The Initial stages of the reintroduction of the population of the species *Dianthus acicularis* Fisch. ex Ledeb. (Caryophyllaceae Juss.), endangered in the I. I. Sprygin Zhiguli State Nature Reserve

M V Shustov¹, A K Mamontov² and M A Zueva²

¹ Moscow State University of Geodesy and Cartography 4, Gorokhovsky per., Moscow, 105064, Russia
² The main botanical garden named after N.V. Tsitsina RAS 4, Botanicheskaya st., Moscow, 127276, Russia

E-mail: mishashustov@yandex.ru

Abstract. The experience of preserving the population of the species *Dianthus acicularis* Fisch. ex Ledeb. (Caryophyllaceae Juss.), which is endangered in the Zhiguli State Reserve. I. I. Sprygin. The initial stages of its reintroduction are considered, the features of the biology and ecology of the preserved natural populations are studied. Seed samples of the species were collected and multiplication under culture conditions was carried out. The resource possibilities of an artificial population for the potential reintroduction of authentic specimens of the species from the botanical garden into natural conditions were studied. A long periodicity of the formation of full-fledged seeds is shown - 1 time in 10 years, low seed productivity - about 30 seeds per individual and their low germination - 13%. The total absolute abundance over 10 years of observations has increased 4 times, has a tendency to stabilize and is not subject to changes over a number of years. The long-term dynamics of the *D. acicularis* abundance under the conditions of the GBS RAS is characterized by a decrease in the number of individuals of vegetative age states and an increase in the number of individuals with a generative age state, which indicates the formation of a mature population. The experience gained in creating an artificial population of *D. acicularis* under the conditions of the GBS RAS provides factual material on the prospects for *ex situ* conservation of the species and the possibilities for further creation of its reintroduced populations in the face of negative dynamics in the abundance of the species in natural conditions of the Zhiguli State Reserve. I.I. Sprygin.

1. Introduction
Since 2013, the Main Botanical Garden named after. N.V. Tsitsina RAS (GBS RAS) and Zhiguli State Reserve named after. I. I. Sprygin conduct joint research on the possibility of preserving rare and endangered species of the Zhiguli flora and the prospects for their further reintroduction [1-2]. Among the endangered Zhiguli species is the stenotopic calciphilic rock-steppe species, listed in the Red Books of a number of regions of European Russia and Siberia - *Dianthus acicularis* Fisch. ex Ledeb. (Caryophyllaceae Juss.). The uniqueness of the population in the Zhiguli State Reserve. I. I. Sprygin, located in the Stavropol district of the Samara region, is due to a number of reasons, and first of all, the fact that the only extreme western locality of the species isolated from the main range is located here [3-4]. The range of the species covers the east of Europe, the Urals, Western Siberia, Central...
Asia, and the Far East [5]. In the region, this species can be classified as a species with an extremely small population, requiring special approaches in protection [6]. It should be noted that in the course of long-term observations of the population since 1977, its area and abundance have repeatedly changed. So, in 1976, 41 plants grew on an area of about 100 m². In 1990, 331 individuals were already noted on an area of more than 1200 m², in 2016 a significant decrease was observed - 75, and in 2017 - 104 [3; 7].

Conservation status of the species in the Red Book of the Samara region: 1 - endangered. Of the 240 taxa of vascular plants listed in the Red Data Book of the region, the need for reintroduction from authentic material is considered only for carnation needlewort [3].

A universal solution to the problem of the reintroduction of endangered stenotopic taxa has not yet been found in world practice. There are different views on the main aspects of reintroduction. Some researchers do not see any expediency in it and consider it only as an extreme measure, when all possible ways of preserving and restoring populations in situ have proved to be ineffective [8]. Other specialists are discussing the minimum number of plants in reintroduced populations necessary for their viability, since the created population must have a sufficient degree of genetic heterogeneity. There is an opinion that to ensure the short-term survival of the population, the number of individuals should be at least 50 plants, and the long-term survival of the population and the continuous development of adaptations can be ensured by about 500 individuals [9].

In countries with high rates of biodiversity destruction, such as China, a special concept has been adopted - The concept of plant species with extremely small populations (PSESP). However, it does not contain a methodology for the conservation and reintroduction of extremely small populations with a disjunctive distribution due to the negative influence of anthropogenic factors for a long time [10]. At the same time, there is experience in the conservation of endangered taxa, for example, Acer yangbiense Chen & Yang, which is represented in nature by only five individuals. An attempt to preserve them in the Kunming Botanical Garden made it possible to obtain more than 1600 seedlings from seeds for further reintroduction [11]. Another example is Vatica guangxiensis S. L. Mo, a critically endangered species for which about 90 individuals from the three remaining populations have been successfully transplanted into Tropical Botanical Gardens. In connection with the above, the purpose of this work is to study D. acicularis ex situ as an object of potential reintroduction with an extremely small natural population.

2. Materials and methods
In GBS RAS, D. acicularis previously passed introduction tests for 10 years. Seeds were obtained in 1963 from the Czech Republic (Brno, Botanical Garden); in 1957 from Omsk (botanical garden) and collected in 1966 in Bashkiria (Zalairsky district, stony outcrops). Vegetation V–IX. Flowering VI–VII. Seeds from VII. When podzimnem sowing blooms and bears fruit in the 3rd year. Stable. Despite the fact that not a single specimen has been preserved to date, the accumulated experience indicates the prospects of the species for ex situ conservation and subsequent reintroduction, since the phenological phases in the culture correspond to natural ones and its artificial seed propagation is possible.

For the implementation of the project of reintroduction of the D. acicularis population in 2013, plant samples were brought from the Zhiguli State Reserve. I. I. Sprygin (Mount Strelnaya) and studied in the culture of GBS RAS. Due to the length of the process of reintroduction of perennial species, it was envisaged to carry out its initial stages. The project of reintroduction of the D. acicularis population includes the following stages: 1) preparatory stage; 2) conducting field research, studying the biology and ecology of preserved natural populations and collecting material for the creation of queen cells; 3) reproduction of the material under culture conditions; 4) choice of habitats for artificial populations; 5) creation of an artificial population; 6) monitoring of reintroduced populations.

The two final phases of the D. acicularis reintroduction project: 5) and 6) are planned for a later period.
3. Results
At the preparatory stage, a plan was drawn up for the proposed reintroduction. The time costs required for its implementation were estimated. Presumably, it may take at least 30 years to complete all stages. Since the duration of the existence of *D. acicularis* did not previously exceed 10 years, the features of adaptation of similar species grown in the GBS RAS and their requirements for special agrotechnical measures in the new weather and climatic conditions of cultivation were studied. As part of the exposition of the calciphilous flora of the Zhiguli, it was decided to create the necessary lithological conditions that are optimal for the growth and development of *D. acicularis*. So, on the slopes of an artificial hill, areas of finely gravelly limestone were prepared for the reproduction of the species and the creation of an *ex situ* population. The approximate amount of seed and planting material allowed for withdrawal without harming the natural population was determined. The prospects for creating artificial populations were assessed. An approximate calendar plan of activities for the project was drawn up.

The study of the state of the population of *D. acicularis* was carried out in the conditions of the Zhiguli State Reserve named after I. I. Sprygina, on the territory of the natural complex of Mount Strelnay during the expedition of 2013, while studying the main features of the biology and ecology of the species.

The life form of *D. acicularis* is a cushion-shaped semi-shrub, extremely rare not only in the natural flora of the Samara region, but also in the flora of all Central Russia, reaches a height of 20–30 cm. The stems are numerous, simple, sometimes branched at the top, bare, grayish-green with bluish bloom. Rhizomes short, strongly branched. Leaves are needle-shaped, 1.5–6 cm long and 1 mm wide, folded lengthwise, almost trihedral, sharp. Flowers mostly solitary, fragrant. The calyx is cylindrical, 20–26 mm long, with lanceolate sharp teeth. Bracts are obovate, covering 1/4 of the calyx. Petals are white, their plate is obovate, with hairs on the upper side, up to 1/3 or deeper fringed-incised into linear lobes. Blooms in June - July. Insect pollinated plant. The fruit is a cylindrical capsule, the seeds are about 2 mm long. Propagated by seeds. On the western macroslope of Mount Strelnay, it grows in communities of the petrophytic steppe on fine limestone.

Due to the oppressed state of the petrophytic flora of the reserve in general, and the population of *D. acicularis* in particular, in agreement with the management of the reserve, only a single specimen of a young vegetative plant *D. acicularis* was selected and 7 seed samples were collected. Thus, a feature of the experiment of species conservation in the GBS was the use of only one individual authentic for the Zhiguli Upland and several seed samples, which unfortunately turned out to be dissimilar. Successful adaptation of the young vegetative plant made it possible to proceed to the next stage.

The *ex situ* breeding stage is the most important among the initial stages of reintroduction. According to observations under the conditions of GBS RAS, the possibility of reproduction of *D. acicularis* in culture conditions strongly depends on a number of factors determined by ontogeny, current weather conditions, and the influence of various pests. The activity of the latter significantly disrupts the annual cycle of shoot development, for example, at the beginning of 2019, the shoots of the mother sample, destroyed by rodents to the ground, recovered only by the end of 2020. However, damage by insects is more tangible systematic, and since 2020, the negative impact of various invasive molluscs has increased. Therefore, over the 10 years of observations since 2013, full-fledged mature seeds were obtained only once, in 2015. In other years, either the fruits or generative shoots as a whole were damaged already at the budding stage. In 30 seed samples obtained under the conditions of GBS RAS, soil germination was 13% - 4 samples sprouted, of which 1 died in the second year of life. Changes in the population size and main age conditions of *D. acicularis* are shown in table 1. As a result of artificial seed propagation, 3 new individuals of *D. acicularis* were obtained, the total population size was 4 individuals by 2022.
Table 1. Changes in the population size of *D. acicularis* in the GBS RAS.

| Age condition | Observation period |
|---------------|--------------------|
|               | 2013 – 2014 | 2015 – 2016 | 2017 – 2018 | 2019 – 2021 | 2022 |
| Vegetative    | 1            | 4           | 3           | 2           | 1    |
| Generative    | 1            | 1           | 1           | 2           | 3    |

The long-term dynamics of the abundance of *D. acicularis* in the GBS RAS shows a short-term increase in the number of vegetative individuals followed by a steady decrease caused by a single drop of a young plant and the transition of surviving individuals to the generative age state. Therefore, the number of generative individuals, on the contrary, increased, which indicates the formation of a mature population. The total abundance tends to stabilize and has not changed over more than 5 years of observations (figure 1). The same dynamics, but against the background of a decrease in numbers, is also shown in the natural population on the western macroslope of Mount Strelnaya. It is characterized by an extremely low number (with a predominance of generative individuals), which has a pronounced downward trend. A stable decline in the number of *D. acicularis* plants indicates the state of the population, which is under threat of extinction. The limiting factors are excessive anthropogenic pressure and changes in the phytocenotic environment [9].

The result of the passage of the initial stages of the reintroduction of *D. acicularis* under the conditions of the GBS RAS allows the creation of reintroduction populations on the territory of the Zhiguli State Reserve, if such a need arises, for example, caused by the further manifestation of the negative dynamics of the species abundance in natural conditions.

![Figure 1](image.png)

**Figure 1.** Dynamics of the number of vegetative and generative individuals of *D. acicularis*.

4. Conclusion
During the initial stages of the reintroduction of *D. acicularis* from 2013 to 2022, the following results were obtained: the state of the population of the endangered, in the conditions of the Zhiguli State Reserve named after I. I. Spryagina of the species, its small number and oppression were noted, the prospects for creating an artificial population under the conditions of the GBS RAS were assessed; optimal conditions have been created for the successful adaptation of only one authentic Zhiguli population of *D. acicularis* and long-term conservation of the species ex situ; for the first time in culture, the resource potential of samples of the species from the Zhiguli for subsequent reintroduction was studied, seed productivity was assessed; revealed a strong susceptibility of the reproductive sphere of plants to pests and, in this regard, extremely rare fruiting, low seed germination, not exceeding 13%.
and the absence of natural seed renewal. Therefore, the success of creating a large population of the species depends on the favorable interaction of abiotic and biotic factors, which was observed extremely rarely. In the long-term dynamics of the abundance of *D. acicularis* under the conditions of the GBS RAS, there is a clear trend towards a decrease in the number of individuals of the vegetative age state and an increase in the number of individuals with the generative age state, which indicates the formation of a mature population and this corresponds to the ratio of age groups in the natural population. The total population size has increased by 4 times over 10 years, has a tendency to stabilize and has not changed for more than 5 years of observations. The initial stages of the reintroduction of *D. acicularis*, carried out under the conditions of the GBS RAS, provide factual data on the possibility of preserving the species and further creating its reintroduced populations in the natural conditions of the Zhiguli State Reserve named after I. I. Sprygin.

**Acknowledgments**

The work was carried out in accordance to Institutional research project № 18-118021490111-5.

**References**

[1] Shustov M V and Mamontov A K 2014 Expedition to Zhigulevsky State Natural Biosphere Reserve named after I I Sprygin. *Council of Botanical Gardens of the CIS countries at the International Association of Academies of Sciences* 2(25) 65-9

[2] Shustov M V, Shvecov A N, Mamontov A K and Zueva M A 2021 Preserving Rare and Endangered Calcific Steppe-Dwelling East-European Species ex situ. *IOP Conf. Ser.: Earth Environ. Sci.* 817 012100

[3] The Red Book of the Samara region. Plants and fungi 2017 (Samara: Publishing House of the Samara State Regional Academy (Nayanova)) I 380

[4] Shishkin B K 1936 *Genus Dianthus L. Flora of the USSR* (Moscow - Leningrad: Publishing House of USSR Academy of Sciences) 6 803-61

[5] Kuzmina M L 2004 *Genus 50. Dianthus L. Flora of Eastern Europe* (Moscow; St. Petersburg: Publishing House of Russian Academy of Sciences) 10 273-97

[6] *Division of Plant Conservation of the State Forestry Administration* 2005 The extremely small populations in China. *Forest and Mankind* 25 14-29

[7] Chap T F and Kiseleva D S 2017 The state of populations of *Dianthus acicularis* (Caryophyllaceae) in the Zhiguli Reserve. *Samarskaya Luka: problems of regional and global ecology* 26(4) 132-40

[8] Vallee L, Hogbin T, Monks L, Makinson B, Matthes M and Rossetto M 2004 Guidelines for the translocation of threatened plants in Australia. *Australian Network for Plant Conservation, Canberra* 245

[9] Volis S 2016 How to conserve threatened Chinese plant species with extremely small populations. *Plant Divers* 38 45-52

[10] Ren H, Zhang Q, Lu H, Liu H, Guo Q and Wang J 2012 Wild plant species with extremely small populations require conservation and reintroduction in China. *Ambio* 41 913-7

[11] Li Q, Xu Z and He T 2002 Ex situ genetic conservation of endangered *Vatica guangxiensis* (Dipterocarpaceae). *China Biol. Conserv* 106 151-6