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Studies on some mountain plants encountered in low altitude refugia from Transylvania

- PhD Thesis Summary -

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Thesis content

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Introduction

The presence of glacial relicts in Romania has had a long debate among botanists and biogeographers. Emil Pop was one of the first scientists to debate the presence of these plants in the Southeastern Carpathians, especially in his comprehensive work on peatlands in Romania (Pop, 1960). In the Carpathian Region, glacial relicts are one of the oldest components of the regional flora in Central Europe (Dítě et al., 2018).

The term "glacial relict" refers to species that were common during the glacial periods and disappeared as a result of the Holocene warming. They survived the interglacial periods in so-called refugia. The refugium is a geographical region or a local microhabitat where these species have found similar ecological conditions to the cold period and a climatic stability that has allowed them to survive for millennia in the same location (Csergő, 2002).

Two types of refugia can be discussed: glacial and postglacial (i.e. interglacial). In recent years, most of the work has focused on glacial refugia. However, the main theme of our study is the second category, namely the postglacial or interglacial refugia. They favored the survival of plant and animal species that were common during the ice age, which, after the climate warming, retreated to the high mountains and the Arctic and boreal regions of the northern hemisphere. Some of them have completely disappeared or survived through a few isolated populations.

In the Central European flora, boreal peatland species and arcto-alpine species are considered glacial relicts (Dítě et al., 2018). At the same time, most paleobotanical evidence is more available for peatland species (Hájek et al., 2011; Hájková et al., 2015). However, there is growing evidence to suggest that many of the current species, common in the Last Glacial, are not only the Arctic-Alpine species or those in peatlands and alkaline fens, but also species characteristic of the steppe region (arid steppe, mesophilous meadows, steppe tundra), compact forest taiga, cold temperate coniferous and deciduous forests, and tall grass communities (Dítě et al., 2018; Horsák et al., 2015; Chytrý et al., 2017b).

Given the vast field of research on these habitats, the present study will only consider the lowland and mountainous wetland habitats, excluding the high altitude marshes of the subalpine and alpine zones. At the same time, the alpine and subalpine species, those of forests, steppes, salt marshes and tall grass communities are excluded. However, some of them are included in the study because their habitat is mainly related to low altitude marshes. The study focuses only on the current types of postglacial refugia in wetlands: peatlands, alkaline fens, peat bogs, springs and alluvial forests, which can be considered some of the most stable and good indicators of the flora of the past. Some of these marshes have survived from the Last Glacial to the present day, preserving a small part of the common archaic species of those times. A large number of relict species in our study, as well as in other European studies, indicate a clear preference and adaptation for ancient alkaline fens and peat bogs. As a result, these species have an extremely limited spread, which prevents them from advancing into new territories.

The present study focuses on the geographical region of the Southeastern Carpathians of Romania, including the Transylvanian Basin and neighboring geographical units that are also part of the Carpathian Region (Kliment et al., 2016; Breman et al., 2020). The northern district of the Western Plain, through the direct connection to the Carpathians and the large number of glacial relicts preserved here, cannot be excluded and will be treated as part of the studied region.

Chapter I. Relicts and refugia

1.1. Glacial relicts

The current species had expansions and contractions of their distribution area. Thus, a taxon is considered relict depending on how much it has reduced its area compared to a certain climatic period (Hampe and Jump, 2011). These authors define relicts as populations of a species that persist in isolated enclaves with favorable local microclimatic conditions, surrounded by large areas with climatic conditions that do not allow the existence of that species. These populations can migrate very little at all, have adapted to changing climatic conditions over a long period of time and continue to survive.

In the present study we accept the concept of Dítě et al. (2018), which defines glacial relicts as common regional heliophilous species in any period of the Glacial before the spread of compact deciduous or spruce forests in the Holocene. Some of these species were more abundant in the Last Glacial Maximum (e.g. some species of peat bogs and arcto-alpine scrub), while others became predominant in milder interstadials, such as Bølling and Allerød in the Late Glacial or Preboreal (e.g. tall grasses, boreal peatland species, cold forest-steppe species and open taiga), therefore a clear distinction is not possible between the relicts of the Last Glacial Maximum and these interstadials, although macrofossils indicate their presence in both periods, only the proportions being variable. Thus, both *Betula nana*, an arctic species that is continuously present until Preboreal, and *Scheuchzeria palustris* or *Menyanthes trifoliata*, generally indicators of wet and warmer periods, are found in the cold stades (Jakab et al., 2010). Therefore, those species are dominant throughout the Last Glacial Maximum, including the Late Glacial and Preboreal.

1.2. Postglacial refugia

Once the climate began to change, these species retreated to northern latitudes and higher mountains, disappearing completely from much of Europe. Some of them have found shelter in several local microhabitats with similar ecological conditions in which they have perpetuated themselves so far. These shelters are generically known as "refugia". Thus, a “refugium” is a geographical region or local microhabitat where these species have found similar habitat and climatic stability that have allowed them to survive for millennia in the same location, despite major changes in the region’s macroclimate (Dítě and colab., 2018).

According to Csergő (2002), these refugia are areas where local climatic conditions, due to the environment, tend to be resistant to changes in the general climate. This climatic resistance prevents the installation and proliferation of vegetation influenced by the general climate, which helps the survival and perpetuation of species adapted to local climatic conditions, many of which are reminiscent of past periods.

For today's glacial relicts, these sites are known as postglacial (i.e. interglacial) refugia (Dítě et al., 2018). The conditions in these refugia are harsher for most current temperate plants, being similar to those in the cold periods. The postglacial refugium is somewhat limited to a smaller area (scattered local micro-habitats or distinct orographic units, mostly swampy plains, valleys and mountain depressions). Birks and Willis (2008) also call them Holocene cryptic refugia.

The topography, the peculiarities of the soil or the structure of the vegetation help to maintain optimal climatic conditions of the refugia. Marshes, lakes and springs are the main thermoregulatory elements of a refugium. The temperature in the center of the marsh is often lower than its edges. Also, the height of the vegetation is another element that can reduce the temperature by a few degrees at the base of the soil. The same can be said about the humidity of the air, a high and compact vegetation preventing evapotranspiration (Hampe and Jump, 2011).

The depressions of the Eastern Carpathians, presenting a high altitude, cool climate, persistent fog and cold waters, favored the survival of the relicts (Pop, 1960; Rațiu, 1971a). The peaty soils of eutrophic fens maintain a lower temperature than the environment. Temperature reversals with low average temperatures are among the determinants of the conservation of glacial relicts in the depressions of the Eastern Carpathians (Sanda et al., 2005a).

Chapter II. Physical and geographical characterization of the researched territory (the Carpathian Region and the northern district of the Western Plain)

2.1. Geographical location and boundaries. The main features of the relief

The present study will focus exclusively on the territory of Romania. The general name of the Carpathian Region includes the Transylvanian Depression, which is a major component of the Carpathian Region (Kliment et al., 2016), as well as the adjacent hills and plateaus outside the Carpathian arc. Therefore, in addition to the three mountain ranges of the Carpathians, the Transylvanian Depression (Plateau), the Western Hills, the Subcarpathians outside the mountain range, the Mehedinți Plateau and the Suceava Plateau, as well as part of the Getic Plateau are included here. This delimitation continues the acceptance of the latest scientific data (Hurdu et al., 2016; Kliment et al., 2016; Breman et al., 2020), the exact delimitations being listed on the interactive map of the consortium "Carpathian Research Network" website (*see Webography*).

The northern district of the Western Plain is a distinct region from a floristic (phytogeographical) point of view. The area is delimited to the south by the interfluve of the rivers Barcău - Crișul Repede according to the forest vegetation (Doniță et al., 1980). Thus, the area of some Mediterranean and Pontic species from the south and center does not cross this corridor, and the relict species in the north are no longer found south of it (Karácsonyi, 1987). The district includes three distinct regions: 1) Someș Plain, which includes the Crasna Basin or Crasna Plain (Ecedea), the site of the former Ecedea Swamp, 2) Ier Plain (Valley) and 3) Nir Plain (Ardelean and Karácsonyi, 2002; 2005).

2.3. The wetlands of the territory

There are five distinct types of wetlands/freshwater marshes known in the literature, the differences of which are too little known in Romania, and are given below.

2.3.1. The eutrophic marsh

It is formed near lakes (basins), rivers (meanders and dead arms), on deep or undulating surfaces or as a result of landslides in hilly and mountainous areas. They are fed by groundwater, stagnant water, drain waters from slopes or flowing waters (Tardy, 2002). They can also be grouped according to the specifics of the vegetation.

2.3.2. The alkaline eutrophic marsh (rich fen)

These marshes receive their nutrients and water source from mineral-rich springs and through groundwater movements, less from rainfall. They have a pH higher than 6. Their characteristic is the formation of peat sediments. They differ from peat bogs in that by being less acidic and having more nutrients, which support a high diversity of plants and animals. The water level can fluctuate or remain constant, not depending on the amount of rainfall. They don`t have tree vegetation. These fens are usually located away from surface waters (lakes, ponds). They are formed near the valleys of some rivers or above some geological faults, where through the ruptures in the rocks and sediments from the substrate, very cold water springs from the deep aquifer reserves come to the surface (Grootjans et al., 2005; Rydin et al., 2013 ; EPA, 2022).

They are characteristic of a colder and very humid climate, where heavy rainfall creates large accumulations of water. The formation of these fens takes up to 10,000 years (EPA, 2022). The Carpathian region is on the periphery of these ecosystems, they are specific to the northern latitudes, being less and less encountered to the southern latitudes (Grootjans et al., 2005).

2.3.3. The oligotrophic marsh (peat bog)

Oligotrophic marshes are fed exclusively by atmospheric precipitation, their evolution being directly influenced by changes in the climate. They are thus deprived of the nutrients needed for plant growth. The amount of water is controlled by precipitation and evapotranspiration, the vegetation cover being variable. The substrate and acidic water, low in nutrients, have led to the adaptation and specialization of many plant and animal species to the related conditions (Pop, 1960; Magyari et al., 2008; EPA, 2022).

The typical oligotrophic marsh stops at the southern Alpine-Carpathian border, hence the numerous glacial relicts that reach their southern border in Transylvania. In the SE Carpathians they are found in a distinct mountain floor at about 900-1200 m altitude in the lower part of the spruce floor or in the upper part of the beech floor, where most of the typical peat bogs on which extensive pine forests have developed are also found (Pop, 1960).

2.3.4. The transitional marsh (poor fen)

These wetlands are intermediate between eutrophic alkaline and oligotrophic acid marshes. Hydrologically, they are similar to eutrophic fens, but in terms of vegetation and chemical composition, they resemble to peat bogs. They are more acidic, with a pH of 5.5 to 4. The deposited peat is thicker, which limits the access of vegetation to underground mineral springs, which also can no longer influence the pH of the poor fen. As a result, in this case the fen becomes dependent on atmospheric precipitation. Peat moss dominates these poor fens, but the species richness is much higher than in the typical oligotrophic peat bog (Rydin et al., 2013).

2.3.5. The alluvial forest and the shrub swamp

Alluvial forests are dominated by swamp trees and shrubs, which form a compact canopy that prevents light from penetrating. This category also includes the shrub swamps, which better withstand water fluctuations by forming aerial roots that follow the water level. Alluvial forests can be temporarily flooded, but the water usually remains constant. Other works include here the forest covered peat bogs, which will be treated in this paper as part of oligotrophic marshes (Tardy, 2002; Rydin et al., 2013).

Chapter IV. Personal contributions. Objectives of the study

During the years 2015-2021, continuing the work of research started in 2010, we conducted several field studies on marshes in the region of Transylvania and the northern Western Plain, where we compiled lists of species and phytocenological surveys. Following this research, we observed a number of species with an abnormally low distribution in the plain and hill areas of the northwest of the country, which are usually found in the high mountain regions. Thus arose the first questions about their appearance in the plains.

The proposed study aims to achieve the following objectives:

1. A ***critical evaluation*** of the species considered in the Romanian botanical literature as possible glacial relicts based on precise criteria;

2. ***Identifying the affinities*** of these species to the plant communities and habitats in the region by conducting phytocenological studies;

3. ***Identification of possible refugia*** in the lowlands of the Carpathian Region where these species survived the warming of the postglacial climate, known as postglacial refugia. Consistent with the conception of several authors (Birks and Willis, 2008), it can be considered that the areas or ecosystems in which these species still live today, can be considered refugia;

4. Phytocenotic investigation of areas that have been ***potential refugia*** by assessing the ecological and biogeographic factors of local species;

5. ***Identify the types of habitats*** in which these species occur, this approach being one of the most important methods used in identifying refugia (Bhagwat and Willis, 2008);

6. Carrying out ***a case study*** from the perspective of the boreal species *Trollius europaeus*, which can provide data on the refugia in which it could have survived;

7. ***Reassessment of the sozological status*** of the species under study in accordance with IUCN criteria;

8. ***Identifying the main factors*** leading to the extinction of marshes and relict species in the region;

9. ***Evaluation of strategies*** for conservation and protection of these species in the studied area;

Chapter V. Materials and methods of work

5.1. Materials

Floristic inventory. Floristic inventories were made for each area visited. The field trips took place over several years (2015-2021), to which were added unpublished data made by the author during 2010-2015. During this last period, vegetation surveys were also carried out.

Chorology. Chorological data were collected by analyzing herbariums and published materials (scientific articles, books, monographs, species sheets, nature magazines, etc.) available online and in libraries. To these were added new data identified by the author of the study following field research.

Macrofossils and pollen. In order to highlight the continuity of these species in the area of the region, all accessible scientific articles containing pollen and macrofossil data were consulted. The database of the Czech and Slovak macrofossils on the Western Carpathians (*see Webography*) was also used because the flora of the two major Carpathian chains was common in the spread of Arctic and Siberian species in the past.

Phytocoenology. For phytocenological data, those surveys from the literature were chosen, as well as from the "Romanian Grassland Database" with the consent of the authors (Vassilev et al., 2018), which contain at least one species from the category of relicts in this study. The total number of surveys is 572. They include 790 species of vascular plants and 143 species of bryophytes totaling 941 taxa. The total number of associations / sub-associations identified in the studied wetlands is 105. These were carried out around 114 localities.

Habitats. Habitat identification was performed based on the vegetation structure, using Doniță et al. (2005) and Gafta and Mountford (2008).

<u>Molecular analysis.</u> To highlight the idea of refugia, a target mountain species was chosen: *Trollius europaeus*. To extract the DNA, 173 individuals from 24 populations in the SE Carpathians region were analyzed.

5.2. Methods

5.2.1. Molecular analysis methods and techniques

The molecular methods addressed in this study were the AFLP (Amplified Fragment Length Polymorphism) technique and the chloroplastic microsatellites (cpSSR) technique.

5.2.2. Candidate species selection method

The current study focuses exclusively on wetland habitats, so all marshy and peatland-specific plants were selected, excluding the high mountain arcto-alpine species traditionally considered as glacial remains (Dítě et al., 2018). These species have also been eliminated due to the fact that they occupy mainly other types of alpine habitats than those of marshes and especially due to the lack of typical oligotrophic peat bogs that cannot form at high altitudes (Pop, 1960). Also included were all continental species whose range extends from Central Europe to Siberia (most of the range being in the area of Siberia) and which are found exclusively in marshes and wet meadows. Continental species were excluded from steppes and salt marshes, as the study only looked at wetlands with fresh water. All the so-called glacial relicts in the literature were selected. At the same time, some mountain species with an unusual distribution in the lowlands that found refuge in these marshes were included. All circumpolar species (usually found in peatlands, taiga and arctic tundra) have been added, as well as mineral-rich alkaline fen species. Thus, 190 candidate species were obtained. Each species was given a score based on its biogeographical and ecological features.

The scores for all individual characters were summed to make the final score. Like Dítě et al. (2018), we used this amount as representative for the probability that a certain species is a glacial relict. The maximum score obtained was 27 (by adding two other features to those of Dítě et al., 2018). Species that reached the value of the final score arbitrarily set at ≥ 13.5 (half of the maximum score, including most species in the literature) were considered likely relicts and therefore included in subsequent analyzes and in the list of putative glacial relicts in Romania. Dítě et al. (2018) arbitrarily choose the final score at ≥ 10 to include most of the glacial relicts in the literature, with a maximum score of 22.

What differs slightly from the method of the authors (Dítě et al., 2018) is the addition of two new features, namely: the southern boundary of the species area and the ecological nature of the affinity for the optimal temperature at which these species grow in Romania. This last character was processed from two different sources: Sanda et al. (1983) and Sârbu et al. (2013), whose average temperature was calculated. Thus, if in one work T_2 appeared, and in the other T_3, the average of the two will be $T_{2.5}$, hence the appearance of decimals in the final score, which also makes a much more distinct delimitation between species.

The categories of traits (symptoms) used for the final scores of each species (adapted from Dítě et al., 2018) are: 1. Continentality; 2. Circumpolarity; 3. Discontinuity of the distribution range; 4. Conservatism of the regional distribution; 5. Rarity; 6. Affinity to dry or saline steppic vegetation; 7. Affinity for tundra vegetation; 8. Affinity to taiga vegetation; 9. Affinity to acidic peatlands (bogs) and non-calcareous arctic-alpine lakes; 10. Affinity to fens and springs; 11. Affinity to mesic forest-steppe habitats (light-coniferous temperate forests, meadow-steppe and tall-herb habitats) and 12. Contraction of the ecological realized niche. The two new features added are 13. The southern areal limit of the species and 14. The temperature affinity (T). Each species received a number from 0 to 3 depending on the degree of affinity for the category (index).

Chapter VI. Results

6.1. Glacial relicts

6.1.1. Species conspect and chorology

The final list of species, selected on the basis of scores, comprises 87 vascular plants (Table 1. Appendix Summary). The results of the final scores (total sums) were between 3.5 and 27. The species that reached the final score ≥ 13.5 were considered to have the most traits to place them in the category of glacial relicts. The species with the highest scores are the safest to remain from the Last Glacial Maximum and the Early Holocene. Thus, 48 species accumulated over 20 points (final score), 26 species between 16 and 19.5 points, 5 species had 15-15.5 points, respectively another 7 species 14-14.5 points and one species 13.5 points.

Of the 87 taxa, 51 already had a consensus in Romanian botanical literature (*Flora R.P.R. Vol. XIII*) as glacial relicts (Pop, 1976) (*see R1 Table 1*). Pop (1960) mentions another 9 species as glacial relicts (*see R2 Table 1*) (*Vaccinium uliginosum* subsp. *uliginosum*, *Salix bicolor*, *Carex hartmanii*, *Betula pubescens*, *Euonymus nanus*, *Eriophorum gracile*, *Pedicularis limnogena*, *Adenophora liliifolia, Carex davalliana*). I. Pop et al. (1962) later include *Crepis sibirica* and *Liparis loeselii* (*see R2a Table 1*). Ciocârlan (2009) introduces a new species (*R3 - Saxifraga mutata* subsp. *mutata*) after Morariu and Negruș (1970) which they consider a glacial relict. Thus, 63 species have been reconfirmed as alleged glacial relicts for the flora of this region. A total of 33 species from this study are mentioned in the comprehensive paper by Witkowski et al. (2003) as relicts for the entire Carpathian region (*see R4 Table 1*). Of these, 6 species are confirmed as possible new glacial relicts for Romania: *Carex chordorrhiza*, *Hammarbya paludosa, Tephroseris palustris, Comarum palustre, Ligularia glauca, Chimaphila umbellata*. Another 16 species have been discussed as glacial relicts for Romania, Central Europe or neighboring regions in recent articles (*see R5 Table 1*): *Carex vaginata* - Romania and the Carpathians (Dítě et al., 2015), *Calamagrostis purpurea* - Romania and the Carpathians (Kobiv et al., 2022); *Pedicularis palustris, Carex hostiana* - Carpathians (Hájek et al., 2011), *Carex capillaris, Cladium mariscus, Schoenus nigricans, S. ferrugineus* - Central Europe (Chytrý et al., 2017a), *Cladium mariscus, Schoenus nigricans* - Romania (Muncaciu and colab., 2010; Cristea, 2014), *Cladium mariscus* - Central Europe (Pokorný et al., 2010), *Menyanthes trifoliata* - Romania (Oprea and Sîrbu, 2010), *Salix pentandra* - Hungary and Bulgaria (Farkas, 1999; Zahariev, 2016), *Ligularia glauca* - Carpathians (Horsák et al., 2010). Others are mentioned as likely to already exist in the region during the glacial period, following genetic or macrofossil analysis: *Dactylorhiza lapponica, D. traunsteineri* (Kenneth et al., 1988; Nordström and Hedrén, 2008), *Malaxis monophyllos* (Jermakowicz et al., 2017), *Carex lasiocarpa* (Magyari et al., 2014a), *Valeriana simplicifolia* (Hájková and Šmerdová, 2022).

Although the list contains endemic species to the region, they have not been eliminated based on their status as glacial relicts in the literature of Romanian botany (*Armeria maritima* subsp. *barcensis, Cochlearia borzaeana*) (*see Discussions*). For this reason, European species that do not reach Fennoscandia or the Arctic area, such as *Salix bicolor* or the hybrid *Drosera* × *obovata*, which is treated as a species in the Romanian flora, have been kept under study.

Compared with the results of Dítě et al. (2018), 48 species were confirmed by this method in both the Western and South-Eastern Carpathians of Romania (*see R6 - Table 1. Appendix Summary*)

The reporting of new species will be done in comparison with the official list of Pop (1976) (*R1 - Table 1. Appendix Summary*), including here species not included by it (*see R2, R2b and R3*), but repeated in various articles. At the same time, the species later proposed as relicts in the Romanian flora or the Carpathians are considered reconfirmed (*see R5*). Therefore, 19 species are proposed as new glacial relicts for the Romanian flora (*see New - Table 1, last column*): *Carex chordorrhiza, Hammarbya paludosa, Trichophorum alpinum, Carex capillaris, Ligularia glauca, Dactylorhiza lapponica, Dactylorhiza traunsteineri, Tephroseris palustris, Chimaphila umbellata, Comarum palustre, Carex lasiocarpa, Malaxis monophyllos, Salix pentandra, Carex hostiana, Juncus alpinoarticulatus* var. *fusco-ater, Schoenus ferrugineus, Pedicularis palustris, Allium ericetorum* subsp. *pseudosuaveolens, Valeriana simplicifolia*, of which five species are completely new in the debate on the status of glacial relict throughout the Carpathian Region: *Allium ericetorum* subsp. *pseudosuaveolens*, *Dactylorhiza lapponica*, *Dactylorhiza traunsteineri*, *Malaxis monophyllos* and *Valeriana simplicifolia*.

From the Romanian literature, 7 species / subspecies mentioned as glacial relicts did not meet the required final score and were eliminated. These are: *Monotropa hypopitys* subsp. *hypopitys* (8.5 points) and subsp. *hypophegea* (only 3.5 points), *Lycopodium tristachyum* (8) and *Lycopodium complanatum* (10), *Rubus nesssensis* (10), *Ribes petraeum* (10) and *Galium boreale* (12), species considered relicts by Pop (1960; 1976) and Ciocârlan (2009). Another five species that are considered by Dítě et al. (2018) as possible glacial relicts did not obtain the necessary scores to be considered on the list in the present study: *Triglochin maritima* (13), *Thalictrum simplex* (10), *Dianthus superbus* (11.5), *Angelica archangelica* (12) and *Scorzonera parviflora* (9). *Trollius europaeus*, given as an example of a glacial relict in numerous works in Hungary (Farkas, 1999; Tardy, 2002) scored only 11.5 points. The current approach was slightly different with the addition of two new features, but these did not change the results, being more or less the same, only the final scores becoming higher, especially for species bordering the southern area in the region and affinity for microthermal and cryophilic habitats.

The first three scores were obtained by *Betula nana*, *Carex chordorrhiza* and *Carex loliacea* (27), followed by *Carex vaginata*, *Ledum palustre*, *Salix myrtilloides* and *Saxifraga hirculus* (26.5), respectively by *Andromeda polifolia*, *Betula humilis* and *Carex magellanica* subsp. *irrigua* (26).

6.1.1.1. Relict and rare species identified in new regions (unpublished chorology)

All chorology is available in the Appendices section of the Phd dissertation (Annex 2). New localities were identified for some species in the study: *Allium ericetorum* subsp. *pseudosuaveolens, Angelica palustris, Betula pubescens, Calamagrostis canescens, Carex appropinquata, Carex diandra, Carex elongata*, *Cnidium dubium, Lycopodiella inundata, Menyanthes trifoliata, Polemonium caeruleum, Spiraea salicifolia, Swertia perennis, Valeriana simplicifolia.*

6.1.2. Study of phytocenoses with relict species

Boreal species dominate by far with 124 taxa, with a percentage of 15.69%. The continental species (55 species - 6.96%) indicate a harsher climate, and the arcto-alpine species (36 species - 4.55%) show again the conservation power of the marsh, in which these species have managed to perpetuate themselves until the present moment. The behavior of the species towards temperature (T) shows the microthermal species (T2-2.5: 21.64%) in second place, with a particularly high percentage which again indicates the conservation power of the marsh.

6.2. Postglacial refugia

6.2.1. Postglacial refugia in the Carpathian Region and the northern district of the Western Plain

1. Bodoc Mts. (60 sp.)
2. The marshy region of the Borsec-Bilbor-Drăgoiasa-Broșteni depressions (52 sp.)
3. Harghita Mts. (51 sp.)
4. Făgăraș and Sibiu depressions (47 sp.)
5. Ciuc Depression (45 sp.)
6. Giurgeu Depression (43 sp.)
7. Nemira-Cotu Buzăului Mts. (42 sp.)
8. Apuseni Mts. (40 sp.)
9. Maramureș Mts. and Depression (40 sp.)
10. Dornelor Depression (37 sp.)
11. Bârsa Depression (36 sp.)
12. Gurghiu Mts. – Praid-Dealu vocanic plateau and Gurghiu Valley upstream of Reghin (35 sp.)
13. Sf. Gheorghe and Târgu Secuiesc Depression (28 sp.)
14. Feleacu Hills (Făget-Valea Morii) (28 sp.)
15. The northern district of the Western Plain (Someș Plain, Nir Plain, Ier Plain) (28 sp.)
16. Oaș-Maramureș volcanic plateau (26 sp.)
17. Lucina - Fundu Moldovei and Obcinele Bucovinei Mts. (25 sp.)
18. The „glimee” from Zagra and Bistrița Hills (19 sp.)
19. Hârtibaciu Plateau (17 sp.)
20. Suceava Plateau (17 sp.)
21. Frumoasa and Tărtărău Valleys – Oașa Depression (16 sp.)
22. Alba-Iulia – Turda Couloir (15 sp.)
23. Lăptici Peat Bog –Lăptici Mt. (Bucegi Mts.) (13 sp.)
24. Neamț Subcarpathians (11 sp.)

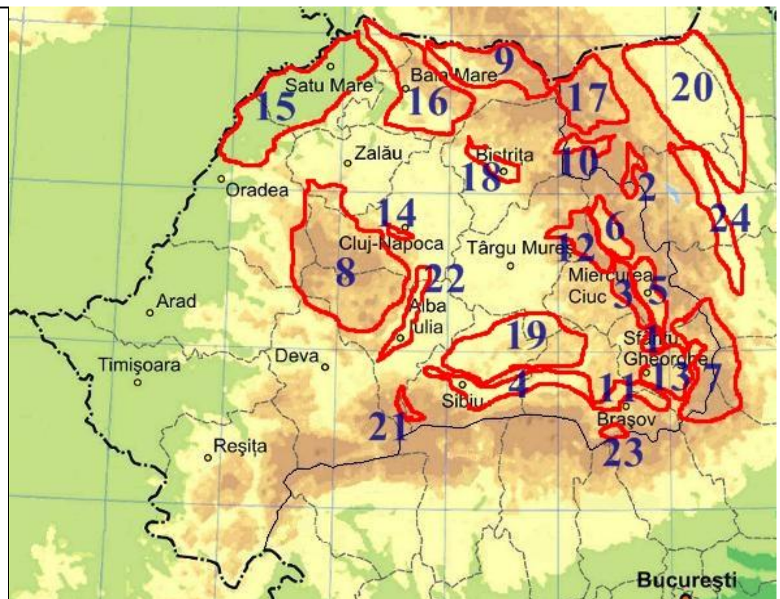

Fig. 1. The identified postglacial refugia overlap on numerous orographic units and distinct marshy regions shown in the table on the left.

The identification of these refugia was done by analyzing the number of possible relicts obtained in this study (87 taxa), which reach a number greater than or equal to 10. These are listed in the order of the number of preserved relict species. Following the distribution of relict species, there is sometimes an almost exact overlap of refugia over a distinct geographical unit. They are shown on the map in Fig. 1, the numbers representing the order in which these orographic units/marshy regions are discussed, and the number of relict species identified is shown in parentheses.

Thus, the existence of 24 marshy areas, often overlaid over distinct geographical units, in which the number of these species is particularly high, has emerged.

6.2.2. Ecological characterization of postglacial refugia

After evaluating the ecological and biogeographical factors of the local species, the phytocenotic investigation of the areas that constituted potential refugia (Fig.1), revealed important features. The large number of helohidatophytes (HH: 18.91%) in the northern district of the Western Plain (Someș Plain, Nir Plain and Ier Plain) shows that these relict species have taken refuge in the plains in much wetter ecosystems, compared to those in mountain areas, which compensates for the lack of rainfall. The same pattern is followed by other regions of marshes in the lowlands (Bârsa Depression, Neamț Subcarpathians, Băgău peat bog). Also, the high number of phanerophytes in the lowlands (Făgăraș Depression, Zagra marsh, northern district of the Western Plain) clearly shows that these relict species survived at lower altitudes near *Salix cinerea* bushes and alluvial forests that provide protection against high temperatures, preventing evapotranspiration and water stress. The circumboreal species (Circ) dominate in the marshes in the mountain areas and the intramontane depressions, where they exceed everywhere 20%, including here the marshes from the extramontane depressions that are influenced by the nearby mountain climate (Bârsa Depression - 20.23%, Făgăraș Depression - 27.47 %, Băgău peat bog - 29.62%). However, it is worth noting the northern district of the Western Plain, where these species reach a remarkable percentage of 15.76% or that of the Neamț Subcarpathians where they reach 19.11%. There is thus a direct influence on them from the neighboring mountain regions. Of note is the Giurgeu Depression, where the percentage of Arcto-alpine species reaches 2.44%. Although there are only 8 species, their number is relevant to highlight the very cold local microclimate of the depression. The plains in the northwest of the country stand out again with a high percentage of boreal species (13.96%), which is exceptional at this altitude. Another high percentage is in the Bârsa Depression (20.8%), Valea Morii Nature Reserve (18.37%), Băgău peat bog (24.39%), Făgăraș Depression (28.57%), Zagra marsh (13.49%) or even Neamț Subcarpathians (19.11%) and Suceava Plateau (6.15%). All this indicates the existence of local microclimates where these species have found a refuge. The Giurgeu Depression (7.03%) is noted by a number of 23 continental species typical of marshes in the continental regions of Siberia. The microthermal species (T2-2.5) are dominant in Nemira-Cotul Buzăului Mts. (37.5%), the region being also considered the “cold pole” in Romania. In the same situation are Maramureș Mts. and Depression (36.36%), the Oaș-Gutâi Plateau (35.18%), Lăptici peat bog (36.36%) and several mountain marshes and intracarpathian depressions whose percentage of microthermal species is close to the micro-mezotherms (Dorna Depression, Frumoasa Valley, Bodoc Mts., Gurghiu Mts.). The number of microthermal species is also high in the lowlands, compared to other regions of marshes that do not preserve glacial relicts: Făgăraș Depression (21.97%), Bârsa Depression (15.02%), Valea Morii Nature Reserve (13.51 %), Neamț Subcarpathians (13.23%), Zagra marsh (12.69%), Suceava Plateau (11.28%) or the northern district of the Western Plain (Someș, Nir, Ier) (10.81%). The number of cryophilic species (T1-1.5) is remarkably higher in Frumoasa Valley (5.35%), Harghita Mts. (1.85%), Dornelor Depression (1.29%) and Giurgeu Depression (1.52%).

The main element of conservation of these plants is facilitated by the existence of peat (oligotrophic or eutrophic). Thus, most of the marshes that do not have peat in their substrate do not preserve any glacial relicts. All 24 regions identified as possible postglacial refugia show peat in the marsh substrate, often of significant age. Peculiarities of the local climate are the second main factor in maintaining these species, such as the harsh and cold continental climate in the intramontane depressions and massifs of the Eastern Carpathians, respectively that of Baltic influences in the north of the Eastern Carpathians and the Suceava Plateau, respectivly Neamț Subcarpathians. At the same time, the abundant precipitations in some western massifs under Atlantic influences (Apuseni Mts., Oaș-Gutâi Plateau, Maramureș Mts. or even the north of the Western Plain) are a determining factor.

Chapter VII. Molecular analysis of the target species *Trollius europaeus*

The boreal species *Trollius europaeus* was chosen for the following reasons:

1) the plant has been identified at particularly low altitudes in several regions outside the Carpathians in the Nir Plain at Urziceni and Bátorliget; Someș Plain at Iojib and Agriș, Valea Morii Nature Reserve near Cluj, Bârsa Depression at Dumbrăvița, Hărman and other areas (Karácsonyi, 1995; Muncaciu et al., 2010; Ularu, 1971). The question arose as to whether these populations survived here from the Last Glacial, when the mountain species descended in altitude to the plains or are of more recent origin (Late Holocene), when due to the wetter and colder climate, a large part of the species from the beech floor have descended into the plains of the northwest of the country.

2) the species is considered a glacial relict in Hungary, most often given as an example of the populations of the Nir Plain at Bátorliget (Tardy, 2002; Farkas, 1999). Molecular analysis could confirm or disprove this concept.

3) In the Carpathians, *Trollius europaeus* is a fairly widespread species and has been researched in Europe, but only punctually. Genetic and molecular studies can provide clues to the long-term local isolation of these populations (distance in time), as well as clues to migration routes to and from neighboring mountain areas (affinity between isolated populations remaining in low-lying marshes and the mountain ones).

Populations that have survived in glacial refugia have high genetic diversity. Thus, the weaker a population is in terms of genetic diversity, the further away it is from the refugium in which it has survived, as many genetic traits are lost along the way to migration. At the same time, the long-term separation of a population will inevitably lead to local genetic differentiation. Comparative studies can highlight certain regions in the range of a species with great genetic diversity, clearly indicating a difference between glacial refugia and postglacial colonized regions (Provan and Benett, 2008). The vast inhospitable regions occupied by the steppe tundra have led to the long-term isolation of several populations in persistent marshes fed by continuous springs. These populations then migrated from glacial refugia to all optimal habitats in the Early Holocene, from and to the Carpathians. The vast majority of them were in turn destroyed again by the warming and arid climate of the Boreal. Although in the next period (Atlantic), the climate becomes very humid, favorable to large marshes, there is a maximum expansion of forests, which form compact ecosystems, leading to the extinction of heliophilous species of marsh and meadows. The most favorable climate of this boreal species settles in the Subatlantic, when it becomes colder and wetter than the previous Subboreal. Recently installed populations are expected to be very close genetically or identical to those in most of the Carpathian chain.

7.1. Assessment of genetic diversity with chloroplastic microsatellite markers of *Trollius europaeus* populations

A total of 12 haplotypes were identified based on the size polymorphism observed at the chloroplastic microsatellite markers (ccmp2, ccmp3, ccmp4, ccmp6 and ccmp10).

The phylogenetic relations between the chloroplastic haplotypes deduced with the help of the inserted inserts and deletions, indicate the genetic proximity relations between the majority haplotype H08 and the haplotype H03, from which it differs by a deletion at ccmp2; haplotype H07 which differs by a deletion in ccmp4; H09 which differs by a ccmp4 insert; and H12 which differs by a ccmp2 insertion. The H01 haplotype identified in the FA12 population of the Făgăraș Mountains indicates a very high phylogenetic distance due to numerous deletions or insertions, being the closest genetically to the H02 haplotype identified in the same population (Fig. 2).

Analysis of the geographical distribution of cpSSR chloroplastic haplotypes identified in *Trollius europaeus* populations (Fig. 3) indicates the existence of haplotypes specific to some Carpathian regions, which may help in identifying glacial refugia. Thus, the fixation of haplotypes (e.g. H01, H02, H03, H06, H07, H09, H10, H11, H12) in *Trollius europaeus* populations is most likely the result of postglacial evolution and adaptation of populations to local conditions.

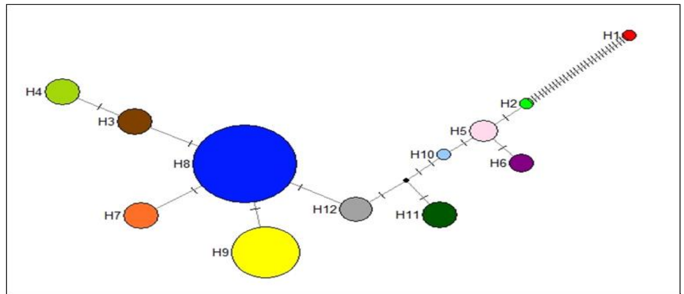

Fig. 2. Phylogenetic relationships between identified chloroplastic haplotypes (Circle size is proportional to the number of individuals in a given haplotype)

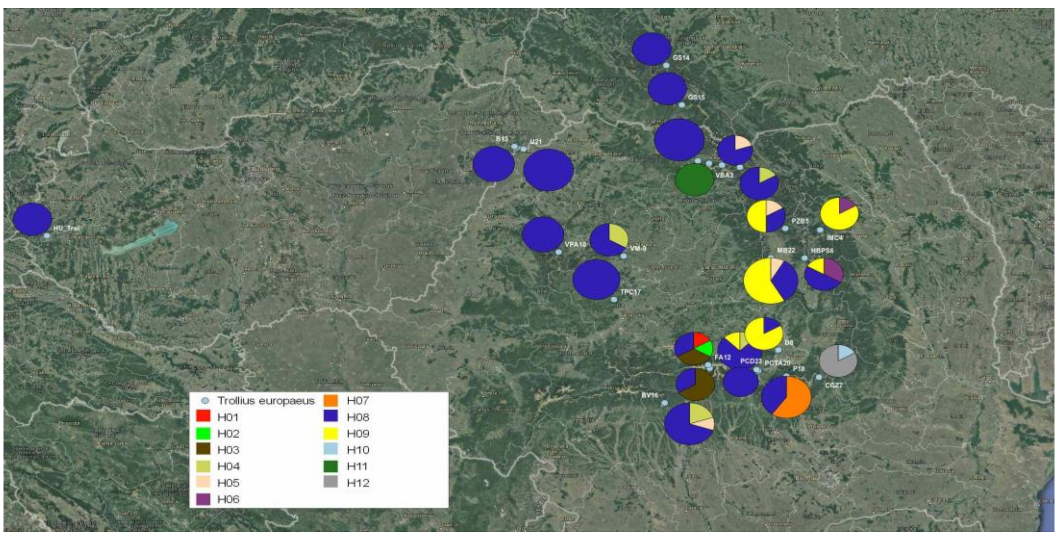

Fig. 3. Geographical distribution of chloroplastic haplotypes (cpSSRs) identified in *Trollius europaeus* populations

7.2. AFLP analysis

Regarding the proportion of variable markers, these are the highest in the PCD23 population (0.42) and the lowest in the ROD1 population (0.08), but in general we can talk about uniform values of the markers. Corresponding to the proportion of variable markers, is the distribution of genetic diversity, relatively uniform in all populations. Based on the DW value, it appears that the populations with the lowest historical genetic isolation are: VM-9, TPC17, MO19, VPA10, ROD1 and GS15, while at the opposite pole is the population of Ceahlău (BMI4) (343.38).

7.3. Evidence of glacial and postglacial refugia from the perspective of the *Trollius europaeus* species

Trollius europaeus had one or more glacial refugia in the SE Carpathians, which also provided support to most of the boreal relict species in the study. Possible glacial refugia were thus identified in: Făgăraș Mountains, Ciucaș Mountains, Rodna Mountains, the central-eastern part of the Eastern Carpathians, Prahova Valley area, the intramontane depressions (Borsec, Giurgeu, Bârsa). At the same time, some haplotypes far removed from the common one and very disjunct reinforce the hypotheses of some glacial refugia in the Transylvanian Basin and the southern slopes of the Southern Carpathians (Schmitt and Varga, 2012).

Chapter VIII. Discussions

8.1. Problems with relict species in the study

Dítě et al. (2018) mentions three aspects that should be considered. **1)** The results should be seen as an overview rather than a firm evidence, as this approach could easily misclassify a species. **2)** The division between the relicts and the rest of the species was done arbitrarily using a value of the final score greater than or equal to 13.5 in our case by adding additional features, 10 being for the Western Carpathians, where this value allowed the inclusion of all species for which there is strong paleoecological and phylogeographical evidence and/or a strong consensus in the literature. Thus, the lowest scores cannot be interpreted as weaker evidence of relict status. **3)** This study should be compared by the fact that some species have been excluded for the Western Carpathians, while others are included for the SE Carpathians and vice versa. Also, their low ecological conservationism and the absence of large discontinuities in their distribution areas could wrongly define the status of the species.

Most species from the Ice Age and Early Holocene have disappeared from the European continent and retreated to high altitudes in the mountains or to northern latitudes. However, it is documented that some species have undergone a change in habitat preference. Species that survived the mid-Holocene warm period joined the new plant cenoses of alkaline fens, giving them invaluable scientific value (Hájková et al., 2015).

<u>8.1.1. Arctic and arcto-alpine species</u>

Most of them have survived only in regions with low temperatures and insufficient nutrients, which have led to a drastic reduction in competitiveness, remaining isolated in peatlands or on high mountain peaks with poor competition between species (Zimmermann et al., 2010). Species that have adopted such behavior are *Betula nana*, taking refuge in oligotrophic marshes at Tinovul Luci or Lucina, *Salix myrtilloides* at Lăptici peat bog, *Salix bicolor* in the marshes of the Sebeș Valley (Frumoasa and Tărtărău), *Trientalis europaea* at Comandău peat bogs, *Vaccinium uliginosum* in the peatlands of Oaș-Gutâi, or in alkaline fens: *Carex vaginata* in Rarău Mts., *Carex capillaris* in Drăgoiasa and in Rarău, *Primula farinosa* in Prejmer and Hărman fens, *Tofieldia calyculata* in Valea Morii fens, *Saxifraga hirculus* in the fens of Harghita Mts. etc.

8.1.2. Continental species

Continental species distributed throughout continental Eurasia were considered to be completely absent from Europe during the glacial period, surviving only in Siberia and Manchuria. They are thought to have arrived in Europe through rapid postglacial migration. This concept began to be questioned as early as 1960-1970, analyzing chorological distributions and intraspecific taxonomic analyzes. As a result, the hypothesis of optimal refugia in the harshest climates of the Last Glacial Maximum has been formulated for the survival of these species. Genetic differences across the European continent have highlighted the presence of several refugia north of southern Europe, indicating that these species already existed in the Last Glacial in Europe and did not arrive here in the postglacial period from Asia. Multiple examples show that continental species have had numerous refugia, performing only local migrations, excluding the possibility of large postglacial migrations between continents (Schmitt and Varga, 2012).

8.1.3. Boreal species (circumboreal)

The boreo-mountain disjunction is similar to the arcto-alpine one, being a biogeographical phenomenon encountered mainly in Europe, but also in Asia (Stevanović et al., 2009). This includes a number of species from the Boreal region (taiga) that are also found in the mountainous areas of the south of the continent. The Preboreal and Boreal are considered the periods of maximum expansion of these species. Due to rising temperatures, the vast majority of them have retreated to mountainous areas or northern latitudes, but some of them have survived in marshes or alluvial forests in the lowlands (Vukojičić et al., 2014).

8.1.4. European and endemic species

Dítě et al. (2018) eliminated *de facto* all European and endemic species from their research, but kept some species that have a consensus in the literature whose populations have a low altitude distribution in obviously relict sites. Some species, even if they have an area limited to the European continent or are strictly located in a certain mountainous area, were taken into consideration in the current study following the example of other relict species from European countries. For example, *Myosotis rehsteineri*, endemic to the Alps, is considered a glacial relict (Zimmermann et al., 2010). On this concept we also included the endemic species that are found in the marshes of the Carpathian Region, but also the European ones. The best example of European species is *Pinus cembra*, which is endemic to the continent and is still considered a glacial relict in Europe (Höhn et al., 2009). The current study follows the same approach for European species with obvious relict distribution in the lower sites (e.g. *Salix bicolor*, *Valeriana simplicifolia*, *Tofieldia calyculata*).

8.1.5. Problematic species

Schoenus nigricans has a high conservatism for the old alkaline fens of Central Europe, therefore in some works it is considered a glacial relict (Bernhardt and Kropf, 2006; Muncaciu et al., 2010; Cristea, 2014). The species has a wide range of distribution in Europe and the southern hemisphere with interesting ecological differences that could indicate the case of a complex of microspecies (Bernhardt and Kropf, 2006). This is also true for *Cladium mariscus*, the species being considered to be a relict from the Early Holocene period (Pokorný et al. 2010). In the Czech Republic, based on paleobotanical discoveries (fossil seeds), Pokorný et al. (2010) find that *Cladium mariscus* survived in alkaline fens throughout the Holocene. As in the Czech Republic and Romania, its current distribution is more restricted than fossil sites, which confirms the previously assumed status of the species *Cladium mariscus* as a relict at least from the Early Holocene (Sádlo, 2000). *Pedicularis limnogena* is found only in the marshes of the Apuseni Mountains, with a disjunct distribution in the Balkans that could suggest an older or more recent migration from north of the Balkans through the Iron Gates. The preference for colder habitats (peatlands) and the association with other relicts such as *Drosera intermedia*, also limited to the Apuseni Mountains, indicate that its arrival here may be related to colder and wetter periods during the Quaternary, the age of the peat bogs here being also very high (Ruskál et al., 2020).

8.2. Postglacial refugia. The marsh as a refuge

According to Hájek et al. (2009) these relict species could survive in 5 types of wetlands: **1)** oligotrophic peat bogs in high mountain areas with acid substrate [such as the whole series of volcanic mountains with acid substrate in the western Eastern Carpathians (Oaș-Bodoc Mts.) or Bucovina mountain region]; **2)** slightly acidic or moderately alkaline poor fens in mountain depressions at medium altitudes (mesotrophic fens – e.g. Ciuc Depression); **3)** alkaline rich fens in intramountain depressions (e.g. Ciuc, Giurgeu, Drăgoiasa, Bilbor, Borsec); **4)** infiltrations of mineral waters rich in limestone at the base of calcareous mountains (e.g. Sâlhoi-Zâmbroslăviile with *Cochlearia borzaeana* in the Maramureș Mountains; the springs under Plaiul Todirescu with *Carex vaginata* in the Rarău Mountains) and **5)** alkaline rich fens from low altitudes around mineral springs [e.g. the depressions of Făgăraș, Sibiu and Bârsa, Valea Morii Nature Reserve, the western foothills of the Harghita Mountains (Vlăhița) and the eastern foothills of the Vlădeasa Mountains (Călățele)].

Chapter IX. Conservation of the glacial relicts and marshes in the studied region

9.1. Updating the sozological categories

As a result of the complex analyzes of these species, the paper proposes a current critical assessment of their conservation status in the studied region, by creating a list that can be the basis for future studies of conservation of endangered species in Romania. The assessment of sozological status is based both on field observations and especially on recent data from the literature.

The results by categories are as follows: EX: 5 sp., CR: 36 sp., EN: 23 sp., VU 14 sp., NT: 5 sp., LC: 4 sp. Of these five species can be considered extinct in the flora of Romania (EX), not being found for more than six decades (*Salix myrtilloides*, *Saxifraga mutata* subsp. *mutata*) or a century (*Ledum palustre, Tephroseris palustris*, *Trichophorum alpinum*).

9.4. Factors leading to the extinction of marshes and relict species in our country

19 factors were identified that lead to the extinction of marshes and relict species in the studied region, the main factors being the artificial drying of marshes, plowing of swampy lands and their inclusion in the agricultural circuit, grazing, irregular mowing, burning of vegetation, deforestation of alluvial forests, exploitation of the peat, the introduction of invasive species, urbanization and chemical pollution.

9.5. Conservation of relict species

The evaluation of the conservation and protection strategies of these species in the studied area can be summarized most concretely by saving seeds in seed banks and growing plants in botanical gardens, being necessary to experience the success rate of acclimatization in case of *ex-situ* conservation.

Conclusions

1. A critical evaluation of the species considered in the Romanian botanical literature as glacial relicts was performed by analyzing well-defined biogeographic traits. Thus, 87 vascular plant taxa were identified as possible glacial relicts in the region. Six decades ago, 63 of them were already considered as glacial relicts in the country's flora by one or more authors, and they were reconfirmed according to the present study. Another five species have been mentioned as glacial relicts for Romania in the last decade (*Carex vaginata, Calamagrostis purpurea, Cladium mariscus, Schoenus nigricans, Menyanthes trifoliata*). A number of 19 species are proposed as new glacial relicts for the Romanian flora (*see Table 1. Appendix Summary*): *Carex chordorrhiza, Hammarbya paludosa, Trichophorum alpinum, Carex capillaris, Ligularia glauca, Dactylorhiza lapponica, Dactylorhiza traunsteineri, Tephroseris palustris, Chimaphila umbellata, Comarum palustre, Carex lasiocarpa, Malaxis monophyllos, Salix pentandra, Carex hostiana, Juncus alpinoarticulatus* var. *fusco-ater, Schoenus ferrugineus, Pedicularis palustris, Allium ericetorum* subsp. *pseudosuaveolens, Valeriana simplicifolia*.

2. The existence of some marshy areas has been outlined, often overlaping over distinct geographical units, in which the number of these species is particularly high. These can be considered postglacial refugia for the concerned species. Most of the relicts are found in the Bodoc Mts., in the marshy region of the Borsec-Bilbor-Drăgoiasa depressions and the Harghita Mts.

3. The phytocenological analyzes carried out in these postglacial refugia highlight some peculiarities for each region. Thus, these glacial relicts benefit in the plain and hilly areas from a much higher humidity than the adjacent mountain areas, which compensates for the lower precipitation. Also, the cold continental climate of the intramontane depressions is indicated by the presence of a series of microthermal and cryophilic species.

4. By analyzing the chloroplastic microsatellites of 24 populations of *Trollius europaeus* from the studied region, it was possible to identify 12 different haplotypes, a very large number for a single region. The analysis of the geographical distribution of the identified cpSSR chloroplastic haplotypes shows the existence of specific haplotypes in some regions of the Carpathians, which may indicate some glacial refugia. Thus, possible glacial refugia were identified in: Făgăraș Mts., Ciucaș Mts., Rodna Mts., the central-eastern part of the Eastern Carpathians, Prahova Valley area, the intramontane depressions (Borsec, Giurgeu, Bârsei). At the same time, some haplotypes far removed from the common one and very disjunct reinforce the hypotheses of some glacial refugia in the Transylvanian Basin and the southern slopes of the Southern Carpathians.

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Appendix Summary

Table 1. List of putative glacial relict species in the Carpathian Region and the northern district of the Western Romanian Plain

No. crt.	Species	Final score	Highest ecological affinity	(Sub) species biogeographic characteristics	The first palynological records and macrofossils recorded in the Carpathian Region (details at Annex 1 in the PhD Thesis)	Cited as glacial relict species in:	Reassessed sozological status (Chapter IX)
1.	*Betula nana*	**27**	Tundra	3, 5a, 13a	Würm-Glacial Maximum	R1, R4, R6	**CR**
2.	*Carex chordorrhiza*	**27**	Tundra, Marshes	5a, 13a	Preboreal	R4, R6, New	**CR**
3.	*Carex loliacea*	**27**	Tundra, Marshes	3, 5a, 13a	-	R1	**CR**
4.	*Carex vaginata*	**26.5**	Tundra	3, 5a, 13a	-	R5, R6	**CR**
5.	*Ledum palustre*	**26.5**	Tundra, Peat bog	5a, 13a	Subboreal	R1, R4, R6	**EX**
6.	*Salix myrtilloides*	**26.5**	Tundra	3, 5a, 13a	Dryas III	R1, R4, R6	**EX**
7.	*Saxifraga hirculus*	**26.5**	Tundra, Marshes	3, 5a, 13b	Glacial Maximum	R1, R4, R6	**CR**
8.	*Andromeda polifolia*	**26**	Tundra, Peat bog	5b, 13a	Preboreal	R1, R4, R6	**EN**
9.	*Betula humilis*	**26**	Taiga	3, 5a, 13a	Würm-Dryas III	R1, R4	**CR**
10.	*Carex magellanica* subsp. *irrigua*	**26**	Tundra, Marshes	5b, 13b	-	R1, R6	**VU**
11.	*Stellaria longifolia*	**25**	Taiga, Marshes	5a, 13a	-	R1, R4, R6	**CR**
12.	*Vaccinium uliginosum* subsp. *uliginosum*	**25**	Tundra, Peat bog	5a, 13a	Preboreal	R2, R6	**CR**
13.	*Achillea impatiens*	**24.5**	Marshes	3, 5a, 13a	-	R1, R4	**CR**
14.	*Hammarbya paludosa*	**24.5**	Peat bog, Marshes (oligotrophic)	5a, 13b	-	R4, New	**CR**
15.	*Vaccinium microcarpum*	**24.5**	Tundra, Peat bog	5b, 13a	-	R1, R4, R6	**EN**
16.	*Vaccinium oxycoccos*	**24.5**	Tundra, Peat bog	5b, 13a	Preboreal	R1, R4, R6	**EN**
17.	*Calamagrostis purpurea*	**24**	Marshes (oligotrophic)	5a, 13a	-	R5	**EN**
18.	*Carex pauciflora*	**24**	Tundra, Marshes (oligotrophic)	13a	Subatlantic	R1, R6	**VU**
19.	*Drosera anglica*	**24**	Tundra, Peat bog (Poor fens)	5a	-	R1, R4, R6	**CR**
20.	*Calamagrostis stricta*	**23.5**	Tundra, Marshes	5b, 13b	-	R1, R4	**EN**
21.	*Carex dioica*	**23.5**	Tundra, Rich fens	5b, 13b	Allerød	R1, R4, R6	**EN**
22.	*Trichophorum alpinum*	**23.5**	Tundra*, Peat bog*, Marshes*	5a, 13a	Subatlantic	R6, New	**EX**
23.	*Drosera × obovata*	**23**	Peat bog (Poor fens)	5a	-	R1	**CR**
24.	*Potamogeton alpinus*	**23**	Tundra, Marshes and watercourses	5a	Dryas I	R1, R6	**CR**
25.	*Primula farinosa*	**23**	Tundra, Rich fens	5a	Preboreal	R1, R6	**CR**
26.	*Empetrum nigrum* subsp. *nigrum*	**22.5**	Tundra, Peat bog	5a	Dryas III	R1, R6	**EN**
27.	*Pedicularis sceptrum-carolinum*	**22.5**	Marshes	5a, 13a	-	R1, R4, R6	**CR**
28.	*Trientalis europaea*	**22.5**	Tundra, Taiga	5b, 13a	Dryas III	R1	**CR**
29.	*Angelica palustris*	**21.5**	Marshes	5a, 13b	-	R1, R4, R5	**CR**
30.	*Carex limosa*	**21.5**	Tundra, Marshes		Glacial Maximum	R1, R4, R6	**VU**
31.	*Drosera intermedia*	**21.5**	Peat bog	5a	-	R1	**CR**
32.	*Salix bicolor*	**21.5**	Alpine tundra, Marshes	5a, 13a	-	R2, R3, R4, R6	**CR**
33.	*Salix starkeana*	**21.5**	Marshes*, Tall-herbs/Continental shrubs*	3, 5a, 13a	-	R1, R4, R6	**CR**
34.	*Scheuchzeria palustris*	**21.5**	Peat bog	5b, 13b	Dryas I	R1, R4, R6	**EN**
35.	*Spiraea salicifolia*	**21.5**	Marshes, Tall-herbs/Continental shrubs	3, 5b, 13b	-	R1	**VU**

36.	*Calla palustris*	**21**	Marshes	5b, 13b	Atlantic	R1, R6	**EN**
37.	*Carex capillaris*	**21**	Tundra	5b	Subatlantic	R5, R6, New	**EN**
38.	*Crepis sibirica*	**21**	Tall-herbs/Continental shrubs	5a, 13a	-	R2a, R4, R6	**CR**
39.	*Ligularia sibirica*	**21**	Marshes	5b, 13b	Late Glacial	R1, R4, R6	**VU**
40.	*Lycopodiella inundata*	**21**	Peat bog, Marshes (oligotrophic)	5a	Dryas III	R1, R4	**EN**
41.	*Viola epipsila*	**21**	Marshes	5b, 13a	-	R1, R4, R6	**CR**
42.	*Ligularia glauca*	**20.5**	Tall-herbs/Continental shrubs	3, 5a, 13a	-	R4, R5, R6, New	**EN**
43.	*Dactylorhiza lapponica*	**20**	Rich fens	5a, 13b	-	R5, New	**CR**
44.	*Dactylorhiza traunsteineri*	**20**	Rich fens	5a, 13b	-	R5, New	**CR**
45.	*Drosera rotundifolia*	**20**	Tundra, Peat bog		Preboreal	R1, R6	**VU**
46.	*Liparis loeselii*	**20**	Rich fens	5a	-	R2a	**CR**
47.	*Swertia perennis*	**20**	Rich fens	5b	-	R1, R6	**EN**
48.	*Tephroseris palustris*	**20**	Marshes	5a, 13b	-	R4, New	**EX**
49.	*Chimaphila umbellata*	**19.5**	Taiga	5a, 13a	-	R4, New	**CR**
50.	*Dryopteris cristata*	**19.5**	Marshes	5b, 13b	-	R1	**EN**
51.	*Lysimachia thyrsiflora*	**19.5**	Marshes	5b, 13b	Atlantic	R1, R4, R6	**VU**
52.	*Rhynchospora alba*	**19.5**	Peat bog	5b	Glacial Maximum	R1, R6	**EN**
53.	*Carex hartmanii*	**19**	Marshes	5b	Subboreal	R2, R3, R4, R6	**VU**
54.	*Cnidium dubium*	**19**	Marshes	5b, 13a	-	R1	**EN**
55.	*Comarum palustre*	**19**	Tundra, Marshes		Würm-Dryas II	R4, R6, New	**VU**
56.	*Betula pubescens*	**18.5**	Tundra, Peat bog		Würm- Glacial Maximum	R2, R6	**LC**
57.	*Eriophorum vaginatum*	**18.5**	Tundra, Peat bog		Allerød-Dryas III	R1, R6	**LC**
58.	*Tofieldia calyculata*	**18.5**	Alpine tundra*, Rich fens*	5a	Postglacial	R1	**CR**
59.	*Euonymus nanus*	**18**	Tall-herbs/Continental shrubs*	3, 5a, 13a	-	R2, R4	**CR**
60.	*Polemonium caeruleum*	**18**	Marshes, Tall-herbs/Continental shrubs	5b	Glacial Maximum	R1, R6	**EN**
61.	*Sparganium minimum*	**18**	Marshes	5b	Glacial Maximum	R1	**EN**
62.	*Viola palustris*	**18**	Marshes	5a	Dryas III	R1	**CR**
63.	*Carex lasiocarpa*	**17.5**	Marshes	5b	Würm- Glacial Maximum	R5, R6, New	**NT**
64.	*Malaxis monophyllos*	**17.5**	Taiga*, Peat bog*	5b	-	R5, New	**CR**
65.	*Salix pentandra*	**17.5**	Marshes		Subatlantic	R5, New	**NT**
66.	*Carex diandra*	**17**	Marshes		Allerød-Dryas III	R1, R4, R6	**VU**
67.	*Carex hostiana*	**17**	Rich fens	5b, 13b	Subboreal	R5, New	**CR**
68.	*Eriophorum gracile*	**17**	Marshes	5b	-	R2, R6	**EN**
69.	*Juncus alpinoarticulatus* var. *fusco-ater*	**17**	Rich fens		-	R6, New	**NT**
70.	*Schoenus ferrugineus*	**17**	Rich fens	5a, 13b	Subatlantic	R5, R6, New	**CR**
71.	*Cochlearia borzaeana*	**16.5**	Marshes and springs	5a	-	R1, R6	**CR**
72.	*Calamagrostis canescens*	**16**	Marshes		Subatlantic	R1, R6	**VU**
73.	*Pedicularis palustris*	**16**	Marshes	5b	Preboreal	R5, New	**EN**
74.	*Sesleria uliginosa*	**16**	Rich fens	5a	-	R1	**CR**
75.	*Allium ericetorum* subsp. *pseudosuaveolens*	**15.5**	Marshes (Rich fens)	5a	-	New	**VU**
76.	*Pedicularis limnogena*	**15.5**	Marshes	5b	-	R2	**EN**
77.	*Adenophora liliifolia*	**15**	Tall-herbs/Continental shrubs	5a, 13b	-	R2, R6	**CR**

78.	*Carex elongata*	**15**	Marshes		Atlantic	R1	**NT**
79.	*Cladium mariscus* subsp. *mariscus*	**15**	Rich fens	5a	Dryas I-Dryas III	R5	**VU**
80.	*Saxifraga mutata* subsp. *mutata*	**14.5**	Alpine tundra*, Rich fens*	5a	-	R3	**EX**
81.	*Armeria maritima* subsp. *barcensis*	**14**	Rich fens	5a	-	R1	**CR**
82.	*Carex appropinquata*	**14**	Marshes		Bølling- Dryas II	R1, R6	**NT**
83.	*Carex davalliana*	**14**	Rich fens	5b	Subboreal	R2	**EN**
84.	*Menyanthes trifoliata*	**14**	Marshes		Würm- Bølling-Dryas II	R5	**LC**
85.	*Ribes nigrum*	**14**	Marshes*, Tall-herbs/Continental shrubs*	5b	-	R1	**VU**
86.	*Schoenus nigricans*	**14**	Rich fens	5b	Subatlantic	R5	**EN**
87.	*Valeriana simplicifolia*	**13.5**	Peat bog, Marshes		Bølling	R5, New	**LC**

Explanations in Table 1: The species are sorted in the order of the final scores from 27 to 13.5 (in alphabetical order for each score). Highest ecological affinity is represented by the largest number of scale 3; if 3 is not available, then the species is evaluated in the second highest category 2* (marked with an asterisk). The following column [(Sub) species biogeographic characteristics] represents the discontinuity of the area (category 3), the rarity of the species (category 5) and the southern limit of the area (category 13). In category 5, the scale 3 (5a) and 2 (5b) are represented; 5a represents a maximum of 5 optimal sites = very rare; 5b represents a maximum of 10 - 20 optimal sites = rare. Category 13a represents the southern European limit of the species in Transylvania (scale 3), and 13b the general southern limit in Romania, with several isolated populations in the Balkans or Turkey (scale 2). In the next column are inserted the oldest evidence of pollen and macrofossils of the species in the region, if they exist. In the literature column the species discussed as glacial relicts in the following sources are found, the current study reconfirming those assumptions: *R1* - species considered glacial relicts by Pop (1976) in *Flora R.P.R., Vol. XIII* (*R2* and *R3* resume most of these species) ; *R2* - Pop (1960); *R3* - Ciocârlan (2009); *R4* –Witkowski et al. (2003) for the Carpathians; *R5* - species considered as relict in various scientific articles in Central Europe and Romania discussed in the Chapter *Results*; R6 - confirmed glacial relicts for the Western Carpathians by Dítě et al. (2018); "New" - other species that meet the criteria of glacial relicts for the South-Eastern Carpathians reported in this study and that are missing either from the lists of the Romanian literature or from the lists from Central Europe. The last column represents the sozological status reevaluated in Chapter IX according to field observations and recent data from the literature (CR: Critically Endangered, EN: Endangered, VU: Vulnerable, NT: Near Threatened, LC: Least Concern, EX: Extinct).