

CONTRIBUTION TO THE KNOWLEDGE
OF *Pontechium maculatum* (BORAGINACEAE),
A SPECIES OF HIGH CONSERVATION CONCERN
IN THE BULGARIAN FLORA

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Abstract

Pontechium maculatum (Boraginaceae) is a species of high conservation concern in the Bulgarian flora. It is assigned a national IUCN category 'Vulnerable', legally protected according to the national Biological Diversity Act, and listed in Annex II of the Habitats Directive (Council Directive 92/43/EEC). Recent studies in one of the largest populations in the country revealed some new data about the species which are reported here. The population studied is situated in Mt Lozenska, Mt Sredna Gora (*Western*) floristic region. Relevant taxonomic literature states that *P. maculatum* is a biennial plant, however, our observations revealed the plants in Mt Lozenska are perennial (possibly short-lived). A great variation in the corolla-colour was observed, most plants having bluish to violet corolla and only a few – reddish to dark red. This can be explained by either natural variation in the population, not reported in the taxonomic literature so far, or by hybridisation with *Echium vulgare* which co-occurs in the same locality. Large fluctuation in the number of flowering plants

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in two consecutive years was recorded, with some 180 blooming specimens in 2019 and only about 25 in 2020. Studies in the genome size of this population revealed the plants have significantly higher DNA-content (1C-value) than plants from the typical populations of the species in Northeast Bulgaria floristic region, e.g. Kabiushka Mogila locality. Further biosystematics studies (chromosome number, genome size, genetic studies, etc.) are currently in progress to explain the observed phenomena within the Bulgarian populations of *P. maculatum*.

Key words: biological type, *Echium s.l.*, *Echium russicum*, genome size, rare species, species of conservation concern

Introduction. *Echium* L. is represented by four species in the Bulgarian flora – *E. italicum* L., *E. plantagineum* L., *E. russicum* J.F. Gmel., and *E. vulgare* L. [1]. Of these, *E. italicum* and *E. vulgare* are relatively widespread across the country, whereas *E. plantagineum* and *E. russicum* have more restricted distribution ranges. Therefore, the latter two species have been assigned national IUCN categories: *E. russicum* – VU [2,3] and *E. plantagineum* – NT [4]. Moreover, *E. russicum* is legally protected according to the national Biological Diversity Act and is included in Annex II of Council Directive 92/43/EEC (Habitats Directive), and thus, it is of high conservation concern.

A series of modern phylogenetic studies during the past decade aimed at revealing the relationships between previously recognised clades and the position of various unplaced genera in Boraginaceae [5–10] has led to a serious change in the modern taxonomic scheme of the family. HILGER and BÖHLE [11] undertook a study to reveal the phylogenetic relationships of *Echium*, *Lobostemon* and *Echiostachys* (Boraginaceae-Lithospermeae). The presence of a bicapitate stigma and plastid *trnL*_(UAA) intron, *trnL*_(UAA)–*trnF*_(GAA) spacer and nuclear (ITS1) DNA sequence analyses confirm an isolated position for *E. russicum* and recognition of a new genus named *Pontechium* Böhle & Hilger. The authors offered the following new combination, which is also followed in the present work – *Pontechium maculatum* (L.) Böhle & Hilger.

As a species of high conservation concern, *P. maculatum* has attracted the attention of Bulgarian botanists during the past few years. In two consecutive years, 2019–2020, a field study was conducted in one of the largest populations of the species, located in Mt Lozenska. The aim was to establish the size and population state, as well as to identify threatening factors.

At the first visit, we noticed that the population in Mt Lozenska differs in some morphological features from the typical representatives of *P. maculatum* which encouraged us to undertake further studies. The aim of the present article is to report some new data about this species which is of high conservation concern in the Bulgarian flora.

Material and methods. Herbarium material and live plants were collected from Mt Lozenska for further morphological and biosystematic studies. For com-

parison, material from typical populations of *P. maculatum* from Northeast Bulgaria floristic region was collected as well. The *Echium* collection in the Herbarium of the Institute of Biodiversity and Ecosystem Research (SOM) was used for a detailed morphological study. Morphological data from the studied specimens were compared with the data in relevant literature (e.g. [1,12]). Data about the size and state of the studied populations was noted in the field.

Nuclear DNA-content of typical *P. maculatum* plants from Northeast Bulgaria (Kabiyushka Mogila near Konyovets village, Shumen Municipality, and Tepichkite locality near Nevsha village, Vetrino Municipality) and of plants from Mt Lozenska were studied by flow cytometry. A small piece of fresh leaf from the target plant was co-chopped with a small piece of leaf of an internal standard (species with already known genome size) with a sharp razorblade using CyStain PI Absolute P reagent kit (Sysmex Partec GmbH, Germany) strictly following the protocol provided by the manufacturer. *Pisum sativum* 'Kleine Rheinländerin' (1C = 4.38 pg, [13]) and *Solanum pseudocapsicum* (1C = 1.295 pg, [14]) were used as internal standards. Two to four plants from each locality were measured.

The following localities have been studied: Mt Sredna Gora (*Western*) floristic region, Mt Lozenska, by a dirt road and open places among shrubs near an abandoned concrete military construction, ca. 1100 m a.s.l., 10.06.2019, coll. V. Vladimirov, S. Bancheva, D. Ivanova & A. Lambevsk-Hristova; loc. *ibid.*, 21.07.2020, V. Vladimirov, S. Bancheva & D. Ivanova, *obs.*; Mt Lozenska, open grassland and by a dirt road in Vlakovete locality below Malka Lalina Mogila peak, ca. 1050 m a.s.l., 10.06.2019, V. Vladimirov, S. Bancheva, D. Ivanova & A. Lambevsk-Hristova, *obs.*; Mt Lozenska, open place by a dirt road close to a picnic area near Mateynata Cheshma (fountain), ca. 960 m a.s.l., 17.07.2019, V. Vladimirov, S. Bancheva & D. Ivanova, *obs.*

Results and discussion. *Pontechium maculatum* (= *Echium russicum*) is a (?) biennial plant with linear-lanceolate leaves (the basal ones pointing more or less upward), covered by soft appressed hairs; narrow spike-like inflorescence up to a half of the stem, with dense curved semi-umbels and dark red corolla. In Bulgaria, the species is locally distributed in most of the floristic regions in the country up to 1200 m a.s.l. The species inhabits dry grasslands in lowlands and low mountain slopes, often in scrublands. So far, it has not been recorded only in the following floristic regions: Mt Belasitsa, Mt Slavyanka, Pirin Mts, Rila Mts [1] and Rhodopi Mts (*Eastern*) [3].

The species was more widespread in the past. Its distribution map in the Red Data Book shows that at least 10 out of 54 localities (18.5%) have become extinct [3]. Among the most important threatening factors were mentioned: establishment of *Pinus* spp. plantations in some localities of the species, enlargement of the settlements and overexploitation of the pastures before the 1990s, whereas, after 1990 abandonment of the pastures and the natural expansion of the shrubs and trees in the habitats of the species have become the major threatening factors [3].

PETROVA [3] mentioned that one of the largest populations of *P. maculatum*, with 100–250 specimens, is situated in Mt Lozenska. During the field work in 2019–2020 to study this population, we noticed some new and interesting facts which are reported in the present article.

The literature sources (e.g. [1, 11, 12, 15–18]) state that *P. maculatum* is a biennial plant. Our observations from Mt Lozenska show that most of the plants are in fact perennial (possibly – short-lived perennials). Careful digging around plants which flowered in the previous year demonstrates that new rosettes are formed in the current year. Most often, only a single new rosette at the base of each specimen is formed (Fig. 1), but in some cases up to three rosettes were observed on more vigorous plants. Some of these leaf-rosettes are rather large and apparently will flower in the next year, i.e. the observed plants are not monocarpic either.



Fig. 1. New leaf-rosette formed on a specimen of *P. maculatum* flowered in the previous year



Fig. 2. *Pontechium maculatum*, red-flowered specimens in Kabiushka Mogila locality, Northeast Bulgaria

Relevant taxonomic literature (e.g. [1, 11, 12, 15–18]) report that the corolla of *P. maculatum* is dark red (Fig. 2). Our visit in Mt Lozenska in 2019 revealed that most plants had bluish to violet corollas (Fig. 3). We were unable to find previous reports of such corolla-colour variation. In fact, we found out that some of the observed plants approached another close taxon, *Echium vulgare*, in the colour and partly in the shape of the corolla (wider corolla in the upper part, with the upper two corolla-lobes clearly longer than lower ones and about 3 mm long). Basically, two hypotheses can explain this phenomenon: 1) normal variation within this population (within *P. maculatum*) due to some specific ecological factors and/or genetic mutations; 2) hybridisation with *Echium vulgare* and consequent introgression events, which have led to ‘transition’ of some characters, e.g. colour and shape of corollas, from *E. vulgare* to *P. maculatum*. In fact, plants of *Echium vulgare* have been observed in the same locality. Both hypotheses require some further studies which have already been undertaken. The second hypothesis is especially important from a conservation point of view. If a natural hybridisation occurred in this population in the past, this has led to disappearance of the typical *P. maculatum* and its replacement by presumable hybrid/introgressive specimens. Such an interspecific (and intergeneric) hybridisation may become the main threatening factor for the extinction of *P. maculatum* in most of its localities especially in the western and southern parts of the country. This phenomenon has been supported by changes in the land use since the political and economic changes



Fig. 3. *Pontechium maculatum*, bluish- and violet-flowered specimens in Mt Lozenska, Sredna Gora floristic region

in the country in 1989, especially abandonment of the pastures, undergrazing or cessation of grazing. These factors lead to strong changes of the habitats of the species in many parts of the country, especially overgrowing with shrubs, trees, and more competitive grasses, closure of the vegetation cover and lack of open and disturbed ground suitable for germination of the seeds of *P. maculatum*. Moreover, climate change, especially unusual weather and extreme weather events, may cause longer overlap of the flowering periods of both parental species, and thus, increase the chance for hybridisation events. Typically, *P. maculatum* blooms a

bit earlier – in May-June, whereas *E. vulgare* blooms in June-July. Observations in Mt Lozenska, both in 2019 and 2020, showed that the flowering times of both species overlap, despite the fact that *P. maculatum* starts flowering earlier. In mid-July, *P. maculatum* is at the end of flowering and formation of young seeds, whereas *E. vulgare* is in full-flowering. So far, no hybrids have been reported within *Echium* in Bulgaria (cf. [1,19]).

In 2019, the total population of *P. maculatum* in Mt Lozenska was estimated to ca. 580 individuals of which about 180 were generative and about 400 – vegetative ones. In 2020, the number of flowering plants was significantly smaller – only about 25 plants. Apparently, large fluctuations in the number of flowering plants are observed in the different years.

Initial studies of the genome size of *P. maculatum* showed that the plants from Mt Lozenska have significantly higher DNA-content (1C-value) than the typical plants of *P. maculatum* from Northeast Bulgaria floristic region. The following results were obtained: for *P. maculatum* from Mt Lozenska 1C = 4.41–4.69 pg (CV = 0.19–0.27%), for plants from Kabiyushka Mogila locality 1C = 2.90–3.05 pg (CV = 0.11–0.18%), and for plants from Tepichkite locality 1C = 2.97–3.19 pg (CV = 0.20–0.32%).

Further biosystematics studies (chromosome number, genome size, genetic studies, etc.) are currently in progress to test the two hypotheses mentioned above and to explain the observed phenomena within the Bulgarian populations of *P. maculatum*.

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