New locality of *Botrychium matricariifolium* in south-eastern Poland – characteristics of the habitat and some plant traits

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Received 22 February 2022 / Accepted 29 November 2022

Abstract. *Botrychium matricariifolium* is a critically endangered species in Poland. In 2020, a new locality of this rare fern was found in the Góra Chełm forest reserve (Strzyżowskie Foothills). The population comprised 58 individuals. The fern was found in the Carpathian beech fertile complex Dentario glandulosae-Fagetum at a forest margin located near an unused quarry. The community comprised 29 vascular plant species. The dense layer of trees (90%) was composed of *Acer pseudoplatanus, Abies alba*, and *Fagus sylvatica*. The greatest coverage in the undergrowth was achieved by *Dentaria glandulosa, Dentaria bulbifera, Galeobdolon luteum*, and *Mercurialis perennis*. The mean height of the ferns was 14.9 cm. The trophophores had 3 branches each and were 1.1-3.4 cm long (mean: 2.1 cm). They had 5 to 9 pinnae. The length of the sporophore ranged from 2.3 to 8.3 cm (mean: 5.1 cm). It had from 12 to 183 sporangia (mean: 62). In 2021, the locality was re-verified, and 30 sporulating individuals were recorded. No factors threatening the species were noted. The population is in good condition and does not require any special active protection measures.

Keywords: Botrychium matricariifolium, threatened species, protected species, Western Carpathians.

1. Introduction

Botrychium matricariifolium (Retz.) A. Braun ex W.D.J. Koch is a circumpolar species (Zając & Zając, 2009) occurring in the temperate and cold zones of Europe and North America. Its geographic range covers the Scandinavian Peninsula, Central Europe, and North-Eastern Europe. The southernmost localities of the species are located in Corsica and the Dinaric Mountains. It reaches the Urals in the east and France in the west (Hultén et & Fries, 1986; Lengyel, 2009; Borovečki-Voska et al., 2011; Stešević & Berg, 2015). In Poland, the main area of occurrence of the fern is the northern and southwestern part of the country. Over 220 localities of the species were recorded mainly in the 19th and early 20th centuries, but most of them have not been confirmed recently (Zając & Zając, 2001; Szczęśniak & Tlałka, 2014). Since 2000 the fern was recorded in only 22 localities, primarily in lowlands (Szczęśniak, 1998; Werpachowski, 2000; Celka & Kasprowicz, 2002; Czarna, 2002; Bobrowicz et al., 2004; Kozak et al., 2005; Kujawa et al., 2005; Gierczyk et al., 2007; Karczewska, 2010; Zarzecki & Wołkowycki, 2012; Beuch & Zawistowski, 2014; Torzewski & Kazienko, 2018). In the uplands, *Botrychium matricariifolium* was noted in Wyżyna Śląska Upland and in Central Roztocze Hills (Tlałka & Rostański, 2008; Szczęśniak & Tlałka, 2014). In the mountainous vegetation belt, this very rare species is known from one locality in the Sudety Mountains (Nowak & Nowak, 2008) and from two localities in the Carpathians (Tlałka, 2011; Wilczek & Zarzycki, 2013).

The fern is a 5-25 cm high perennial plant with overwintering rhizomes. Its fronds composed of two parts: the trophophore and the sporophore are produced in spring. The trophophore is pinnate or bi-pinnate and has elongated oblong or blunt sections. The bi- or 3-pinnate sporophore is larger than the trophophore. It bears numerous yellow or brown sporangia. The plant reproduces through spores or in a vegetative way through fragmentation of the rhizome. The spores mature in June and July. The development of the gametophyte requires the presence of endotrophic mycorrhiza. The emergence of the mature sporophyte above the ground may take place 10 years after the fertilisation of the gametophyte (Muller, 1993; Tutin et al., 1993; Williams & Waller, 2015). Botrychium matricariifolium exhibits high morphological plasticity. This morphological diversity does not accompany genetic differences, which confirms its status as a genetically distinct species (Williams et al., 2016). The fern can be spotted for a short time during the year, as it grows in May and dies off in June or July after release of spores. It is characterised by irregular occurrence; most often, it produces a frond every year or every two years. The dormancy period may sometimes last 4 years. This results in changes in the population size from year to year. In some years, the species is difficult to spot at all (Muller, 1993; Johnson-Groh & Lee, 2002; Williams & Waller, 2015).

Botrychium matricariifolium is a geophyte preferring moderate light and thermal conditions. It grows on fresh, nutrient-poor, slightly acidic soils, most often on sandy or clay substrate (Zarzycki et al., 2002). It occurs in nutrientpoor grasslands, forest clearings, fringe communities, light forests, and coniferous forests. It has been recorded most often in the communities of poor grasslands and heathlands of the class Nardo-Callunetea (Muller, 1999; Stosik, 2011; Wilczek & Zarzycki, 2013) and in sandy grasslands of the class Koelerio-glaucae-Corynephoretea canescentis (Muller, 1999; Karczewska, 2010; Beuch & Zawistowski, 2014). Less frequently, it was recorded in transitional communities between xerothermic sandy grasslands and fringe vegetation (Nowak & Nowak, 2008; Zarzecki & Wołkowycki, 2012; Torzewski & Kazienko, 2018), forest communities undergoing succession (Celka & Kasprowicz, 2002), forest margins, and forests representing the Dicrano-Pinion and Quercion roboripetraeae alliances (Szczęśniak, 1998; Bobrowicz et al., 2004). However, this species was recorded much less frequently in Molinio-Arrhenatheretea meadows and pastures (Muller, 1993, 1999; Lengyel, 2009), oak-hornbeam forests (Czarna, 2002; Tlałka, 2011), and beech forests (Motyka, 1956). Interestingly, it can also be found in anthropogenic forest monocultures (Kujawa et al., 2005; Gierczyk et al., 2007).

In Poland, *Botrychium matricariifolium* is under strict species protection (Rozporządzenie, 2014). It has been included in the *Polish Red Data Book of Plants* (Szczęśniak & Tlałka, 2014) and the *Polish Red List of Ferns and Flowering Plants* (Kaźmierczakowa et al., 2016) in the group of critically endangered species (CR). It also has a status of a rare species in neighbouring countries, e.g. it has been classified as a critically endangered species in the Czech Republic (Grulich, 2012) and Slovakia (Turis et al., 2014). In Europe, it is covered by international protection under the Bern Convention (Convention, 1979) with the near-threatened NT category (Bilz et al., 2011). Additionally, the species is included in the IUCN World Red List with the NT category (IUCN, 2022).

In 2020, a rare Botrychium matricariifolium fern was found in the Góra Chełm reserve in the Strzyżów Foothills. The reserve protects the extrazonal locality of the Carpathian beech fertile complex Dentario glandulosae-Fagetum, which is the dominant community here. The moist areas and those along streams are occupied by submontane Carici remotae-Fraxinetum ash-alder riparian forest, whereas transitional communities between the oak-hornbeam forest and the beech forest have developed at the foot of the slope. In the top part of the reserve, there is an unused quarry with two rock shelves formed after excavation of rocks. At present, the excavation area is occupied by fresh Arrhenatheretalia elatioris meadows and spontaneous Epilobietea angustifolii scrubs. Investigations conducted so far in the reserve have reported the presence of over 250 vascular plant species and highlighted the presence of numerous well-preserved populations of 15 protected species, e.g. Cephalanthera longifolia, Platanthera bifolia, Lilium martagon, and Arum alpinu (Wójcik et al., 2018; Wójcik & Ziaja, 2019; Wójcik & Kostrakiewicz-Gierałt, 2020).

The aim of the study was to describe a new *Botrychium matricariifolium* locality in south-eastern Poland. Detailed goals included: (1) the phytosociological characteristics of the habitat, (2) determining the population number, (3) analysis of some traits of the fern individuals.

2. Material and Methods

The research was conducted in the years 2021–2022. The location of the site was given in the ATPOL map system (Zając 1978). During two field studies, their number was precisely determined and a phytosociological relevé was made with the Braun-Blanquet method (1964). The syntaxonomic affiliation of the species was determined as in Matuszkiewicz (2001). Additionally, some parameters of *Botrychum matricariifolium* were measured as in the study conducted by Williams and Waller (2015). The following traits were analysed: overall height, length of the trophophore, length of the sporophore, number of pinnae per trophophore.

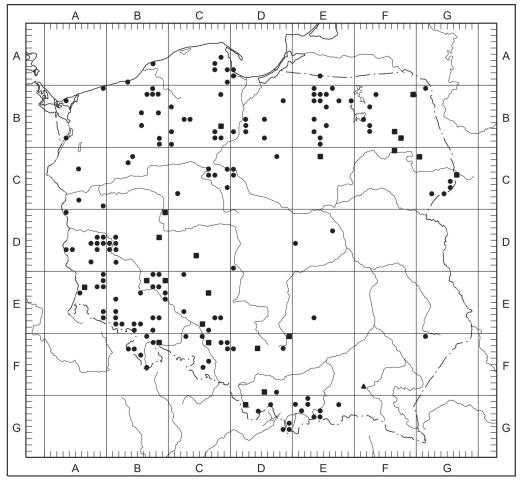
3. Results

In 2020, *Botrychium matricariifolium* was found in the Góra Chełm reserve in Strzyżowskie Foothills in the ATPOL FF81 square. The fern was located in the highest parts of Góra Chełm at an altitude of 517 m a.s.l. in the Carpathian beech fertile complex *Dentario glandulosae-Fagetum* (Fig 1). The locality is situated at the forest margin near an unused quarry. Hence, the forest floor is well-lit and dry in summer. It is currently the south-easternmost locality of *Botrychium matricariifolium*. Its distribution in Poland is shown in Fig. 2. The closest existing localities are at a distance of about 160 km (Mały Beskid Mts) and 180 km (Silesian Beskid Mts).

In order to characterise the habitat, a phytosociological relevé of a 100 m² area was made. The patch exhibited high species richness with 29 recorded vascular plants. The tree stand was very compact (90% coverage) and was composed of *Acer pseudoplatanus* (dominant species), *Abies alba*, and *Fagus sylvatica*. The layer of shrubs covering 50% of the relevé area comprised *Corylus avellana* and tree saplings. The undergrowth was dominated by species characteristic



Figure 1. Occurrence of *Botrychium matricariifolium* (Retz.) A. Braun ex W.D.J. Koch in the Góra Chełm reserve (photo T. Wójcik)



Fiure. 2. Distribution of *Botrychium matricariifolium* (Retz.) A. Braun ex W.D.J. Koch in Poland (according to Zając & Zając 2001; completed and changed). ● – localities prior to 2000, ■ – localities found after 2000, ▲ – new locality

of the order *Fagetalia sylvaticae*, in particular *Galeobdolon luteum* and *Mercurialis perennis*. There were numerous *Dentaria glandulosa* and *Dentaria bulbifera* individuals; hence, the community was characterised as the *Dentario glandulosae-Fagetum* complex. The full composition of this phytocoenosis is presented in the phytosociological relevé shown below:

Date: 24.06.2020, area of relevé: 100 m², cover of tree layer a: 90%, cover of shrub layer b: 50%, cover of herb layer c: 60%, number of species: 29. Ch.Ass. Dentario glandulosae-Fagetum: Dentaria glandulosa 2, Euphorbia amygdaloides +, Salvia glutinosa +; Ch.All. Fagion sylvaticae: Fagus sylvatica a 1, Fagus sylvatica b 1, Dentaria bulbifera 2; Ch.O. Fagetalia sylvaticae: Acer pseudoplatanus a 4, Carex pilosa +, Carex sylvatica +, Carpinus betulus b +, Galeobdolon luteum 2, Galium odoratum 1, Lilium martagon +, Mercurialis perennis 2, Paris quadrifolia +, Polygonatum multiflorum 1, Pulmonaria obscura +, Stachys sylvatica +, Viola reichenbachiana +; Ch.Cl. Querco-Fagetea: Aegopodium podagraria +, Anemone nemorosa 1, Corylus avellana b 2, Poa nemoralis +; Others: Abies alba a 2, Abies alba b 2, Botrychium matricariifolium +, Dryopteris carthusiana +, Mycelis muralis 1, Rubus hirtus +, Senecio ovatus +, Veronica officinalis +.

Fifty eight *Botrychium matricariifolium* specimens were found in the new locality. They all had a frond consisting of a trophophore and sporophore part. Six parameters were measured in 30 randomly selected individuals (Table 1). The mean height of the plants was 14.9 cm. The trophophore had on average 3 branches and was 1.1-3.4 cm long (mean: 2.1 cm). It had from 5 to 9 pinnae (mean: 7). The length of the sporophore ranged from 2.3 to 8.3 cm (mean: 5.1 cm). It contained from 12 to 183 sporangia (mean: 62). The locality was re-verified in 2021 and 30 sporulating individuals were recorded.

 Table 1. Analysis of individual traits of Botrychium

 matricariifolium

	Min	Max	Mean
Overall height [cm]	9.6	19.0	14.9
Length of trophophore [cm]	1.1	3.4	2.1
Length of sporophore [cm]	2.3	8.3	5.1
Number of trophophore	1	5	3
branches			
Number of sporangia	12	183	62
Number of pinnae per	5	9	7
trophophore			

4. Discussion

Species of the genus *Botrychium* are rare plants forming dispersed and highly isolated small populations. For this

reason, populations of these species are seriously threatened by natural factors (inbreeding, genetic drift), which result in loss of genetic variability, lower reproduction performance, and higher mortality rates (Johnson-Groh & Lee, 2002; Mesipuu et al., 2009; Williams et al., 2016). This may threaten their survival and reduce their competitiveness in the changing environmental conditions. As a result, their populations become more susceptible to other threatening factors, e.g. anthropogenic effects. Hence, it is difficult to take appropriate protective measures, and the proposed strategies often turn out to be insufficient or ineffective (Muller, 1993; Williams & Waller, 2015).

The Polish Botrychium matricariifolium populations are strongly isolated and dispersed in various regions of the country. Therefore, it has been included in many local red lists: with the CR category in Central Poland (Jakubowska-Gabara & Kucharski, 1999), the Sudety Mountains (Fabiszewski & Kwiatkowski, 2002), the Lower Silesia region (Kacki et al., 2003), Gdańsk Pomerania (Markowski & Buliński, 2004), Opolskie Province (Nowak et al., 2008), and Wyżyna Małopolska Upland (Bróż & Przemyski, 2009) and with the EN category in the Wielkopolska region (Jackowiak et al., 2007), Śląskie Province (Parusel & Urbisz, 2012), and Lubelskie Province (Cwener et al., 2016). The rarity of Botrychium matricariifolium is surprising in comparison with its wide ecological tolerance. It do not exhibit a close relationship with a specific type of plant community. Surprising is the large divergence of phytocoenoses, from grassland, heathland, and meadow communities to forest habitats, in which Botrychium matricariifolium occurs. Their common trait is the substrate type, as it usually grows in organic matter-poor and acidic habitats. Therefore, the species has mostly been recorded in grasslands and heathlands as well as forests and forest margins located on nutrient-poor substrate (Szczęśniak, 1998; Bobrowicz et al., 2004; Nowak & Nowak, 2008; Karczewska, 2010; Zarzecki & Wołkowycki, 2012; Wilczek & Zarzycki, 2013; Beuch & Zawistowski, 2014; Torzewski & Kazienko, 2018). Nevertheless, several recent reports indicate that this species can grow in more fertile habitats (Czarna, 2002; Tlałka, 2011) and even in anthropogenic forest monocultures (Kujawa et al., 2005; Gierczyk et al., 2007), which proves its high ecological plasticity. This is confirmed by the results of analyses conducted by Muller (1999), who investigated the relationship between the floristic composition and the occurrence of Botrychium lunaria and Botrychium matriciifolium in lowland areas of Central Europe. Both species are associated with acidic and oligo-mesotrophic grasslands with an intermediate floristic composition between the classes Nardo-Callunetea and Molinio-Arrhenatheretea. In France, they are associated with Festuco-Brometea and Sedo-Scleranthetea communities. In terms of phytosociology, the newly discovered locality differs from those reported by other authors, as *Botrychium matricariifolium* was found in the Carpathian beech fertile complex *Dentario glandulosae-Fagetum*. The only confirmation of the presence of ferns in this community was provided by Motyka (1956). This phenomenon may be explained by the fact that the fern was found on the forest margin neighbouring the steep rock wall of the unused quarry, which undoubtedly contributed to the greater amount of light in the forest floor and the dryness of the substrate. Indeed, the unused quarry has provided new open microhabitats in the initially dense forest landscape. This resulted in an increase in the phytocoenotic and species diversity in the reserve (Wójcik et al., 2018; Wójcik & Ziaja, 2019).

The analysis of the individual traits of Botrychium matricariifolium in the new locality showed their mean height of 14.9 cm. Similar results were recorded in other forest communities. Gierczyk et al. (2007) reported a height of fern individuals of 14.7 and 16.8 cm in a study conducted in two periods at a margin of Fraxinus excelsior monoculture in a landscape park in central Wielkopolska. Similar results of measurements of two individuals (12 and 15 cm) were reported by Szczęśniak (1998), who examined a fern population growing in an acidophilic oak forest in Lower Silesia. In turn, the highest fern individuals (over 20 cm) were found by Zarzecki and Wołkowycki (2012) on a side of a sandy path in a pine coppice in Turczyński Forest. In turn, Celka and Kasprowicz (2002) reported a substantially lower height ranging from 7 to 9 cm (sporophore: 3-5 cm; trophophore: 3.3-4 cm) in fern individuals growing in a dense and homogeneous stand with Acer platanoides. In the new locality discovered in the Góra Chełm reserve, the sporophore was similar in size (5.1 cm), but the trophophore was smaller (2.1 cm).

In Poland, Botrychium matricariifolium populations usually comprise from a few to several dozen individuals (Celka & Kasprowicz, 2002; Bobrowicz et al., 2004; Kujawa et al., 2007; Tlałka, 2011; Stosik, 2011; Zarzecki & Wołkowycki, 2012; Beuch & Zawistowski, 2014; Torzewski & Kazienko, 2018). There are also extremely small populations with only two plants (Szczęśniak, 1998; Wilczek & Zarzycki, 2013) and sometimes only one individual (Bobrowicz et al., 2004; Nowak & Nowak, 2008). Currently, the largest known populations of the species in the country are found in two localities in Wielkopolska with 120 and 274 individuals (Czarna, 2002; Gierczyk et al., 2007) and a locality in the Białowieski National Park comprising over 200 individuals (Karczewska, 2010). In this respect, the newly discovered locality in southeastern Poland should be regarded as moderately numerous, as in the localities of the species in other European countries. For example, the populations of the fern in France usually comprise from a few to several tens of individuals (Muller, 1993, 1999). In turn, the southernmost localities in Europe

are extremely small and strongly isolated. For instance, in Hungary, the species was considered extinct until recently. It was rediscovered in 2008 in one locality, where only one specimen was found (Lengyel, 2009). Similarly, only one locality with a single individual has been reported from Montenegro (Stešević & Berg, 2015) and from neighbouring Croatia, where only one *Botrychium matricariifolium* locality has been confirmed with a population size not exceeding 50 individuals (Borovečki-Voska et al., 2011). Another species, i.e. *Botrychium multifidum*, which is characterised by similar abundance, is worth mentioning. Its population size in two localities in Estonia ranges from 14 to 104 individuals (Mesipuu et al., 2009).

The size of Botrychium matricariifolium populations not only is small but also changes with time. Five-year observations carried out in France have proved the high dynamics of a population comprising from 15 to 23 individuals, with only some appearing each year. Other individuals were observable only in some years or disappeared completely. New individuals emerged in the subsequent years of the study, but the population was ultimately shown to be stable throughout the research period (Muller, 1993). Similar results were obtained by Williams and Waller (2015), who studied the demographic dynamics of seven Botrychium species in the Michigan Peninsula in the United States in 2008-2010. In the case of Botrychium matricariifolium, they showed reappearance of only 32-39% of labelled individuals. Changes in the population size were observed also in the case of other Botrychium species in multiyear studies (Johnson-Groh & Lee, 2002; Mesipuu et al., 2009). The difficulties in accurate determination of the population dynamics are related to the specific biology of *Botrychium* species. The growth of Botrychium species depends primarily on the heterotrophic association with specific mycorrhizal fungi. Moreover, Botrychium species can remain dormant for several years and may thus be difficult to observe (Muller, 1993). Additionally, plant development may be disordered through mechanical damage, herbivore activity, diseases, and growth arrest caused by insufficient resources (Mesipuu et al., 2009; Johnson-Groh & Lee, 2002). Drought has been shown to exert a significant effect on Botrychium matriciifolium, which is highly sensitive to long periods of water shortage in May (Muller, 1993). A similar observation was reported in a study of Botrychium multifidum. Low rainfall was found to result in a reduction in its abundance in the following year (Mesipuu et al., 2009). In turn, a significant decline in the Botrychium mormo population was caused not only by drought but also by an invasion of earthworms (Johnson-Groh & Lee, 2002).

Changes in habitat conditions may also pose a threat to *Botrychium*. Studies of large grassland habitats in France have shown that soil fertilisation facilitates development

of competitive and highly productive grasses, e.g. *Arrhenatherum elatius*, *Dactylis glomerata*, and *Phleum pratense*. The growth of lower plants, such as *Botrychium* species, is suppressed or they can be removed before production of spores through early grass mowing. In turn, rapid growth of shrubs (*Sarothamnus scoparius* and *Pinus sylvestris*), which causes disappearance of such less competitive species as *Botrychium*, is observed in abandoned lands. The best way to protect these relatively rare species is to maintain extensive (no fertilisation) farming based on late mowing and to introduce measures to restore the pioneering habitats of the species (Muller, 1999, 2002).

In the light of the above-mentioned studies, it can be concluded that the newly described locality of *Botrychium matricariifolium* in south-eastern Poland is not endangered at present. Although the high compactness of the tree stand may limit the access of light, the location of the population at the forest margin at the unused quarry provides better lighting conditions. It was observed that most of the individuals occurred in more open areas. The proximity of hiking paths and penetration of the forest by visitors may pose a certain threat to the population of this rare species. However, it should be emphasised that the locality is part of the reserve, which ensures effective protection of the species. Additionally, constant monitoring of the locality has been planned.

Acknowledgements

We would like to thank prof. Adam Zając for the list of sites of *Botrychium matricariifolium* and dr Agata Stadnicka-Futoma for assistance in the preparing of Figure 2. The research in the Góra Chełm reserve was granted a permit from the Regional Directorate for Environmental Protection in Rzeszów: WPN.6205.54.2017.ŁL.2.

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