

Analysis and suggestions on monitoring of the population of *Botrychium virginianum* (L.) Sw. in remote parts of the geographical range (based on the examples of Pennsylvania (USA) and Kostroma region (Russia))

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Abstract. In this analysis we present the characteristics of the habitats of a species rare in Europe - *Botrychium virginianum* (L.) Sw., in remote parts of the geographical range on the territories of USA and Russia. The analysis confirms the possibility of the growth of this species in naturally recovering forests, gives an update of the information about a variety of environmental conditions of growth of this species and shows its similarity in different parts of Europe. At the time of research conducted in Bethlehem this species appeared to be rare, their populations small, which is connected with the cryptic character of the populations of this species as well as with the ability of showing a wide range of polyvariety of ontogenesis. Only in one of the examined populations the age spectre is fully represented and we can see all the basic states of ontogenesis. This species is also rare on the territory of the Kostroma region while the populations are larger and contain all states of ontogenesis of sporophyte. The phenology of the species was examined. The analysis points to the vista of creation of new populations by introduction of spores in new areas and shows the necessity of monitoring in order to develop mechanisms of protection if the species.

Introduction

Botrychium virginianum (L.) Sw. belongs to one of the rarest and the least researched and archaic Paleozoic fern of the classis Ophioglossopsida. The literature on the subject that we have studied does not have any monographic research of its population-based biology and phytocoenotic confinedness. Previously we have researched other types of the class *Botrychium* Sw. [3,4,5]. *B. virginianum* has a cosmopolitan scattered geographical range. It can be seen in Central and Eastern Europe from Sweden to Slovenia, Tirol and Eastern Switzerland. In the relation to latitude – from Alps to Amour in the Far East. In North America – in south-eastern states of the USA, New Scotland, Quebec, the islands of Prince-Eduard and in Central and South America, Australia and New Zealand [1,6,11]. In some parts of Europe this species is considered extinct, in Hungary, Litva, Belarus and Ukraine it's considered an endangered species and is listed in the Red List of Threatened Species [1,7,12]. The described species is also rare in Russia. The reasons of the reduction of the species are unknown [1,6]. In North America *B. virginianum* is not considered rare [6,9,11,14].

In order to determine the reasons of the dying-out of this species and the strategies for its protection special population-based studies are needed, including monitoring in different parts of its habitat. Therefore the aim of this research is to study the population of this species in remote parts of Pennsylvania (USA) and Kostroma region (Russia)

Object, materials and methods

The studies were conducted during the operational season of 2013. In the USA it was conducted in the suburbs of the city of Bethlehem, Pennsylvania. We have researched the forests restored after the Great Clearcut of 1890-1930s along the valley of river Lehigh, South Mountain, as well as the hills near river Lehigh around the hospital of St.Luke. The population of *B. virginianum* is only discovered in the small area of South Mountain and on two hills near river Lehigh (Lehigh Mountain Uplands Preserve).

In Russia the researched area included the Manturov region of the preserved area "Kologriv forest" (Manturov, Kostroma region). The reserve is situated on the left bank of river Oonza and partially included overgrown clearcuts of the beginning and middle of the XX century and burned-out forests of 1972. The research shows that *B. virginianum* here is connected with broadleaved forests on former burned-out areas, clearings, meadows and overgrown roads. To research the condition of its population we have examined a gentle slope of the south exposure near river Ivanchikha, on the ridge of rivers Pouminna and Ivankovo along the clearing. This territory is covered by aspen and birch trees with occasional interjection of *Salix caprea* L. In both the examine regions in habitats containing *B. virginianum* we conducted geobotanical descriptions for the study of the vegetation, and the evaluation of the species amount was conducted according to the Brown-Blanke scale. The type of flora in the USA is presented according to [14], the type of flora in the Russian Federation is presented according to the summary of S.K. Cherepanov [8].

The research

During the research of the population, the following demographic features were taken into consideration: density, number, ontogenetic composition, the regional position in comparison with edificators. To determine the age of the population we described the ontogenesis of sporophyte based on the macro morphological characteristics. We have used a diagram which we had previously created for other genus of *Botrychium* Sw. [3,4,5]. The markers of ontogenetic states are as follows: the level of heterotomy, the shape and size of trophophore (the steril segment of the leaf) and sporophore (spore-forming segment) as well as the presence of absence of sporophore. In the ontogenesis of *B. virginianum* we have distinguished the following ontogenetic states: J, Im, V, Sp₁, Sp₂, Sp₃, Sp₁₋₃V, Ss. Sp₁₋₃V is not a special ontogenetic state but a form of polyvariety of ontogenesis when the development of sporophore of the specimen in the reproductive period stops at early stages. They can be distinguished from the virginal (V) specimens by the leftover rudimentary sporophore situated between phylomophore and the stem of trophophore. Such sporophytes can be found in all the researched population of the genus *Botrychium* Sw. [3,4,5].

The habitat in the South Mountain (USA) is situated on top the ridge covered by broad-leaved forests (N40°35'47,72" W75°22'26,39"). The geobotanical description (01.06.2013): tree stratum: 1 story *Quercus rubra* L., *Q. alba* L., *Liriodendron tulipifera* L., 2 story: *Carpinus caroliniana* Walt., *Acer platanoides* L., with intermixture of *Carya ovata* (P. Mill.) K. Koch., *Sassafras albidum* (Nutt.), and *Pinus strobus* L. Total crown density - 50%. Undergrowth: *Aesculus flava* Marshall, *Amelanchier arborea* (Michx. f.) Fernald., density - 10%. Bush story: *Berberis canadensis* Mill., *Euonymus alatus* (Thunb.) Sieb. - 60%. The story of low undergrowth: *Robinia pseudoacacia* L., *Gleditsia triacanthos* L., *Acer rubrum* L. - 10%. Total projective grass canopy - 50-70%, we have counted 15 species. Moss story is not prominent.

The population covered an area of 40 m². Number of specimens is 22, most of them growing along a bicycle path or 3-5 meters away from it. Sporogenous specimens are situated near *Berberis canadensis*. The age composition of the population is shown in Table 1. The sporophores mature by mid May and are shed in the beginning of June.

The population that existed on the territory of the Preserve (Russia) was situated in a birch grove with linden nemoralis along an overgrown timber road. Geobotanical description (09.08.2013). In the tree stratum there is *Betula pubescens* Ehrh., *Tilia cordata* Mill., with intermixture of *Salix caprea* and *Ulmus laevis* Pall., crown density - 40%. In the second story there is *Daphne*

mezereum L., *Salix aurita* L., *S. caprea*, *Sorbus aucuparia* L., *Ribes nigrum* L., *Lonicera xylosteum* L. The density of forest stand is uneven up to 60%. Undergrowth - *Betula pubescens*, *Ulmus laevis*, *Piceae abies*, *Salix caprea*, *Tilia cordata*, *Sorbus aucuparia* L., *Acer platanoides* L. Understory - *Ulmus laevis*, *Salix caprea*, *Tilia cordata*, *Ribes nigrum* L., *Lonicera xylosteum* L., *Daphne mezereum* L. There are quite a lot of dead standing trees, fallen trees, mainly *Salix caprea*.

The grass canopy is very rich and represented by nemoralis. Total projective coverage is 80% and counts 48 species including *Cyripedium calceolus* L. (1), *B. virginianum* (1). The moss coverage is not prominent. The characteristics of the plant life displays a high mineral saturation in the area. The total expansion of the population along the road, with insignificant fragmentation, is 1100 meters and 20-30 meters wide in both directions. The total number of specimens wasn't counted, but not less than 1000 specimens. The density of the examined area 136 specimens per 100 m², the age range is represented in Table 1. Sp₁₋₃V – the sporohpytes in the vicinity of trees and near *Cirsium oleraceum* and *Angelica sylvestris*.

Table 1.

Age range of the population of *Botrychium virginianum* in the South Mountain, Bethlehem – 1, and on the territory of Preseved area «Kologriv forest» - 2

	J	Im	V	Sp ₁	Sp ₂	Sp ₃	Sp ₁₋₃ V	Ss
1	1	0	3	4	1	-	13	-
2	2	11	37	18	32	-	36	-

The conducted geobotanical descriptions present a picture of wide diversity in which *B. virginianum* can grow. In Sweden and Finland this species grow in spruce forests, in Poland – in deciduary and mixed forests [17,18,19]. In Belarus it is considered to be meadow-forest, preferring spruce and broad-leaved forests with introductions of *Myliobatis*, *Malus silvestris*, *Aegopodium* and huckleberry areas near eutrophic marshes [1]. In Russia this species is also found in similar ecological conditions [2, personal observations]. In Ukraine it is coenotically connected with broad-leaved forests (beech, oak, hornbeam, aspen and birch) and their clearings [6,7,8]. Such variety of plant life signifies a high valence of this species [10]. For all territories it is characteristic that it prefers fresh soils rich with nutrients and shaded areas, preferring pristine forests with fertilized soils rich in carbonate.

The analysis of condition of vegetation of *B. virginianum* in Pennsylvania and Kostroma region suggest a possibility of its introduction into the forests which are being restored after interventions. The species is considered usual for broad-leaved forests and damp slopes in Pennsylvania, however the inspection of significant areas of woodland in 2013 around Bethlehem shows that the species is represented by small populations or individual specimens.

B. virginianum, as well as other types of *Botrychium* Sw., has a high ability to go into the state of secondary dormancy under unfavorable conditions. In some years up to 100% of the population can go into anabiosis, and that explains its rare occurrence.

As a result the populations have a “pulsating” behaviour and show cryptic character [3,4,5]. Perhaps the low number of the examined populations in the USA is the result of anthropogenic influence in a densely populated area. The existing populations can both be relics that survived the Great Clearingin Pennsylvania in 1890-1920 [16], or *B. virginianum* has “colonized” its destroyed territories again introducing itself into new forming forests. This fern has an ability to show elements of life strategy of explerent while being a typical patient which avoids unfavorable factors, thus showing a wide specter of polyvariency [3,4,5]. The introduction of its populations happen in areas of low competition and low level of destruction of plant and particularly moss coverage [3,4,5,14,17,18], since moss is the most important obstacle in implantation of spores and their further development which first doesn't allow the spores to get into the soil and then inhibits the sprouting and the growth of gametophyte [3]. Our observations show that this species as well as other rare species favors the sides of potential corridors of expansion – forest paths. In Belarus and

Russia it is linked to overgrown forest skirts, paths and old forest roads, while they are not found in deep forest.

What may be the reasons for different density of *B. virginianum* populations on two northern continents? This species could have survived the ice age in separate refugiums or else it may have moved south with the forests nemoralis. Later on with gradual expansion of the nemoralis forests to the north, it reestablished its previous areas, and the mass anthropogenic transformation of biotopes placed this naturally rare type on the brink of extinction. The modern disjunctive habitat and small distance to which the spores can be carried signify that this species has existed in compact locus while the vegetative spread is absent. The research shows that the spores of *B. virginianum* that are planted on agar have the viability of 90% [13], and if we take into consideration that the sporophytes of this species can contain their territories then from the existing populations we can witness the further spread of this species. To intensify the process and promote the number of specimens in local populations it is possible to introduce the spores in new habitats but it is very difficult to control the result of it since the development of gametophytes Ophioglossaceae takes 20 or more years.

The observed paucity of populations in Pennsylvania in 2013 and the domination of Sp₁₋₃V sporophytes reminds us of the situation with European populations. In Europe the number of known populations is very low, it mostly grows as individual specimens or in small groups on a few square meters [1,2]. The only verified population in Ukraine in Chernigov Polesy counts 12 sporophytes [6] while the population in Kostroma region counts 136 sporophytes. More numerous populations are found in Sweden [18] however the supposition of better environmental conditions for the existence of the species in Northern Europe need monitoring and research in different parts of the area.

Sp₁₋₃V specimens dominate both American and Russian populations while J, Im and V specimens are rare. This can be explained by the fact that this species belongs to the genus *Botrychium* Sw., whose types have an ability for anabiosis, especially young specimens (J и Im) while mature sporophytes (Sp₁, Sp₂ и Sp₃) more often go into periods of temporary dormancy (Sp₁₋₃V). In the populations of this type a natural collection of Sp₂-sporophytes occurs since this period of ontogenesis is the longest one [3].

The populations in Pennsylvania which are situated south of their analogous populations in Russia, Ukraine and Belarus have an earlier phenological development. The plants have mature sporophores by the end of May and spores are planted in the beginning of June. In European populations leaves appear in June- July and spores are discharged in July and August [1, 24].

The types with cryptic character of populations their real structure and number remain unknown since their precise research is impossible without destruction of the consistence of population and environment. According to published sources and personal observations *B. virginianum* as well as other types of this class, do not tolerate replanting very well and are incapable of spore reproduction without symbiotic fungi [14]. This points to the importance of preservation of currently existing populations as well as areas potentially suitable for introduction of spores. The markers of suitable areas are other obligate micotrophic plants.

Conclusion

In conclusion we can say that *B. virginianum* in both researched areas is a components of self-restoring forests. The populations prefer the vicinity of forest paths and roads and other areas where there has been intervention in the past. The populations in Pennsylvania during the time of research were small while the researched population in Kostroma area was numerous and spread over a large area. In both regions the populations are mostly represented by mature sporophytes most of whom was in the state of spore discharge (Sp₁₋₃V). The key to the understanding and preservation of the populations of this species is monitoring and protection of the existing populations as well as creation of new stable populations which could in the future colonize both natural and restored forests. Considering the high fertility of *B. Virginianum* it is possible to plant its spores in other protected and suitable areas.

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