In locality 6, a few hundreds of flowering *Legousia* specimens were observed. The common species in close proximity were: *Achillea clypeolata*, *Adonis vernalis*, *Asphodeline lutea*, *Convolvulus cantabrica*, *Iris pumila*, and *Paeonia tenuifolia*.

**Conservation considerations:** Ponto-Sarmatic steppes and similar grassland habitats are relatively widespread along the Black Sea coast in the area between Cape Shabla in the north and Cape Kaliakra in the south. However, *L. hybrida* is rather rare and found only in a few places, where it forms local populations occupying relatively small areas (from a few square meters to a few hundred square meters) with scattered individuals. Many of the observed individuals have been small in size (about 5 cm), with unbranched stems and only few flowered (Fig. 5). Therefore, the species deserves to be evaluated according to the IUCN Red List criteria and possibly receive conservation status at national level.

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