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Population study of some rock endemic and vulnerable species from Romanian Carpathians

PhD Thesis

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CONTENTS OF THE SUMMARY OF THE DOCTORAL THESIS

Contents of the PhD Thesis

INTRODUCTION

In Europe, rocky areas are the main habitat for species of *Draba, Saxifraga, Sorbus, Daphne, Dianthus, Campanula* and *Androsace* genus, and more than one-third of the endemic taxa in the alpine regions of Europe grow only in cracks on steep slopes. The areas populated by rocks are one of the habitats least affected by the anthropic impact, and the biodiversity at their level is impressive.

From the point of view of the specific wealth, Romania is ranked 6th in Europe in terms of the existence of endemic plants. Their presence is closely linked to the numerous transformations suffered by the Carpathian Mountains, especially during the glacial periods.

On the rocks in the alpine areas, the plants have managed to colonize a wide range of places suitable for life, many of them being the habitat of species that are nowhere in the world.

In Romania, of the 11 species of the genus Draba, 4 are endemic to the Carpathian Mountains. All are named after 4 exceptional botanists who contributed to the knowledge of the genus: *D. dorneri* , an endemic species for the Retezat massif, *D. haynaldi*, endemic to South-East Carpathian with distribution in the Ceahlău massif, Bucegi , Piatra Craiului, Piatra Mare, *D. kotschyi*, endemic to the South-East Carpathians, *D. simonkaiana*, which grows only in the Cozia and Parâng massifs.

The present paper is intended to be an incursion into the study of populations ecology of these unique plants. Little known, the life of these species growing on the cliffs of the Carpathian alpine area is extremely fragile, mainly due to the vulnerability stemming from rarity, endemism or human action, which is why the importance of ecology studies will help to better protect them.

Keywords:

Draba, endemic taxon, alpine ecosystems, cliff habitats, fruit and seed morphology, geographical distribution, population ecology, demographic study, genetic variability, alpine plant associations, endemic species conservation.

In Romania four species of the genus *Draba* are endemic to the Carpathian Mountains, as shown above, and *D. fladnizensis*, a critically endangered species is at the southern limit of distribution. Among the many endemic species present in the Retezat Mountains, *Draba dorneri* Heuff. occupies a special place, this being the first endemic species reported for the Retezat Mountains, the first National Park in Romania. Piciorul Colțului (Colții Prelucelor), *locus classicus*, a rocky place located at the upper limit of mountain pine, harbours the small population of the endangered species. Despite the reputation it carries, few studies have had it as a subject.

Therefore, in this study, we tried to validate the existing information and to contribute to the knowledge of the biology of these species. Thus, by filling in the existing gaps in understanding the habitat characteristics and distribution patterns we can find out about the history and evolution of the species. Knowing how they reproduce, testing the viability of seeds through germination experiments, to which a long-term demographic study will be added, will result in a better understanding of their life cycle.

Although there are numerous morphological descriptions of the species of the Brassicaceae family in Europe, there was a lack of information regarding the morphology of the seeds and indirectly the relation between them and the morphology of the fruit in the endemic species of the genus *Draba* from the Carpathians. Thus, this study aimed to evaluate the taxonomic value of these parameters.

Of particular interest was understanding the relationship between 2 very similar species: *D. dorneri* and *D. siliquosa* var. *glabrata*. The first, an endemic species, distributed in the Retezat Mountains, *locus classicus* Colții Prelucelor, the second with a wide spread area. In the immature stage the two are very similar, especially if only the rosette leaf characters are taken into account. In order to clarify the distribution of the *D. dorneri* species, several approaches were considered: the analysis of the ecological characteristics, the establishment of the phenotypic characters with a role in the clear differentiation of the species by reviewing the herbal materials but also in situ, the morphology of the seeds and the fruit, molecular biology studies, phytocenological analysis.

ABSTRACT OF THE CHAPTERS OF THE DOCTORAL THESIS

The present doctoral thesis, named "The population study of endemic and endangered species of rock in the Romanian Carpathians" consists of 9 chapters, within which there is a theoretical framework that deals with the critical analysis of knowledge and an application section.

Chapter 2 covers aspects related to the habitat requirements of endemic and endangered species of the genus *Draba*, with an emphasis on substrate and climate characteristics.

The analysis of the altitudinal distribution showed a grouping of endemic and endangered *Draba* species in the subalpine / alpine vegetation floors, in the range 1500-2500 m (Fig. 1).

The habitat requirements of the species studied showed that it prefers a certain type of geological substrate: granite for the species *D. dorneri*, limestone or calcareous conglomerates (in the Romanian Carpathians), limestone marls (in the Tatra) for *D. fladnizensis*; limestone or limestone conglomerates for *D. haynaldi*, *D. compacta*. The species *D. kotschyi*, *D. siliquosa* have a wide ecological optimum, being able to support populations on both basic and acid rocks such as shale or granite (Fig. 2).

The pedological parameters studied (pH, the content of organic matter) reflect the specific trophic requirements of representatives of the *Draba* genus, and come to emphasize the existence of environments dependent on physical factors.

The climatic conditions that define the habitats of Carpathian *Draba* species are characterized by annual average temperatures between the minimum of -1.81 ° C at the Omul Peak and a maximum of 9.38 ° C at the Petroșani station. If at Parâng, Sinaia and Cuntu stations which are located at approximately close altitudes, the amplitude is low, without significant differences between years, at stations at high altitudes such as Omu and Țarcu, an alternation between years with temperatures below 0 ° C is observed in the years cold and over 5 ° C in the following year. The months with the highest annual average temperatures in the high alpine floor are in the months of July-August, the lowest being recorded in January-February.

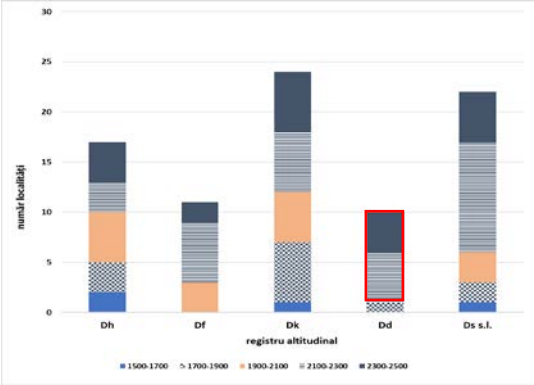

Fig. 1 *Draba* species distribution on a hight gradient according to the number of places where the species was found (personal data from field, and herbarium)

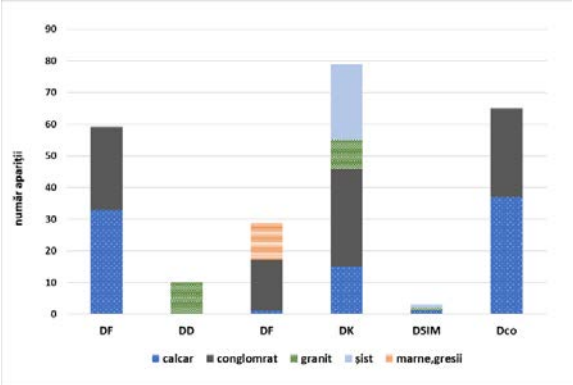

Fig. 2 *Draba* species distribution on a geological substrate gradient according to the number of places where the species was found (personal data from field, and herbarium)

Regarding the average monthly precipitations, they vary depending on the altitude and the advancement of the wet air masses. At the weather station from Omu Peak located at an altitude of 2505 m at the highest point of the Bucegi massif, values between 65-117 mm were recorded, the lower areas on the subalpine or mountain floor from Sinaia and Cuntu being easy higher. At Omu Peak, the highest amount of precipitation is recorded in the summer season, May-August. In the Ceahlău massif, the average amount of precipitation was very low during our research period (below 80 mm).

In the alpine floor the snow persists until May, on sheltered slopes or chimneys this may remain even in June, this having implications in the phenology of alpine species of the genus Draba.

In the Carpathians, over the upper limit of the forest, at altitudes above 1800 m the prevailing winds are the western ones. In the alpine areas above 2500 m (Omu Peak, Bucșoiu Peak) a dynamic of the air currents similar to the conditions in the free troposphere takes place, here the western winds generally prevail 8 months a year (Micu *et al.*, 2014).

Analysis of the spatial distribution of *Draba* species revealed that *D. fladnizensis* according to the slope exposure revealed its presence only on the slopes on the NW, SW and W slopes in the direct prevailing winds and in the ridge / plateau area, its highest points, where it is exposed to all the air currents that cross the massif.

In the climate specific to the alpine area, the microclimatic conditions created by the solar exposure have an important significance, determining the distribution of the saxicole species

according to the amount of solar radiation that the rock receives. The species belonging to the Aizopsis section (*D. haynaldi, D. compacta, D. lasiocarpa*) grow only on the sunny rocks of the subalpine-alpine floor, being part of an extreme group of mesophyte-mesoxerophyte character. These are not found on sunny crevices with evidently higher humidity reserves than the cracks of a massive rock, even if they are near the rock.

Within **Chapter 3**, from the distribution point of view, 3 critically endangered taxa of the genus *Draba* are analysed: 2 endemic *D. dorneri* and *D. haynaldi* and one rare taxa located at the south-eastern limit of the European area, *D. fladnizensis*. Taxa selection was motivated both by the existence of the chronological news and by their biogeographic importance.

Assessment of the distribution of the *Draba dorneri* species in the Carpathians

In the specialized literature, the geographical distribution of the species *D. dorneri* has been debated by botanists for more than two centuries. Starting with Baumgarten (1816) which place it in several massifs of the Southern Carpathians (Piatra Mare, Bucegi, Postăvaru, Retezat) and continuing with those who support its presence in Făgăraș Massif, Parâng or even Ceahlău, *D. dorneri* had over time various variants of distribution. Some have considered it an endemic of the Retezat Mountains, while other botanists believe that the species has a wider distribution area, until now a number of 12 localities distributed in the subalpine and alpine areas of the Southern Carpathians have been reported.

In the field studies carried out by us during the period 2009-2019, all the areas mentioned in the literature were investigated as follows: in Bâlea glacier caldera the species identified correspond to the researches of Drăgulescu (2000), to which we also add the species *D. compacta*; in the ridge area adjacent to the Capra glacier circus: Căprăreasa Peak and Capra Peak we could identify only the widespread species *D. kotschyi*; on the Buteanu Peak the only species present was *D. kotschyi*; along the Buteanu ridge, on the Netedu peak, no species belonging to the genus *Draba* was found.

In 1954, Nyarady A. collects species of *Draba* from the alpine area of the Bucegi Mountains near Omu Peak, the specimens being deposited in Herbar CLA. Our analysis of the herbarium shows that the plants designated as *D. dorneri* actually belong to the species *D. kotschyi* and *D. siliquosa* var. *glabrata*.

The flora of the Bucegi Mountains was carefully documented by Beldie (1967), but *D. dorneri* was not mentioned in any of its publications.

Sârbu and Lupu (1989) indicate from the watershed between Cerbului Valley and Ialomiţa Valley on limestone rocks, at the attitude of over 2000 m. The review of the material from the herbarium collection highlighted the erroneous attribution to *D. dorneri*, the herbalized specimens belonging to the species *D. siliquosa* var. *glabrata*.

Sârbu (2006) mentions *D. dorneri* present in the Important Area for Plants (IPA) Valea Gaura, Bucşoiu Peak, Mălăieşti Valley. Moreover, the presence of the species in the Bucegi Mountains, in the northern part of the massif is all the more unlikely as the habitat of the species from the *locus classicus* is extremely different from that of the Bucegi Mountains (altitude, substrate, cenotic association, climatic conditions). In 2011 *D. dorneri* was listed in the Standard Form of the Natura 2000 Site ROSCI0013 Bucegi as a species in Annex II of the Habitats Directive present on the site. We consider that most likely *D. dorneri* was confused with one of the species commonly encountered on the rocks in the Alpine area of the Bucegi, namely *D. siliquosa* var. *glabrata* or with *D. kotschyi*. In the absence of a herbarium voucher or an exact location, the presence of the species both in the Valea Gaura area, Bucşoiu Peak, Valea Mălăieşti as well as in the area of the Jepii Mari and Jepii Mici mentions in Managemenet Plan of Bucegi Natural Park, remains questionable. Following the inventories and personal observations made during the period 2009-2018, the species was not found in the places mentioned above.

Assessment of the distribution of the *Draba haynaldi* in the Carpathians

During our field visits we were able to confirm the species in all the mountain ranges where it was mentioned.

In the Bucegi massif, 4 historical distribution points were confirmed, 7 unconfirmed, to which 4 new points are added (Caraiman, slope with western exposure; Coștila, entrance to the White Valley; Creasta Morarului (Fig. 3); Șaua Hornurile - Mălăiești Towers).

In the Piatra Craiului massif, the species was confirmed in the points distributed in the ridge area (3), to which 2 are added in the sector between Vlădușca Peak and Țimbalul Mare, but 2 points remain unconfirmed.

During our field trips we were able to confirm the presence of the species *D. haynaldi* in the high area of the massif, where it vegetates on the limestone blocks in the ridge area of the Piatra

Mare massif. The presence of the species could not be confirmed at Piatra Mare Peak. This may be due in part to the very large influx of tourists accessing the peak and possible recent installation of a monument even in the potential habitat of the target species.

Fig. 3 Colții Morarului seen from Valea Morarului (up), the newly discovered population at the base of Creasta Ascuțită and on Acul Mare (in white circle) (a); *D. haynaldi* Crestei Morarului cliff (b); the summit on which *D. haynaldi* is installed, in the white circle are shows the tufts installed in the cracks in the rock (c) (original photo)

During our botanical visits to the Ceahlău Mountains (Panaghia Rock) in 2013 and 2014 we could not reconfirm the presence of the species in this location, but nearby, under Toaca Peak, on conglomerate rocks, we found a significant population of this species (Ion and Ion, 2014). Based on our expertise on the habitat of the species, we believe that, although mentioned only in this part of the massif, the species could be present in other locations with reduced accessibility.

Assessment of the distribution of the species of *D. fladnizensis* in the Carpathians

D. fladnizensis is one of the most widespread species of *Draba*, having a circumpolar distribution in the lower Arctic area and reaching to the mountains of Central and Eastern Europe, the Rocky Mountains and the Himalayas. In the Carpathians we can find populations of this species in Belianske Tatra, a mountain range in the eastern part of the Tatra massif and in the Eastern Carpathians in Rodna and Bucegi (the southeastern limit of the European area).

Following our field trips in 2014 we reconfirmed the presence of the species in the Tatra, the Belianske Tatry limestone massif, at the base of the Ždiarska Vidla Peak, and at several points on Mount Hlupy.

Regarding the presence of the species in the Romanian Carpathians, 5 of the 6 historical distribution points in the Bucegi Massif have been confirmed, to which a new one is added (Cerbului Rock-Saturn Rock). Since the last mention in the Rodnei Mountains on the Ineu Peak (Andreanszky, 1942 – herbarium data), the species has not been found. Following our intense searches conducted in 2014 in the Rodna Mountains, the species could not be identified in the rocky habitat on Ineu Peak.

Chapter 4 includes a detailed study of the morphological characters with a role in the differentiation of the species: trichomes, the fruit and seed morphology.

Based on the types of trichomes on the leaf's margins, the perennial species of *Draba* from the Romanian Carpathians could be delimited in 2 sections:

1. The Aizopsis section for which there are characteristic rigid, simple trichomes, present only on the edge of the leaf. This type of hairs can be found in the species: *D. haynaldi* (Fig. 4, 5), *D. compacta, D. lasiocarpa, D. aizoides*.

2. The Leucodraba section that brings together the species with simple or branched trichomes, present both on the edge of the lamina and on the upper or lower surface of the leaf (Fig. 6). Of the species that belong to this section, only *D. fladnizensis* has simple trichomes present on the edge of the lamina, the rest of the species having branched trichomes.

Within the Leucodraba Section, *D. dorneri* is clearly delimited by *D. fladnizensis* by the presence of the classic pattern of trichomes on the edge of the leaves: branched in the upper half and simple towards the base (Fig. 7).

Both species have glabrous leaves on both sides, with hairs present only on the margin. This model of hairs considered characteristic for the species *D. dorneri* was first illustrated in the work of Štúr (1861) and could be confirmed in the specimens (correctly identified) present in the herbarium collections.

Fig. 4 Rosette leaves and specific type of hairs on *D. haynaldi* (original photo)

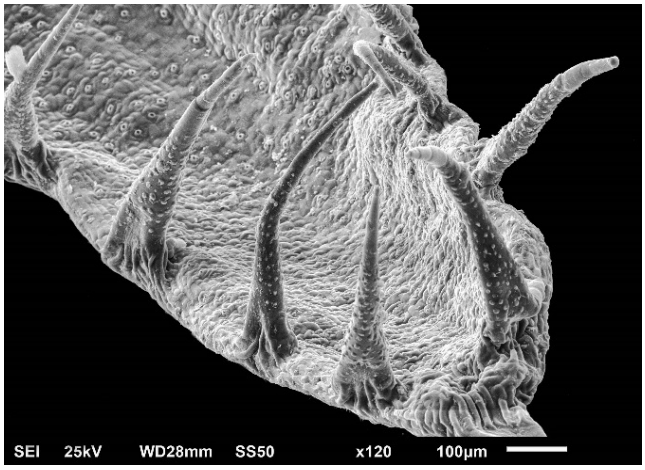

Fig. 5 Detail of simple trichomes on the edge of the leaf at *D. haynaldi* (foto SEM)

Fig. 6 Rosette leaves and specific type of hairs on *D. dorneri* (original photo)

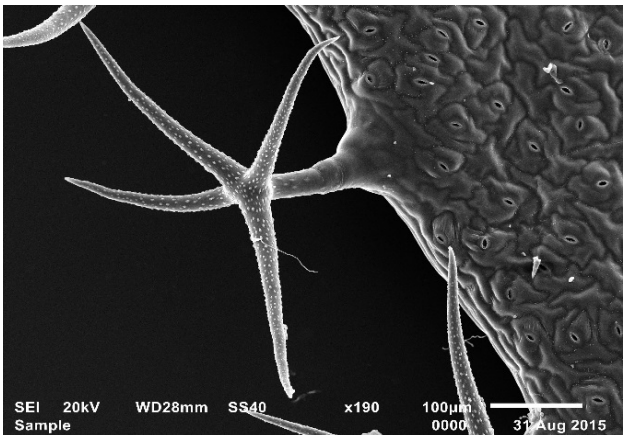

Fig.7 Detail of branched trichomes on the edge of the leaf at *D. dorneri* (foto SEM)

However, this model of hairs *is not unique*, being reported for other subspecific species or taxa within the Leucodraba Section.

D. siliquosa is known for the hairiness from the rosette leaves, which are covered with numerous branched hairs. The leaf lamina is elongated in elliptic shape, slightly narrowed towards

the base and with entire margins. Plants that have these characteristics have been assigned to var. *genuina* Štúr. Koch describes in 1857 a variety of the species *D. siliquosa* (syn. *D. joannis* Host.), namely var. *glabrata* Koch. It has glabrescent or glabrous leaves, with hairy margins, the type of trichomes on the edge of the leaf being simple towards the base and branched towards the tip, similar to the *D. dorneri* pattern.

D. kotschyi is also a representative of the Leucodraba Section, where we can see this pattern of hairs. The typical form of this species being represented by var. *flexuosa* characterized by the presence of simple, Y-shaped or stellate hairs on the edge of the lamina, and the upper face is glabrous, or sometimes has branched hairs, the plant is up to 10 cm high. *D. kotschyi* is separated from the rest of the species by the deep and sharply toothed leaves, hairy stalk and stems, short-styled silicule and smooth valves (Nyárády, 1955). Plants attributed to var. *robusta* presents hard-toothed leaves, and the plant is small in size.

Using for determination only the pattern of trichomes on the edge of the leaves, the glabrous silicule and the obvious style led to the erroneous attribution of some specimens from Bucegi, Făgăraș, Parâng to *D. dorneri*. This fact could be demonstrated by reviewing the herbarium materials and the in situ taxonomic assessment.

Fruit and seed morphology and seed coat micromorphology; implications for species taxonomy

The Brassicaceae family is known for the difficulty of separating the tribes, especially the order of the genera from their composition. The fruits in this family, however, are so diverse that they offer sufficient usefulness in successfully delimiting tribes, genera and even subspecific species or taxa (Al-Shehbaz, 1984).

There were investigated 8 species belonging to the genus *Draba* from 15 populations in the Southern Carpathians in terms of fruit and seed morphology (Fig. 8).

The length of the fruit was closely correlated with the width ($r = 0.6872$, $p < 0.001$). The smallest fruits stand out for the species *D. fladnizensis*, with average values of 2,971 mm in length and 1,643 mm in width. In contrast, the largest fruits were observed in the species *D. haynaldi*, for the plants from the Piatra Craiului population, an average of 7.88 mm being recorded, followed by *D.dorneri* with 6.04 mm.

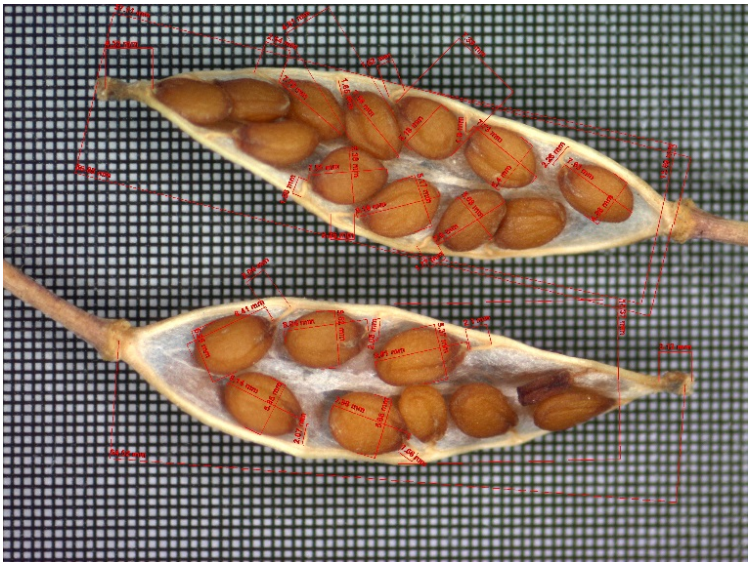

Fig. 8 Stereomicroscope measurements: seeds inside the silicules on *D. siliquosa* (original photo)

The shape of the fruit varied from elliptical elongation, even lanceolate to *D. siliquosa* (L / l> 3; max. 4,595) and *D. siliquosa* var. *glabrata* (L / l> 2,569; max. 4,345) at the elliptical-rounded *D. fladnizensis* (L / l> 1,235; max. 2.5).

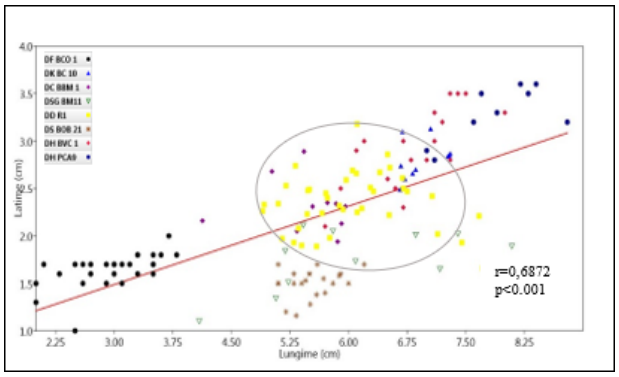

Fig. 9 Linear correlation between length and width of fruit in Draba species from the Southern Carpathians; In the oval shape the species *D. dorneri* is highlighted

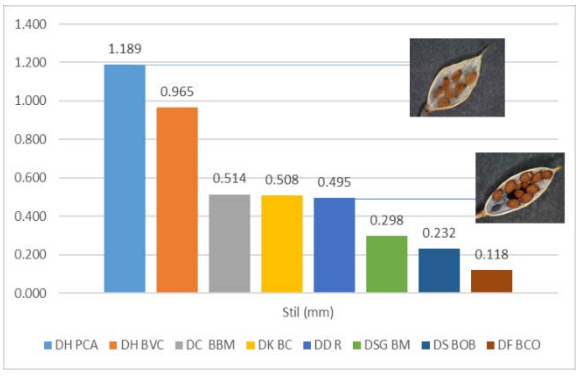

Fig. 10 Comparison between the length of the style in Draba populations in the Southern Carpathians

Although no significant differences are observed between the species *D. dorneri* and *D. siliquosa* var. *glabrata* related to the length and width variables, the 2 species differ in the shape

of the fruit, narrowly elliptical to *D. siliquosa* var. *glabrata* due to the small and elliptical mean width in *D. dorneri* (Fig. 9).

The analysis of the similarities between the analyzed species showed a significant correlation between the size of the fruit and the length of the style. The longest style species was *D. haynaldi* (1.1-1.5 mm), the smallest being noted in *D. fladnizensis* (0.1-0.2 mm). In the D. *dorneri*, the style recorded values between 0.4-0.728 mm, the average being 0.495 mm. Unlike *D. siliquosa* in which the style is very small (0.2-0.3 mm, average 2.232 mm), in *D. siliquosa* var. *glabrata* style has higher values (0.13-0.39 mm, mean 0.298 mm) (Fig. 10).

Related to the average number of mature seeds present in silica, the species with the most seeds were *D. siliquosa* (20 seeds / silica) and *D. siliquosa* var. *glabrata* (16,182 seeds / silica). At the opposite end is *D. fladnizensis*, which due to the reduced length of the silicule, the average number of seeds was 6,286 seeds / silicule.

In the fruiting stage, the pedicel of the fruit may be an important taxonomic character (Khalik, 2002). A significant correlation was recorded between the size of the fruit and that of the pedicel of the fruit, the species with the longest pedicel, *D. haynaldi* (average 7.004 mm) being the one with the longest fruit.

The analysis of similarities between the analyzed species showed a significant correlation between seed length and width ($r = 0.86212$; $p < 0.001$). The smallest seeds are highlighted in the *D. siliquosa* complex, the *D. siliquosa* var. *glabrata* (DS BO) having average values of 0.808 mm long (sd = 0.533) and 0.474 mm wide (sd = 0.038). In contrast, the largest seeds were observed in the *D. haynaldi* species, for the DH BVC population with an average length of 1.405 mm (sd = 0.673) and a width of 0.838 mm (sd = 0.517)

The size and mass of the seeds was poorly correlated with their degree of ploidy. This can be seen in the Leucodraba Section, where the tetraploid species *D. dorneri* and *D. kotschyi* had the highest weight in the group. The species from the Aizopsis Section, although diploid, had the highest mass, with *D. haynaldi* recording an average of 0.217 mg / seed.

A negative correlation between the size of the seeds and their number in fruit was observed. In the case of the Draba species analyzed, there is a trade-off between these two variables: *D. siliquosa* species with the smallest seeds, also produces the highest number of seeds (22,429 seeds / fruit).

The analysis of the main components (PCA) of the measured variables (length, width, length / width ratio) in *Draba* populations in the Southern Carpathians shows a grouping of species according to the two major sections. The group consisting of the species of the Aizopsis section comprises species that differ in larger seed sizes, long pedicel and large mass. The second group includes the species of the Leucodraba Section characterized by smaller dimensions, small pedicel and obviously reduced mass as opposed to the previous group. The endemic species *D. dorneri* has a greater similarity to *D. siliquosa and D. siliquosa* var. *glabrata*.

The primary micromorphology of the seed coat is glabrous is rough and composed of cells of different shapes. The density and size of cells vary depending on their location. In the endemic species *D. haynaldi* on the edges of the seed and on the apex are observed the cells with the largest size (Fig. 11).

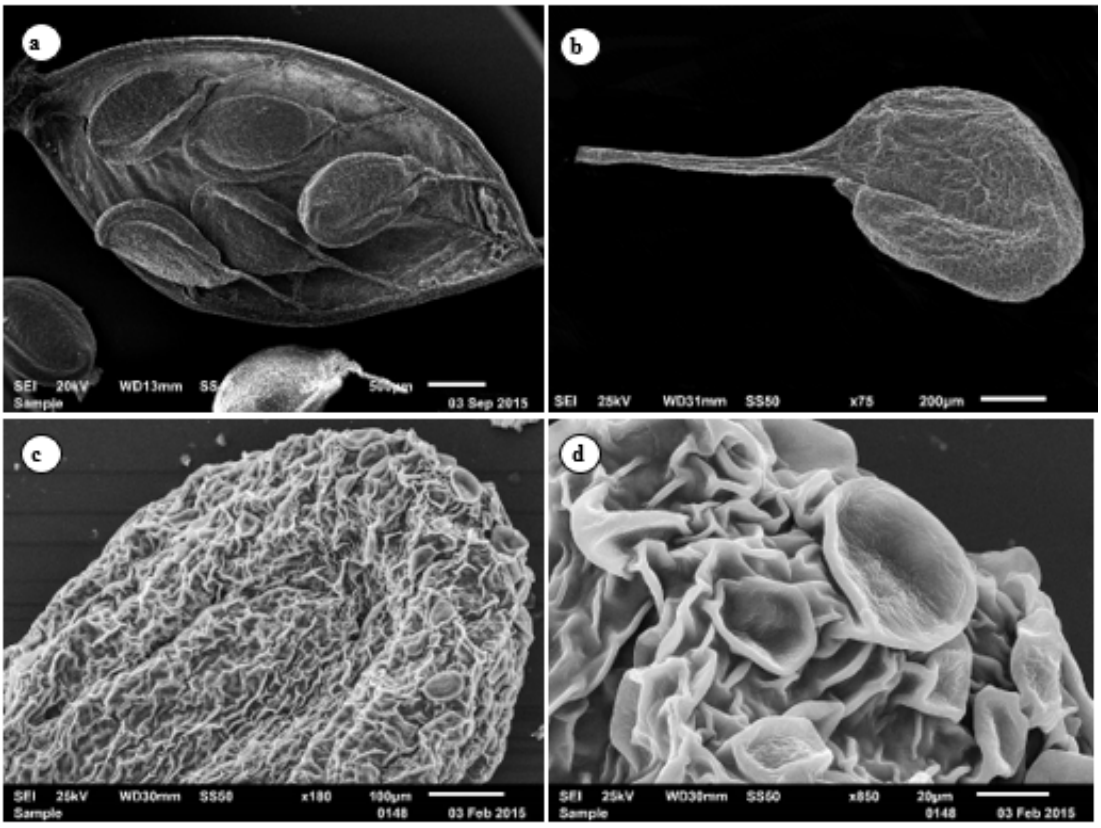

Fig. 11 a-d SEM Photo Micromorphological aspects of the seed coat of D. *haynaldi*

The yellow-flowered species of the Aizopsis Section have a similar secondary structure, with irregularly shaped anticlinal walls delimiting large ornamentations (max. 675 μm²), following a reticulate-papillate pattern in the *D. haynaldi* species and reticulate in *D. compacta*. In the species of the Leucodraba Section, the cellular arrangement follows a cross-linked pattern with thickened cell walls and a central area with a rounded, flat appearance, collapsed in *D. siliquosa* and *D. dorneri* or convex in *D. kotschyi* and *D. fladnizensis*. *D. siliquosa* var. *glabrata* of the cell walls is thickened and the papillae are absent.

Chapter 5 outlines reproductive traits as a way of adapting to life in the alpine environment.

Most species of the genus *Draba* are autogamous, but if the weather conditions favor the activity of pollinators, cross pollination can also be achieved (Mulligan and Findlay, 1970; Brochmann, 1992). The flowers of individuals of the *Draba* genus are adapted for cross pollination (Schulz, 1927). The species *D. dorneri*, *D. fladnizensis* and *D. haynaldi* are considered to be autogamous and allogamous through entomophilia (Dihoru and Negrean, 2009).

Protogyny is considered a mechanism that favors cross-breeding (alogamy) by maturing the stigma before the pollen is released into the same flower. Protogyny is important for the survival of the population when other mechanisms favoring allogamy are lacking or ineffective or when the gene bank is reduced. In extremely autogamous species, even a small percentage of the type of pollination resulting from protogyny, it may be all that is needed to produce some genetic variation based on which natural selection may work (Al-Shehbaz, 1977).

Our observations in the field involved the recording of the following specific features of the allogamy: protogyny, the presence of pollinators, the structure and position of the floral elements. The phenological specificity, the floral characters that encourage the attraction of insects, were also followed.

Based on studies on individuals of the *Draba* genus belonging to 12 populations, we can conclude that most of the flowers were identified as protogines *sensu* Kerner, the stigma being exposed only by incomplete open flower, no cases of sensu Al-Shehbaz protogyny being observed. In both the diploid species *D. fladnizensis* and the tetraploid *D. kotschyi*, the type of protogyny is similar to that observed in *D. dorneri*. In *D. kotschyi* at the opening of flowers, the position of the stamens is at the same level as that of the stigma, the proportion of stigma covered with pollen after self-pollination being about 50%.

D. compacta and *D. haynaldi* have non-protogynous flowers, the length of the petals larger than that of the previous species does not allow the stigma to be exposed, being available for pollination only after the flowers have opened. The superior position of the anthers to that of the stigma was observed in both species, even after flower opening. In *D. haynaldi*, shortly after the opening of the flowers (yellow) the stamens become dehiscent. After pollination occurs, the petals turn white. This unique change in color can lead to taxonomic identification errors if the phenology of the species is not sufficiently known.

Insect attractants. For the length of the petals, the average in the population ranged from 2.55 mm in *D. siliquosa* to 4.91 mm for *D. haynaldi*, and for the width of the petals from 1.21 mm to *D. kotschyi* to 1.90 mm for *D. haynaldi*. Thus, the maximum surface area of the petals was recorded in the species *D. haynaldi* (37.56 mm2), and the minimum one in *D. kotschyi* (13.12 mm2). The average number of open flowers / inflorescence ranged from 1.2 for *D. haynaldi* to 6.2 for *D. compacta*. This last parameter influenced the surface of the petals / inflorescence so the maximum exposure of the petals was for *D. compacta* (42.65 mm2 / inflorescence), and the minimum one for the species *D. haynaldi* (11.26 mm2 / inflorescence).

In the case of the *D. dorneri* species, we observed with an increasing frequency representatives of the Formicide family, the genus *Myrmica* visiting the flowers. They are attracted by the nectar produced by the nectariferous glands at the base of the short stamens. Analyzing both the floral traits of the species *D. dorneri* and the behavior of the ants in its habitat, we can say that the ants can be pollinators, being simply a case that reflects the local ecological conditions.

From our field observations, the species of the genus *Draba* taken in the study, bloom early, at the beginning of the vegetation season, shortly after snow melting and defrosting of the soil. The average flowering time varies between 18 days in the species with distribution at very high altitudes and 31 days in the species *D. dorneri* and 26 days respectively in *D. haynaldi* in the population of Piatra Mare, the one at the lowest altitude. We consider that the early development of flowers and the duration of flowering is short because in early spring, on the alpine floor, pollinators have access to a reduced number of plant species, visiting the studied species more often. Later, more species of plants bloom, which makes the offer for pollinators much larger, and they could avoid *Draba* species that are small and insignificant in flower density.

Our germination experiments showed that seeds of *D. dorneri* subjected to preliminary treatments on MS + 30g / L sucrose with GA3 resulted in 70% germination on 80mg / L GA3 and MS + 30g / L sucrose at different pH values.

In **Chapter 6** the population of the species *D. dorneri* was analyzed under demographic aspect.

Characterizing the demographics of a population is the first step towards understanding how it persists over time and is the way to identify key stages of plant life that contribute to population growth and maintenance (Larkin and Salzar, 1992; Morris and Doak, 2002). Rare plants usually grow in small populations, which is why any fluctuation in the number of individuals represents a potential threat to the survival of the species.

From our measurements, the population comprises a fairly stable number of individuals (128 - 134). Thus, the population size is stable, with relatively low variations over the years: 134 tufts counted in 2010, 132 in 2014 and 128 in 2019. The total number of rosettes (ramets) in the tufts (genets) inventory varied from 4277 in 2010, to 4791 in 2014 to 5749 in 2019 (to which we added new tufts recently discovered).

The number of juvenile individuals is very small, the successful installation of new plants being a rare event, the population being thus maintained by mature individuals found in each inventory. Non-reproductive adults make up a constant percentage (21-29%) of the total number of individuals and are made up of 15-17 vegetative rosettes, the tufts with the largest number of rosettes (86) is installed on the western face of the rocky blade, in a shaded area for most of the day.

From our data it follows that reproductive adults represent the main category that forms the population of the species *D. dorneri*. Their number remains constant over the years of observation.

The number of rosettes that form the tufts can vary between 1 and 230, both the number of fertile rosettes and that of vegetative rosettes is correlated with the total number of rosettes that make up a tuft in 86%, respectively 82% of cases.

The division of individuals (tufts) according to the number and type of rosettes (vegetative or fertile) components revealed that fertile rosettes represent a high percentage of the total rosettes in all size classes. About 50% of the total number of bushes fall into the first size class (1-20

rosettes). Plants in the upper classes, although in small numbers, contributed over 40% of the total number of fertile rosettes.

The entry of new individuals into the population, respectively the number of juveniles, is very low, in 2010 being observed only 11.

Outflows of individuals from the population occur rarely, during the observation period the number of bushes remains constant. Possible causes can be both natural (strong winds, cracking and dislocation of rocks, accidental deterioration caused by the presence of bears in search of ants' bites) but also anthropic (rock climbing by tourists). There is a more obvious variation in the number of juveniles who may have a high mortality rate. Thus, the entry of new individuals into the population is not maintained, so the longevity of the population depends on the mature individuals already established.

The analysis of the coordinates of all the individuals (tufts) of D. dorneri identified and located on the rocks at Colţii Prelucelor showed that they have an aggregate spatial distribution. Given that the cenotic environment is preserved, their density was independent of the type of plant association. In contrast, this density depended on the horizontal extension of the tufts, the diameter of the tuft being correlated with the total number of rosettes in the tuft ($r2 = 0.74$, $p < 0.005$).

Reproductive success. The average number of viable seeds / fruit (11.87) compared to the average number of ovules / fruit (14.23) in the context of a low number of seeds (0.31) and completely undeveloped ovules (2.37), indicates a high ratio S: O = 0.85, thus a high reproductive success.

The analysis of the correlations between the characteristics of the habitat, the fruit and the seed with the reproductive success of the species, showed a positive correlation between the length of the fruit and the reproductive success, $r = 0,508$, $p < 0.001$. Also, as expected, the number of viable seeds is correlated with a great reproductive success $r = 0.635$, $p < 0.001$.

A positive correlation can also be observed between the length of the fruit and the number of viable seeds $r = 0.780$, $p < 0.005$, so the cost of larger seeds obviously implies the investment in a larger fruit. The length of the fruit was negatively correlated with the number of eggs aborted. Pre-abortive abortion (aborted eggs) was observed in 71% of the fruits, and the post-zygotic (aborted seeds) was observed with a frequency of 21%. 8% of the analyzed fruits contained only viable seeds. The abortion rate of ovules was 88.43 (50% <ROA <100%), *D. dorneri* spices having fruits with more aborted ovules than aborted seeds).

The higher rate of abortion of eggs has reduced the costs of fruit development, fruits that abort only seeds are more expensive than those that have aborted only eggs (Calvino, 2014). Studies of fruit from *Arabidopsis* have shown that abortion of eggs before fertilization allows the plant to redirect conserved resources to other floral organs (Sun *et al.* 2004).

Chapter 7 discusses the characterization of the integrating communities of the species *D. dorneri, D. haynaldi* and *D. fladnizensis*. These were analyzed under the ecological aspect (indices of soil moisture, air temperature and soil reaction), the spectrum of bioforms and their lifespan, geo-elements, karyological spectrum and vegetation cover.

The studies by Doniță *et al.* (2005), Onete and Ion (2010), Nicoara *et al.* (2019) includes the association of plants with *D. dorneri* in *Asplenio trichomani-Poetum nemoralis* Boșcaiu 1971.

The specific richness of this phytocenosis is reduced. The association is made up of few species, among them *Poa nemoralis* as the dominant species. The species with high constancy and characteristic of the Class *Asplenietea trichomanis*, *Silene nutans* subsp. *dubia*, respectively *Thymus praecox* subsp. *polytrichus*. Beside this are species adapted to the habitat conditions of the rock with the prevalence of the species *Juncus trifidus, Sedum annuum, Thymus praecox* subsp. *polytrichum, Silene nutans* subsp. *dubia, Symphyandra wanneri.*

From a phytogeographic point of view, the species identified on the rocks at the Colţii Prelucelor present a variety of geo-elements that reflect the climatic, geographical and ecological conditions of this region. The origin of the species with general distribution in the circumpolar area had the highest percentage, reflecting the past of these mountains from the glacial periods.

Following the ecological study, it was pointed out that *D. dorneri* grows in communities where the vegetation cover varies between 10-90%, depending on the heterogeneity of the habitat. In the areas where the individuals were installed in the cracks of the high slopes, on the vertical or even overhanged faces, the vegetation cover had low values. In the "ridge" area of the rocks, or where the rock material has strongly degraded and deposited on the small cracks and shafts, so where the slope was less than 60 °the coverage was higher.

Of the 6 biological forms identified, we note the dominance of hemicryptophytes with 52% representativeness, followed by chamefites with 20%. In the investigated area the species present on the rocks of the Colții Prelucelor have a wide ecological optimum, these being distributed from the oak forest area to the subalpine and alpine area. The height of the vegetation in the analyzed

valleys was divided into size classes, the category comprising the species with the height between 10-30 cm and 30-70 cm has the most numerous species. Depending on the humidity index, the highest weight was in the category of xero-mesophytes (44%), followed by that of mesophytes (40%) and xerophytes (4%). 8% of the species are meso-hydrophytes and are represented by the *Asplenium viride* species present on the northern facing rock faces near the shrubs and the *Hypericum maculatum* species characteristic of the juniper trees but which penetrates to the limit of the rock.

In terms of temperature indices, most species are microthermal (44%). There is a slight affirmation of the species hekistotherms (28%) and mesotherms (20%). From a karyological point of view, there is a slightly higher percentage of polyploid species (44%) compared to diploid species (40%). The ploidy index is 0.90.

The CCA analysis highlights a close relationship between the terrain parameters, such as the rock type, the rock exposure and the slope. The species *D. dorneri* is often associated with *Festuca ovina*, mainly present on the test surfaces, *Symphyandra wanneri, Poa nemoralis.*

In the Piatra Mare Mountains, *D. haynaldi* grows within the association *Seslerio haynaldianae -Caricetum sempervirentis* Pușcaru et al. 1956, installed on the limestone rocks distributed at altitudes of over 1600 m. In the Piatra Craiului massif, the association was found on the rocks and the cracks of rocks in the ridge area. The characteristic and dominant species is *Sesleria haynaldiana*. It grows in compact and very thick tufts. The maximum height of the vegetation is about 30-50 cm, the most vigorous species being *Sesleria haynaldiana*. Also, in Piatra Craiului *D. haynaldi* is mentioned in *Saxifrago demissae-Gypsophilletum petraeae* Boșcaiu and Täuber 1977. In Bucegi and Ceahlău the species vegetates in *Artemisio erianthae - Gypsophiletum petraeae* Pușcaru et al. 1956.

Phytocenological surveys in *D. fladnizensis* habitats were located in the alpine area of the Bucegi massif, in the central sector. We note the presence of the species *D. fladnizensis* for the first time in the associations *Artemisio erianthae - Gypsophiletum petraeae* Pușcaru et al. 1956, where it was found vegetating alongside *D. haynaldi* and *Oxytropido-Elynetum* (Pușcaru *et al*., 1956) Coldea 1991. On the northern peaks, *D. fladnizensis* can rarely occur within the *Saxifrago moschatae - Drabetum kotschyi* unit Pușcaru et al. 1956.

The vegetation cover has a low percentage. This signifies both the preference for a certain cenotic environment and the fact that the species is not competing *sensu* Grimme (1979) but rather a stress-tolerant.

In **Chapter 8** the intra-populational variation of the species *D. dorneri* is treated and the investigation of the phylogenetic relationships between several related species of the genus.

Using the RAPD method, we evaluated the genetic diversity for *Draba dorneri*, a critically endangered species in Romania. From the 12 selected primers, we obtained a total of 77 reproducible bands, with an average of 6.41 bands per primer. The similarity between the samples collected from the two sides of the rock is 67%. The large number of polymorphic loci obtained indicates an increased genetic polymorphism of individuals of the species *D. dorneri* from the analyzed population, compared to other endangered plants. Despite this, *D. dorneri* has a very poor dissemination, the genetic polymorphism being very large for a population composed of a small number of individuals.

Investigation of phylogenetic relationships between representatives of the genus Draba in the Alpine Carpathian area. From our analysis it was found that *trn H* is a suitable marker to be able to distinguish between representatives of different sections. In the present case, the Aizopsis Section, characterized by leafless stalk, yellow flowers, narrow and stiff leaves, oval silicules with acute valves, is delimited by the Leucodraba Section defined by foliar stems, white flowers, without nectariferous glands on long stamens and silica in obvious style. Also, within the Aizopsis section it can separate *D. haynaldi* from *D. compacta* with a high degree of certainty.

The exploration of phylogenetic relationships within the Leucodraba Section, highlighted the separation of the species *D. dorneri* from the species *D. siliquosa* var. *genuina* (typical species) and *D. siliquosa* var. *glabrata*.

This is of particular taxonomic importance as it comes to confirm the hypothesis launched at the beginning of this work, namely the presence of the species *Draba dorneri* only in the Retezat massif.

Chapter 9 discusses conservation strategies for endemic and endangered *Draba* species in Romania, restrictive factors that may affect populations and recommendations for improving management measures in protected natural areas, where *D. dorneri* can be used as a case study for others rare, endemic and vulnerable species that have similar conservation needs.

Emblematic for the Retezat Massif, *D. dorneri* is the first reported endemic species for the first national park in Romania, Retezat National Park, established in 1935 at the initiative of Professor Alexandru Borza. Since 1979, at the international level, the park has been nominated Biosphere Reserve.

D. haynaldi and *D. fladnizensis* are protected within the sites of community importance within the European network Natura 2000 ROSCI0013 Bucegi and Bucegi Natural Park, ROSCI0194 Piatra Craiului and Piatra Craiului National Park, ROSCI0195 Piatra Mare and ROSCI0129 Ceahlău and Ceahlău National Park.

As reported in the specific assessment of Article 17 of the Habitats Directive, from the conservation point of view, for *D. dorneri* two protected areas are defined at European level, and these are found in Romania, respectively Retezat ROSCI0217 and Bucegi ROSCI0013.

The evaluation of this statute was carried out with the application of the methodological guide and of the specific tools made available by the European Commission. In the synthetic report of Romania published in 2015 (Fig. 12), the assessment of the conservation status for the alpine bio-region is presented, where the 4 specific parameters are evaluated: the distribution area, the population, the species habitat and the evolution prospects. As for the distribution area, they are integrated with the presence of species 4 of the 10x10 km^2 reporting unit, considering a potential area of 400 km^2 that is in a favorable state. As for the population, it is considered to be inadequate due to the small number of locations and individuals. The status of the characteristic habitats is also evaluated by experts being located in the alpine area where no activities other than those specific to tourism are conducted.

2113 *Draba dorneri* Heuff.
Denumirea populară: Flămânzică
Regiunea biogeografică: CON
Directiva Habitate: Anexele IIb şi IVb
OUG 57/2007 (Legea 49/2011): Anexele 3 şi 4A

Evaluarea generală a stării de conservare în România:
Inadecvată cu tendință necunoscută

Bioregiunea / Parametrul	ALP	CON	PAN	PON	MBLS	STE
Areal (km²)	400 FV	n/a	n/a	n/a	n/a	n/a
Populație	UI	n/a	n/a	n/a	n/a	n/a
Habitatul speciei	FV	n/a	n/a	n/a	n/a	n/a
Perspective	UI	n/a	n/a	n/a	n/a	n/a

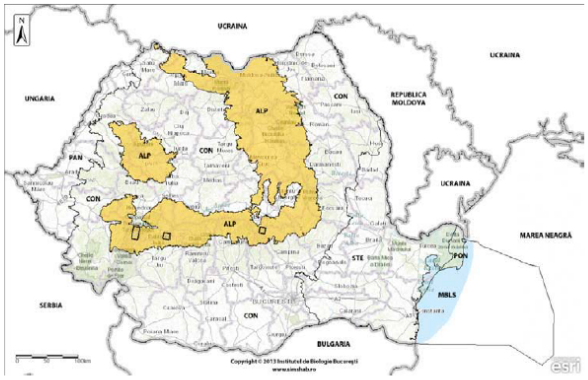

Fig.12 Excerpt from the synthetic report of Romania regarding the 2007-2012 reporting according to Art. 17 for the species *D. dorneri* (Mihăilescu *et al.*, 2015)

Inadequate conservation status is mainly due to the confirmation of a stable population, but with a very small spatial distribution and consisting of a few individuals.

The identified pressures that also give the classification inadequate are due to the development of the access roads and the poor management of the existing ones (tourist paths in the alpine area). And in terms of threats, we can mention those represented by the development of access roads and recreational means, the expansion of native species and the adaptation to climate change.

Currently, Romania has finalized the report for the period 2012-2018 which will allow us when it will be published to compare and evaluate the evolution of the population of the species *D. dorneri*.

Proposed conservation measures:

• Management of tourist routes within the protected natural areas

• In the case of the *D. dorneri* species, the restriction of access to the rocks from the Colţi Prelucelor. This can be achieved by diverting the tourist route and installing new indicators.

• Monitoring of endemic and endangered species of the *Draba* genus and monitoring the demographic tendency to signal possible threats

• Initiation of *in vitro* conservation programs for endemic and endangered *Draba* species from the Carpathians

• Maintenance of areas with characteristic alpine rock vegetation

• Restricting the collection of endemic and endangered species of the genus *Draba* from the alpine area for personal collections

• Management of native species with invasive potential (*Pinus mugo, Juniperus communis*) in the perimeter of the *D. dorneri* population

• Regulation of the building construction in the potentials rock habitats for species of the genus *Draba*

GENERAL CONCLUSIONS

The alpine cliffs are a biodiversity refuge for the native flora, representing ancient, relics, stable habitats that have provided over time shelter but also a place suitable for the emergence of new species.

Communities in which *Draba* species vegetate are often exposed to significant climatic extremes. *Draba*, along with accompanying species, cope with both the lower temperatures on the slopes exposed to wind, lacking the protective layer of snow in winter, but can tolerate the drought of summers in the harshest environment that the mountain can offer.

In the Carpathian habitat of *Draba* species, the climate is moderately cold and humid, typical of the vegetation floor, the annual average temperatures at the Omu peak station are often negative, the total amount of precipitation is over 1100 mm / year, with lower values on the slopes. Norse.

We can view the colonization of these adverse habitats as occupying free niches by *Draba* species, which not only can tolerate the severe stationary conditions but use this as an advantage. Producing seeds of small size and without special structures for dispersion, growing on the highest points of an area and braving strong winds helps them disperse the seeds through anemochory. Moreover, in the case of *D. fladnizensis*, the distribution according to the sun exposure showed that it only grows on the slopes exposed to the prevailing winds.

D. haynaldi, an endemic species for the Romanian Carpathians has a more or less discontinuous distribution, the suitable habitat is in the subalpine / alpine floor of the Carpathians. Its distribution includes a group of populations grouped in the SE part of the Carpathians (Bucegi, Piatra Mare, Piatra Craiului) and a peripheral population, in the Ceahlău massif.

The presence of the species was confirmed in all the massifs where it was mentioned, where it appears sporadically on the limestone and sunny cliffs of the alpine floor. In Bucegi, 4 new points were added, thus completing the range of distribution of the species within the massif.

D. fladnizensis. The presence of the species could be confirmed in the historical resorts of the Bucegi massif, in 5 distribution points, (of which a new one - the Șaua Cerbului, Saturn Rock) and 2 in Belianske Tatra. On the Ineu peak of the Rodnei Mountains, from where it was first noted in the Romanian Carpathians, the species was not found.

This study supports the use of morphological characters of fruits and seeds in differentiating Draba species. The shape and size of the fruit, the length of the style and the pedicel, have proved to be important characters in distinguishing Draba species with similar morphologies. The description of these characters together with the presence /absence and the type of bristles will help accurately border the species within the genus.

In conclusion, the most important characteristics of the fruit in the classification and delimitation of *Draba* species from the Eastern Carpathians are: fruit length, ratio between length and width, style dimensions, number of mature seeds, pedicel of the fruit, number of immature seeds.

Identifying the species of the genus *Draba* can be made only with complete plant material that includes ripe fruits, in the absence of which the species can be mistakenly identified.

The SEM visualization of the seeds of the species of the genus *Draba* from the Eastern Carpathians revealed a cross-linked cellular organization. The micromorphology of the seed surface with aspects related to the arrangement of cells and their particular details and even their size can be used to successfully diagnose the two groups studied, the Aizopsis and Leucodraba Section. At a specific level, however, this delimitation must be done with some caution.

All 7 species taken in the study reproduce sexually, self-pollinate, and occasionally cross pollination is observed.

Of the traits that promote allogamy, the protoginy *sensu* Kerner was observed in the species *D. dorneri, D. kotschyi*, *D. siliquosa* s.l, the stigma being exposed by the incomplete open flower with stamens positioned at it. The flowers of the species *D. haynaldi* and *D. compacta* are non-protogynus, the stigma is exposed only by the fully open flower.

Species of the genus *Draba* with distribution in the Carpathians have an early flowering, as soon as the snow layer melts. The average flowering time is very short and varies from 18 days in the species on the upper alpine floor to 31 days in species distributed in the subalpine floor. This highlights the adaptation of these species to the harsh conditions of the rocks and the alpine / subalpine environment.

The size of the petals varied substantially within the genus, with the maximum area being recorded at *D. haynaldi*. However, this species has a reduced petal exposure due to the small number of flowers open at the same time / inflorescence. However, *D. haynaldi* was the species

with the most intense smell of flowers, which led to the visit of many pollinators, despite the short flowering duration (18-26 days).

Petal exposure registered an intermediate value for the species *D. dorneri* and the smell of the flowers was weak, but the effect of the altitude allows the development of bushes with a large number of flowering stems , so with an increased density of flowers, which attracts insects from Family Formicidae, close to the population of *D. dorneri*. Previously reported as inefficient pollinators, our observations advocate flower pollination by ants, the plant breeding system correlating with habitat specificity.

The germination experiments showed that the seeds of the species *D. dorneri* prefer a medium with acidic pH, confirmed by the pH analysis of the soil collected from the growing area of the species. The fact that the seedlings resulting from the seeds germinated in laboratory conditions did not survive regardless of the nutritional environment they were transferred, further studies are needed in the future.

Our studies have shown that the population of the endemic species *D. dorneri* that grows on the rocks of the Colţii Prelucelor consists of an extremely small number of individuals, but that remains constant in each inventory. Adult reproductive plants represent over 70% of the total number of individuals. The population of the species *D. dorneri* registers a small number of juvenile plants which indicates a failure of seed installation and a high mortality rate of juveniles, the persistence of the adult individuals being the key factor in the survival of the species.

The ecological causes of population size are reflected in the small surface area of the habitat (100 m^2), and the reduced availability of places for the establishment of new individuals.

The only known population (Colţii Prelucelor) is located in a relatively isolated location, separated by large distances from the potential habitats of the species, and the colonization of new places is either above the dispersal power of the plant or the transport capacity from the site is low.

Despite high reproductive success (large number of viable seeds relative to the total number of eggs), both the dispersal capacity of the plant and the establishment of new plants is low.

D. dorneri is a perennial species, our studies show that it can persist for a long time in the same resort which characterizes it as a stress tolerant species and with a long life in relatively stable habitats.

Our research of the area allowed the identification and classification of the species taken into study in associations of alpine rocks, being indicated the belonging to new associations, which have not been recorded so far in the literature.

The plant associations of these endemic and endangered species of rock are also endemic or with a limited distribution in the Carpathians. A large number of species that are part of these associations are local or Carpathian endemic, rare or vulnerable. The mountainous massifs in which these associations are distributed represent endemic and conservative areas of great scientific importance.

The main components of the grass layer on the cliffs, in terms of bioforms are hemicryptophytes and chameftes, and the majority of species are perennial. The species in these associations are xeromezophile and mesophilic, cryophile, microterme and amphitolerant.

In the distribution areas where the species *D. fladnizensis* is present, we could highlight the presence of a large number of mesophilic species up to the mesohigrofiles, distributed on the rocks with various exposures, but with the predominance of W, SW, NW in the prevailing mass winds.

The study of intra-population genetic variability performed on individuals of *D. dorneri* from the Retezat massif revealed that from a genetic point of view the species has a high polymorphism. Demographic studies in the case of the *D. dorneri* species have shown that although the population size is small, it remains constant from year to year. The monitoring of the only known population so far, isolated on granite rocks from the Retezat massif, has highlighted the persistence of mature elderly individuals despite a low seed dispersal capacity. This explains the great genetic diversity observed in the case of the species, to which is added the isolated position of the Peak Hills within the massif, the events that took place during the glacial periods that could have caused a separation of the population and therefore the opportunity for speciation or refuge.

Our analyses showed 2 groups of Draba (Aizopsis Section and Leucodraba Section) were clearly separated according to the traditional mode of recognition (classical taxonomy) by means of determination keys (Sârbu *et al*., 2013, Ciocârlan, 2009, Nyarady, 1955). Within the Aizopsis Section *D. compacta* and *D. haynaldi* were clearly delimited as well-defined and closely related taxonomic species.

Molecular data support the separation of species *D. dorneri* from the rest of *Draba* species related to a high degree of certainty.

The results of our molecular analysis by sequencing some plastid regions fed the international database, thus providing researchers with the possibility of using barcoding to identify new species of *Draba*.

The lack both in the flora of our country and in the whole Carpathian of *Draba* species with high degree of ploidy (maximum degree of ploidy: tetraploid) and the small number of existing polyploid species (3 species) come to reinforce the idea of existence in this region. of some ancestral species, which contributed to the diversification of the present species.

We mention that the current doctoral program being a sustained program for a longer period of time and totally focused on *Draba* species will unequivocally contribute to the consolidation of information for better protection of them.

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