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***IN-SITU AND EX-SITU CONSERVATION OF PONTechium
MACULATUM (L.) BÖHLE ET HILGER (BORAGINACEAE) IN
REPUBLIC OF MOLDOVA***

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ABSTRACT

This study presents the results of the *in-situ* and *ex-situ* activities aimed at the effective protection of *Pontechium maculatum* (L.) Böhle et Hilger (= *Echium russicum* J. F. Gmelin) populations, the vascular plant species of community interest growing in the spontaneous flora of the Republic of Moldova, but which is in danger of extinction. The habitat of the species *Pontechium maculatum* includes xerothermic meadows, petrified slopes with calcareous substrate but also thermophilic forest glades with downy oak. The decrease in the number of populations and the disappearance from the natural sites is most likely caused by the degradation of specific habitats by changing the structure of xerothermal meadows: increasing their density, forming a compact layer of dead plants, preventing the development of seedlings, but also the excessive growth of shrubs in the glades of downy oak forests. Other threats, which contributed to the disappearance of many populations of *Pontechium maculatum*, are the afforestation of grasslands and steppe slopes and the overgrazing of specific habitats. We estimate that the population in the "Bugeac multifunctional management area", in the south of the republic, has high potential for conservation actions, this being the "mother population" from which seeds were collected at the beginning of the project and seedlings were obtained, with the aim of later repatriation into the natural habitats. The main activities carried out within the project were the identification of two natural habitats to which plants, obtained in *ex-situ* conditions, were transplanted. At the same time, an *ex-situ* plant collection was created within the "Alexandru Ciubotaru" National Botanical Garden (Institute).

Keywords: *Pontechium maculatum*, rare vascular plant habitat, conservation, Republic of Moldova.

INTRODUCTION

Pontechium maculatum, one of the plant species of community interest occurring in dry grasslands and steppes of south-eastern and central Europe, including habitats such as (*62C0) – Ponto-Sarmatic steppes, and (*6240) – Sub-pannonian steppe

grasslands (Interpretation ... 2013), and is included in the Habitats Directive, Annex IIb, IVb (Convention ... 1979). Its distribution range includes Central and South-Eastern Europe, the Balkan Peninsula and Western Siberia. Among European countries, this species grows spontaneously in Bulgaria, the Czech Republic, Hungary, Poland, Romania, Slovakia, Austria, Serbia, Bosnia and Herzegovina and further to the east – in Russia and Turkey (Figure 1) (POWO). In most of these countries, it is a rare species included in the national red lists, and in some countries, it is even included in the Red Books, with different category of threat, such as Critically Endangered (CR) in the Czech Republic (Holub and Procházka, 2000) and in Poland (Chmielewski *et al.*, 2014), Endangered (EN) in Slovakia (Turis *et al.*, 2014) and Vulnerable (VU) in Bulgaria (Petrova and Vladimirov, 2009).



Figure 1. The distribution range of the species *Pontechium maculatum*

The decrease in the number of populations and the disappearance from natural sites is most likely caused by the degradation of specific habitats by changing the structure of xerothermal grasslands: the increase of their density, the formation of a compact layer of dead plants preventing the development of seedlings, but also the excessive growth of shrubs in the glades of downy oak forests. Other threats, which have contributed to the disappearance of many populations of *P. maculatum*, are afforestation of grasslands and steppe slopes and overgrazing in specific habitats. The biological peculiarities and specific ecological requirements of the species, together with the existing threats to the populations and their habitats, determined the need to take prompt measures to protect it. Thus, in the framework of the project “Research and conservation of vascular flora and macromycobiota of the Republic of Moldova”, we intend to achieve effective *in situ* and *ex-situ* protection of the species *P. maculatum* by creating new populations in natural habitats and/or by repatriating it to previously known growing sites.

MATERIAL AND METHODS

The present study was carried out in the period of 2020-2023 and based on the *P. maculatum* population from the "Bugeac multifunctional management area", a rare species for the territory of the Republic of Moldova. The seeds collected from natural habitat and germinated in Petri dishes without prior processing. After germination they were transferred to a chernozem (black soil) mixed with peat in a ratio of 3 : 1, in seedling trays for a month, after which they were taken outside the glasshouse, for growth and acclimatization. The exact location of the sites was determined using Google Maps. Population parameters were established on site according to accepted methods (Akeroyd and Jackson, 1995; Godefroid *et al.*, 2011; Gorbunov *et al.*, 2008), and the detailed description is based on the live plants collected but also on the basis of the literature data (Ionița, 2021). The general distribution, chorology, ecology and phytocenology of the species *P. maculatum* were established by the authors, supported by data from the literature (Gibbs, 1972; Dobrochayeva, 1981; POWO).

RESULTS AND DISCUSSION

As the result of our investigation, the *Pontechium maculatum* in the spontaneous flora of the Republic of Moldova, according to the literature and the data from the National Botanical Garden (Institute) (NBGI) Herbarium, it occurred sporadically in most steppe sectors but also in the associations of phytocenoses of *Quercus pubescens* subarid forests (Postolache, 1986). The collection of the NBGI Herbarium contains 123 specimens, the last specimens dating from 2013. *P. maculatum* was indicated and collected from more than 35 places, but only in two of them survived, these being: The representative sector with steppe vegetation "Bugeac multifunctional management area" and on a steppe slope near the village of Trif ne ti (Flore ti district). In the Republic of Moldova, it is not protected in any way, being included in the preliminary Red List of vascular plants (Ghendov *et al.*, 2012) as well as in the operational lists of the Emerald network as protected at the European level.

The real situation in natural habitats, in recent years, differs significantly from the data in literature and in the Herbarium, the distribution of the species being much more limited than what the available data indicate, but it is also much more vulnerable and threatened with extinction.

Pontechium maculatum (L.) Böhle et Hilger (Boraginaceae family) is a biennial or perennial rhizophyte that in the first year produces a rosette of lanceolate leaves up to 25 cm long and a taproot. In the second year, the plant develops an erect, unbranched stem, 20-80 cm tall, with blackish spots, densely leaved downwards. Basal and lower cauline leaves linear-lanceolate, acute, 6-10 cm long and 5-9 mm wide, narrowed into a short petiole; the cauline leaves – sessile, linear-lanceolate, towards the top of the stem gradually turning into lanceolate bracts. Entire plant covered with bristle-shaped, white, stiff, tuberculate hairs, mixed with short, soft hairs. The spike inflorescence, 10-30(40) cm long, consists of numerous bracteate cymes. Flowers pentamerous, slightly zygomorphic, short pedicellate. Calyx about

7 mm long with linear-lanceolate lacinia, acute, with white setaceous hairs. Corolla 12-15 mm long, burgundy-red, funnel-shaped, pubescent; corolla tube straight, without fornix, 2 times as long as the calyx. Stamens 5, unequal, exerted from the corolla, filaments reddish. Style exerted, pubescent, stigma capitate or very slightly bilobed. Nutlets ovoid-triangular, tuberculate, black. It blooms in May-June for a period of 2-3 weeks. The inflorescence stalk dies after fruiting, and the following year, new rosettes may develop at the base of the parent rosette, which produce flowering shoots the following year.

The habitat of the species *P. maculatum* includes xerothermic grasslands, stony slopes with calcareous substrate but also thermophilic forest glades with downy oak.

In the summer of 2020, a series of field expeditions were undertaken in order to identify populations of *P. maculatum*, a population being identified in "Bugeac multifunctional management area", one of the two extant well-preserved population. Site location: N 46°23'50.9", E 28°41'46.5". The population is located within the boundaries of the Ponto-Sarmatic steppes – *62C0, on a North-Eastern facing slope, occupies an area of 1000 m² and consists of 30 generative and over 100 vegetative specimens that grow in small groups of 2-3(-5), but also isolated, from which seeds were collected. The habitat of the species is represented by primary steppe communities with *Stipa dasyphylla* (Lindem.) Trautv., *S. lessingiana* Trin. et Rupr., *S. capillata* R.Br. and *Festuca valesiaca* Gaudin and as accompanying species, we would like to mention: *Poa angustifolia* L., *Koeleria cristata* (L.) Pers., *Teucrium chamaedrys* L., *Plantago lanceolata* L., *Galium humifusum* Bieb., *Adonis vernalis* L., *Helichrysum arenarium* (L.) Moench, *Crambe tataria* Sebeók, *Veronica jacquinii* Baumg., *Phlomis tuberosa* L., *Salvia nutans* L., *S. nemorosa* L., *Linum austriacum* L., *Thymus marschallianus* Willd., *Centaurea trinervia* Steph., etc.

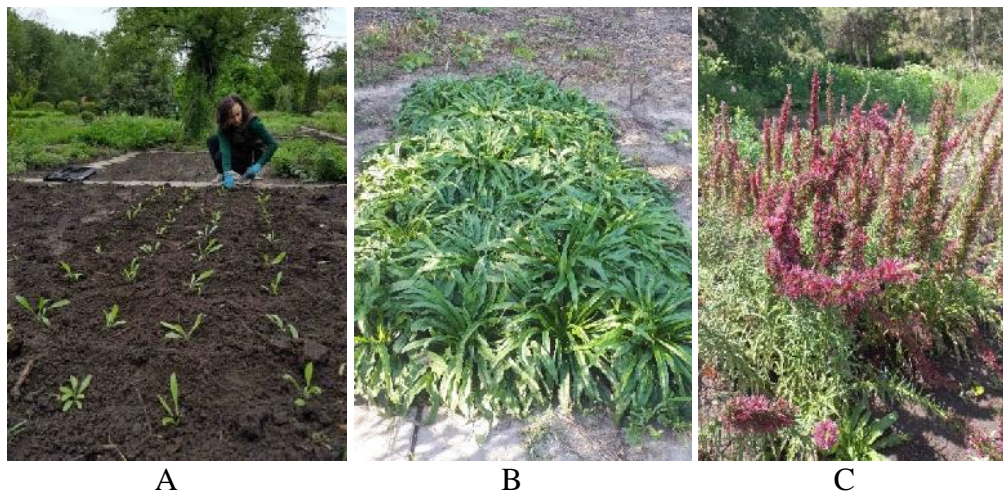
In the spring of 2021, the seeds germinated in Petri dishes on filter paper soaked in distilled water. In each Petri dish 100 seeds were placed, and after 5 days all seeds germinated (the germination rate was 100%) and the seedlings were transferred to a substrate black soil and peat in ratio of 3 : 1. As the seedlings developed, they were placed in the shade and regularly watered until adaptation. After a month of growth and adaptation, the seedlings were transferred into two natural habitats. The habitats were selected according to the needs of the species, taking into account several criteria: the area of distribution of the species; the bioecology of the species; the history of detection of this species in the respective locations or at least in the area; and last but not least, the degree of our accessibility for subsequent monitoring was taken into account as well as the status of these habitats (the locations with protection status were prioritized).

Thus, we chose the Sector with steppe vegetation near the village of Cîzlar, Leova district, geographical coordinates: N 46°38'11.6", E 28°32'11.8", where 20 seedlings were transplanted. The identified habitat is very valuable from the floristic and phytocenotic point of view and represents a slope with Ponto-Sarmatic steppe vegetation, with North-Eastern exposure and inclination of 15-20°, with

primary communities of steppe plants containing over 200 species of vascular plants, including rare species for the territory of the Republic of Moldova, such as: *Stipa dasyphylla* (Lindem.) Trautv., *Hyacinthella leucophaea* (C.Koch) Schur, *Iris variegata* L., *Ornithogalum refractum* Schlecht., *Adonis vernalis* L., *Astragalus dasyanthus* Pall., *Polygala major* Jacq., *P. sibirica* L., *Goniolimon besserianum* (Schult.) Kuhn., *Crambe tatarica* Sebeok, *Helichrysum arenarium* (L.) Moench, *Colchicum arenarium* Waldst. et Kit., *Bellevalia sarmatica* (Georgi) Woronow, *Crocus reticulatus* Steven ex Adams. We visited the seedlings after two months, and out of the 20 specimens we identified 12 that survived the harsh weather conditions with temperatures of up to 38 °C and severe deficiency of precipitation. In the spring-summer of 2022, 6 specimens were identified, of which 2 specimens flowered, but the other 4 remained in the vegetative stage. In the spring of 2023, the site was visited again and two more specimens in the flowering stage were detected. Their monitoring will continue in the upcoming years. We do not exclude the option of adding of new seedlings in the next period to strengthen the population and increase the number of individuals.

The second location where we have taken measures on the reintroduction of the species *P. maculatum* is near the village of Speia, Anenii-Noi district, geographical coordinates: N 47°00'36.7", E 29°18'57.9", in a glade with primary steppe vegetation at the edge of the forest of *Quercus pubescens* Willd. with *Cotinus coggygria* Scop. and *Acer tataricum* L., on a South-West facing calcareous slope with an inclination between 30°-45°. The grass layer is dominated by *Festuca valesiaca* Gaudin, *Stipa pulcherrima* C.Koch, *Achillea ochroleuca* Ehrh., *Teucrium chamaedrys* L., *Elytrigia intermedia* (Host) Nevski. The plant community includes about 180 species of vascular plants including rare species protected by law in the republic: *Centaurea marschalliana* Spreng., *Helichrysum arenarium* (L.) Moench, *Asparagus verticillatus* L., *Achillea ochroleuca* Ehrh., *Valerianella brachystephana* (Ten.) Bertol., *Scorzonera mollis* M. Bieb. In this habitat, in the spring of 2021, 20 seedlings were transplanted. In the spring of 2022, we found that only 4 specimens survived (the meteorological conditions of 2021 were extremely harsh), two of which flowered, and the other two remained in the vegetative stage. In the autumn of 2022, 10 more specimens were planted on the site. During the monitoring carried out in the spring of 2023, we found the presence of two specimens in the generative phase, and the 10 specimens planted in the previous autumn survived, remaining in the vegetative stage and most likely will flower the following year.

For the *ex-situ* conservation of the species *P. maculatum*, in the spring of 2021, a collection was created in the experimental plot of the NBI (Figure 2).



A B C
 Figure 2. *Pontechium maculatum* (L.) Böhle et Hilger in *ex-situ* collection
 A – seedling planting, B – first year vegetative plants, C – second year generative plants.

The plants in this collection can be used to strengthen the existing populations of *Pontechium maculatum*, but especially as "seed source plants", from which seeds are collected and kept for the production of new seedlings with the aim of reintroducing and repopulating the habitats from where the species has disappeared in recent decades. We have planned a long-term monitoring of the state of the populations and their numbers, as well as the identification and establishment of the limiting factors that led to the decline of *P. maculatum* populations in natural habitats.

CONCLUSIONS

As a result of the present study and the *in-situ* and *ex-situ* activities, two incipient populations of *Pontechium maculatum* have been initiated, as the numbers of the representatives in natural habitats of this have decreased year by year during the last decades. The decrease in the number of populations and the disappearance from the steppe vegetation is most likely caused by the degradation of specific habitats by the disturbance of the steppe sectors and their conversion into arable land, a process that reached its climax in the mid-70^s of XXth century, then the steppe sectors that remained insular, suffered from changing the structure of xerothermal meadows: increasing their density, forming a compact layer of dead plants, preventing the development of seedlings, but also the excessive growth of shrubs in the glades of downy oak forests. Other threats, which contributed to the disappearance of many populations of *P. maculatum*, are the afforestation of grasslands and steppe slopes and the overgrazing of specific habitats. We estimate that the population in the "Bugeac multifunctional management area" has high potential for conservation actions, this being the "mother population". Two new populations of the species *P.*

maculatum have been created and will be carefully monitored over the next 10 years to be able to make an eloquent conclusion about the success of this initiative. Moreover, an *ex-situ* plant collection was created within the "Alexandru Ciubotaru" National Botanical Garden (Institute). Because the species is represented in the Republic of Moldova by a very small number of populations with small numbers of specimens, *P. maculatum* needs inclusion in the next edition of the Red Book, but also in the list of species protected by law in the Republic of Moldova.

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