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ECO-COENOTIC CONDITIONS AND THE GENETIC STRUCTURE OF THE *CRAMBE TATARIA* SEBEÓK POPULATIONS IN ROMANIA

PHD THESIS SUMMARY

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INTRODUCTION

In the last decade, grassland communities have become one of the most endangered ecosystems in Europe. This biome represents over 40.00% of the earth's surface, with various endangered plant species. In this context, identifying conservation measures for these species is a priority area of study (CONANT *et al*., 2017).

In the context of the above, one of the endangered plant species of the Brassicaceae family is *Crambe tataria* Sebeók, a species present in the Continental, Pannonian, Pontic, and Steppe biogeographical regions of Romania (SÂRBU *et al*., 2007). Moreover, this species is considered a thermophilic relict in Europe (SOÓ, 1942) and a steppe postglacial relict in Romania (BÉRES, 1996).

C. tataria is a rare species in the xerophilous grasslands of Central Europe, South-Eastern Europe, and Western Siberia. In Romania, the species is spread mainly in the regions of North-East (Moldova), North-West (Transylvania), Center (Transylvania), and South-East (Muntenia), on sunny hills and slopes with moderately steep slopes. The species can grow less often in orchards, agricultural lands, vineyards, and mowed grasslands.

In Romania, the species was reported in 1816 in Sibiu County. From then until now, the gradual decrease in the number and size of populations has been visible. Thus, 70.00% of the local populations known in the literature have not been identified in the last three decades. The main cause of the extinction of local populations is the degradation and fragmentation of habitats, which is caused indirectly and directly by human activities.

The problem of the study consists of the distribution of the species, the eco-coenotic conditions, and the genetic structure of the species *C. tataria*. Thus, the species is endangered by inadequate grassland management, in which its habitats face various anthropogenic factors. Compared to field studies, the largest populations were identified in Cluj, Iași, Mureș, and

Vaslui counties. Most of these populations are in protected areas and include herds ranging from a few individuals to several hundred individuals.

Identifying the eco-coenotic conditions and the genetic structure of *C. tataria* populations is essential because they can explain the probabilities of their survival in current habitat conditions and their ability to occupy new favorable habitats. Populations with high and low genetic diversity can also be identified, and the factors that negatively influence the analyzed species. It is also essential to save this species from extinction, as it provides habitat for many species of insects in the families *Anthribidae* Billberg, 1820; *Chrysomelidae* Latreille, 1802; and *Curculionidae* Latreille, 1802, and food for many species of birds in the family *Passeriformes* Linnaeus, 1758. If no protection measures are developed in the case of the analyzed populations, both *C. tataria* and insects and birds are in danger.

The scientific novelty and originality consisted in the following results: thus, it is the first interdisciplinary study of the species *C. tataria* in Romania, in which were analyzed: the distribution of the species, as well as the causes that determined its decline in Romania; analysis of population characteristics, relationships between them and variables studied; moreover, soil samples were collected to perform chemical soil analyzes; the plant associations and variables studied that influence the floristic composition of *C. tataria* phytocoenoses were identified; also, the ISSR - PCR technique was applied, to evaluate the genetic variation of the investigated populations; and investigations were performed on the micromorphology of leaf areas from *C. tataria*, using scanning electron microscopy (SEM).

PURPOSE, HYPOTHESES AND OBJECTIVES

The purpose of the research undertaken is to identify the eco-coenotic conditions and analyze the genetic structure of some populations of *C. tataria* in Romania to establish measures to protect the species.

In accordance with the purpose and objectives of the study and the information from the specialized literature, it was wanted to verify three hypotheses:

Hypothesis 1 - Currently, *C. tataria* has a lower distribution than that presented in the literature;

Hypothesis 2 - The abiotic variables highlight the variation of the morphology and demography of the species, as well as the variation of the floristic composition of the phytocoenoses of *C. tataria*;

Hypothesis 3 - Genetic and environmental variables, and vegetation characteristics, are prerequisites in establishing measures for conservative management of *C. tataria* populations in Romania.

The following specific objectives have been set to achieve the goal:

Specific objective 1 - Analysis of the distribution of the species and identification of the causes that determine the restriction of *C. tataria* populations in Romania;

Specific objective 2 - Analysis of the characteristics of habitats and populations of *C. tataria* in Romania;

Specific objective 3 - Eco-coenotic analysis of *C. tataria* populations in Romania;

Specific objective 4 - Analysis of the genetic structure of natural populations of *C. tataria*;

Specific objective 5 - Elaboration of measures for the protection of populations of *C. tataria*.

THESIS STRUCTURE

According to the purpose, objectives, and hypotheses, the paper is structured in two distinct parts:

The first part, entitled *Synthesis of data from the literature*, comprises a single chapter, namely **Chapter 1**: *Current state of field research*, which presents a critical analysis of the current context of research on the species *C. tataria* and related information on morphology, biology, ecology, phytocoenology and distribution of the species *C. tataria*.

Part II - The presentation of my research is structured into two chapters: the materials, working methods, and results obtained in this study. In **Chapter 1**: *Materials and methods* have presented the materials used and the methods applied for each objective pursued. **Chapter 2**: *Results and discussions* include the research results on the distribution of the species ecological, coenotic and genetic analyzes of *C. tataria* populations investigated. Finally, conservative management measures for *C. tataria* populations in Romania were developed.

For each of the three chapters was formulated partial conclusions. Finally, the general conclusions, contributions, perspectives for further research, scientific activity, and the list of bibliographic references are presented.

PART I SYNTHESIS OF SPECIALTY LITERATURE

CHAPTER 1 CURRENT STATE OF THE RESEARCH IN THE FIELD

INTRODUCTION

In the last three decades, biodiversity has significantly declined locally and globally due to habitat changes induced by climate change and anthropogenic activities (POSCHLOD and WALLISDEVRIES, 2002). One of the plant species threatened by anthropogenic activities is *Crambe tataria*. The current level of knowledge of this species has been analyzed through the literature. It sought to highlight the knowledge about the analyzed species through various studies that describe the flora of Romania.

This chapter presents detailed information on the ecology, morphology, and biology of *C. tataria*. In this context, was analyzed the information published in the literature to achieve the following objectives: (1) identification of pressures and threats on the species *C. tataria*; (2) identification of the conservation status of the species *C. tataria* in Romania; (3) identification of the scientific importance of the species *C. tataria*; and (4) presentation of the current context of studies on the species *C. tataria*.

1.1 General considerations for Crambe tataria

1.1.1 Conservation status

In Europe, according to the criteria mentioned in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, the status of *C. tataria* is "least concern" (LC; BILZ *et al*., 2011). In the Romanian flora, the species is considered vulnerable and rare (OLTEAN *et al*., 1994). At the European level, this species has protection status through the following national and international legal instruments and agreements: Annex I revised in 2011 of the Bern Convention (Bern Convention, 1998); Annex IIIb of the Government Emergency Ordinance no. 57 of June 20, 2007

(Government Emergency Ordinance, 2007); and is listed in Annexes IIb and IVb of the European Union Habitats Directive (Council of Europe Directive, 1992).

1.1.2 Taxonomy, etymology, and varieties

According to the lineane taxonomic classification and monographs, *C. tataria* is taxonomically classified as follows: regnum Plantae Haeckel 1866; subregnum Tracheophyta Sinnott 1935; phylum Magnoliophyta Cronquist, Takhtajan & W. Zimmermann ex Reveal; class Magnoliopsida Brongn.; subclass Dilleniidae Takhtajan ex Reveal & Takhtajan; superorder Rosanae Takhtajan; order Brassicales Bromhead; family Brassicaceae Burnett; genus *Crambe* L. and species *Crambe tataria* Sebeók (MARHOLD, 2011).

The etymology of the word "Crambe" has its origin in the Greek term, "krambe" = cabbage, which refers to the relatively small flowers with four petals and the specific aroma of edible cabbage leaves (BRÎNZAN *et al*., 2013). Instead, the Latinized word "tataria, tatarica" = Tatar refers to the Tartars (POPESCU, 2013).

The variability of the species was presented by Traian SĂVULESCU (1955), Rezső SOÓ (1968), Vladimir Leontyevich KOMAROV (1970), etc.

1.1.3 Origin, evolution, and ethnobotany

The history of the tartar goes back in time to antiquity, but its presence was first indicated in a study by the botanist Leonhardus FUCHSIUS in 1542 (HORVÁTH, 2005). The first written mention of the plant name is quoted in 1590 in Szikszai-Fabricius BALÁZS's dictionary (MOLNÁR, 1998a). However, the first scientifically essential reference belongs to Carolus CLUSIUS, a botanist who studied plants in Austria and Pannonia. He mentioned the species in the study "Rariorum plantarum historia" published in 1601 (SEBEÓK, 1781; WILKES, 1810; RAPAICS, 1938; BÉRES, 1996; KERESZTY and GALÁNTAI, 2001).

Alexander Sebeók de Szent-Miklós (Sàndor SEBEÓK) examined the plant based on specimens from the grasslands of Eger (Hungary), which in the end was the subject of his doctoral thesis. Thus Sàndor SEBEÓK is the first descriptor of the species *C. tataria*.

C. tataria has uses in agriculture, food, beekeeping, pharmacology, phytotherapy, human medicine, and ornamental purposes and could lead to new promising sources for the discovery of biofuels, but also in highlighting large amounts of α-linolenic acid (PÂRVU, 2005; PUSHKAROVA *et al*., 2016).

1.1.4 Morphology, biology, and ontogeny

C. tataria is a bluish-green species that can reach considerable heights (between 60 cm and 150 cm). The root is long, vigorous, fleshy, and sweet, up to 150 cm long. Stem straight or ascending, thick, hairy, and rigidly branched (SEBEÓK, 1781; NYÁRÁDY, 1955; BELDIE, 1977; HORVÁTH, 2005; PÂRVU, 2005).

The leaves are large and have several narrow and elongated or linear-elongated lobes. The lobes have a wavy edge with large rounded teeth (NYÁRÁDY, 1955; POPESCU, 2013). The basal leaves that appear first are small, then become rhombic, large, hairy, or pubescent. The inflorescence is rich, spherical, and very large, consisting of dense racemes (DOMOKOS *et al*., 1999; HORVÁTH, 2005; PÂRVU, 2005; BRÎNZAN *et al*., 2013). The fruit is an articulated silicula, with a length of 5 mm - 7 mm, with a sterile lower part of approx. 1 mm long and a globular upper (rostral) part, with a length of approx. 4 mm - 5 mm (HORVÁTH, 2005; MIHĂILESCU *et al*., 2015).

1.1.5 Ecology, phytocoenology, and distribution

C. tataria is a rare species in Europe (KUPRIIANOV *et al*., 2020), with a discontinuous distribution from the Pannonian Plain to the grasslands north of the Black Sea (European Environment Agency, 2007 - 2012). It is a xerophilous and moderately temophilous species that grow in steppe phytocoenoses (DONIȚĂ *et al*., 2005; GAFTA and MOUNTFORD, 2008; OROIAN *et al*., 2017; BĂDĂRĂU, 2019)

1.2 The current context of research on the species C. tataria

Following the evolution and dynamics of research conducted so far in the case of *C. tataria*, it was found that internationally, was observed the most significant interest in the analysis of the species by Hungarian researchers (KERESZTY and GALÁNTAI, 2001; HORVÁTH, 2005), who monitored the populations of *C. tataria* in Hungary and elaboration concrete measures to protect it. Great interest has been given to the species *C. tataria* by Ukrainian (PUSHKAROVA *et al*., 2019) and Turkish researchers (TARIKAHYA-HACIOĞLU, 2016). They studied the chemical compounds, the antioxidant capacity, the cytogenetic activity, the morpho-anatomical aspects, and the characterization of the habitats in which *C. tataria* can be identified. At the national level, there are a very small number of studies targeting the species *C. tataria*. Most of them focused on general aspects of its description, ecology, chorology, and phytocoenology (OROIAN *et al*., 2017; BĂDĂRĂU, 2019; MÂNZU *et al*., 2020).

PARTIAL CONCLUSIONS

C. tataria is a perennial species widespread from the Pannonian Plain to the northern Black Sea steppes, protected by various national and international agreements and conventions. Currently, the habitat of this species is disturbed by some limiting factors, which has led to an unfavorable conservation status of the analyzed species.

Studies in the field of ethnobotany have shown that the species *C. tataria* is considered a food plant that can also be used in beekeeping, agriculture, phytotherapy, pharmacology, and decorative purposes, with economic and scientific importance that has attracted the attention of researchers in various fields such as chemistry, biochemistry, molecular biology, ecology, and biotechnology.

The reference works of ecology and phytocoenology have shown that *C. tataria* prefers open microhabitats, alkaline and nutrient-rich soils, and eroded and sunny slopes of steppe and forest-steppe areas.

Regarding the molecular research on genetic relationships, in which were studied different species of the genus *Crambe*, including *C. tataria*, the importance, and usefulness of microsatellites were highlighted, which led to the differentiation and grouping of genotypes in clusters to the generation of clear, amplified, reproducible fragments but also to the generation of non-reproducible fragments.

Regarding the biotechnological research that included the species *C. tataria* or other species of the genus *Crambe*, it was found that in the last decade, with the extent of biodiversity conservation measures, the conservation of these species has been considered using different techniques *in vitro* cultures, such as micropropagation, direct organogenesis, and somatic embryogenesis by callus or propagation.

PART II PRESENTATION OF OWN RESEARCH

CHAPTER 1 MATERIALS AND METHODS

INTRODUCTION

Over time, the genetic and ecological methods used in various studies have proven to be the most representative techniques, essential for identifying biodiversity conservation measures and in simulating and analyzing long-term dynamics, conservation support, and management *ex situ* and *in situ* conservation actions on rare or declining plant species (ŁASKA and SIENKIEWICZ, 2018). To investigate the mechanisms responsible for the endangerment of an endangered species, widely applied statistical tools can be used, such as regression and multivariate analyses (GHOLAMI and FAKHARI, 2017) or genetic analyses.

The following objectives have been set in this chapter: (1) establishing a method for collecting data on the chorology of the species, population characteristics, and floristic composition of the species *C. tataria*; (2) selection of statistical analyzes on the relationship between floristic composition, population characteristics, and environmental variables; and (3) selection of primers and a molecular method for determining the genetic structure of *C. tataria* populations.

1.1 Selection and delimitation of study areas

In the period 2019-2021, *C. tataria* was analyzed in 15 localities (**Fig. 1.1**): Alexandru cel Bun, Bădeni, Ceplenița, Horlești, Miroslava, Popricani, Tăutești, Rediu, Vânători, Vulturi (Iași County); și Glodeni (Vaslui County), Pâclele (Buzău County), Valea Glodului (Mureș County), Slimnic (Sibiu County) and Bunești (Brașov County).

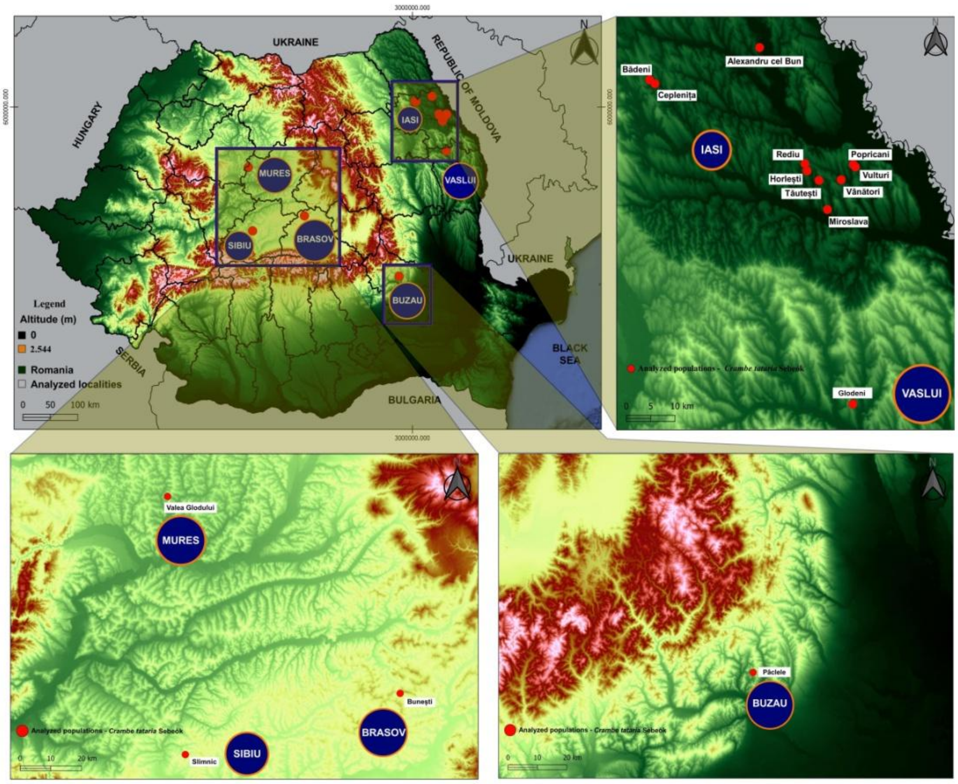

Fig. 1.1 - The study areas investigated.
(**vector data source**: **http://geo-spatial.org/vechi/download/romania-seturi-vectoriale**; **raster data source**: **http://srtm.csi.cgiar.org/srtmdata/**).

1.2 Analysis of the distribution of the species and identification of the causes that lead to the restriction of the populations in Romania

The purpose of this subchapter was to provide an overview of the presence of *C. tataria* in Romania by confirming and completing the distribution with newly identified populations and identifying the causes of the restriction of *C. tataria* populations. The data collected from both the field and the literature were organized in a tabular and spatial format

1.3 Analysis of the characteristics of populations and habitats of C. tataria

1.3.1 Recording demographic and morphological parameters

In the study, demographic parameters were represented by population density, population size, and the proportion of flowering individuals.

Regarding the biometric analysis of the morphological parameters, it consisted in performing the following measurements: plant height (cm); inflorescence circumference (cm); the number of leaves per plant; leaf length and width (cm).

1.3.2 Chemical analysis of soil samples

1.3.2.1 Collection and preparation of soil samples

Soil sampling was performed in each investigated sample area from a floristic and phytocoenological point of view. These were selectively placed in each investigated locality, from which a single soil sample was taken from its center using a soil sampler. Soil samples were analyzed in the soil chemistry laboratory, from the Research Institute for Agriculture and Environment (ICAM), within the University of Life Sciences "Ion Ionescu de la Brad" in Iași.

1.3.2.2 Determination of pH

The pH was determined using a pH meter with a combined electrode, according to SR ISO 10390 (2015). At the analytical balance, 10 g of soil was weighed. The soil was then placed in a Berzelius beaker.

1.3.2.3 Determination of total organic carbon

Determining organic carbon (LĂCĂTUȘU, 2016; LUNGU and RIZEA, 2017) involved weighing the analytical balance of 0.2 g - 1 g of soil. Subsequently, 10 mL of 1 N potassium dichromate solution and then 20 mL of concentrated sulfuric acid were added.

1.3.2.4 Determination of total nitrogen

The Kjeldahl method performed the determination of total nitrogen (LĂCĂTUȘU, 2016; LUNGU and RIZEA, 2017). The first stage of this method was the mineralization of the solution, and the second stage consisted of the dosing of nitrogen.

1.3.2.5 Determination of mobile phosphorus and potassium

In ammonium acetate solution, the spectrometric determination of mobile phosphorus and potassium (LĂCĂTUȘU, 2016; LUNGU and RIZEA, 2017) was performed according to the Egner-Riehm-Domingo method (ISO 11263: 1994). For phosphorus analysis, samples were read six minutes after staining (after Nikolov). For the analysis of potassium, the solution used was that obtained in the case of the study of mobile phosphorus by using ammonium acetate.

1.3.2.6 Determination of X-ray fluorescence chemicals

Determination of total concentrations of chemical elements: aluminium (Al), arsenic (As), calcium (Ca), iron (Fe), magnesium (Mg), oxygen (O), lead (Pb), silicon (Si), and sodium (Na), was performed by the method of X-ray fluorescence (XRF; SR EN 15309: 2007).

1.3.2.7 Statistical analysis of data

Descriptive statistical analysis (mean and standard deviation) was performed in the PAST program version 4.03 (HAMMER *et al*., 2001). Kruskal-Wallis and Mann-Whitney tests were applied to identify significant differences. Regarding the soil, the genetic type of soil was presented, which was identified in QGIS, based on the vector data downloaded from the DSMW database (Digital Soil Map of the World, 2007) and the soil class (Romanian Soil Taxonomy System, 2003).

1.3.3 Analysis of the relationships between population characteristics and environmental variables

1.3.3.1 Setting and selecting variables

An important step in terms of changes in the characteristics of *C. tataria* populations depending on the changes in the variables studied was establishing the set of variables. In this context, the set of variables studied includes biotic, abiotic, and anthropogenic variables (**Table 1.1**).

Table 1.1

Variables used in ecological modeling of population characteristics and floristic composition.

No. crt.	Variable	Abbreviation
A	**Biotic variables**	
1	Vegetation cover (%)	AVEG
2	Vegetation height (cm)	ÎVEG
B	**Abiotic variables**	
B.1	**Topoclimatic variables**	
1	Elevation (m)	ALT
2	Slope (º)	Pa
3	Aspect (º)	EXP
B.2	**Macroclimatic variables**	
4	Annual Mean Temperature (℃)	BIO1
5	Mean Diurnal Range (℃)	BIO2
6	Isothermality	BIO3
7	Temperature Seasonality (%)	BIO4
8	Max Temperature of Warmest Month (℃)	BIO5
9	Min Temperature of Coldest Month (℃)	BIO6
10	Temperature Annual Range (℃)	BIO7
11	Mean Temperature of Wettest Quarter (℃)	BIO8
12	Mean Temperature of Driest Quarter (℃)	BIO9
13	Mean Temperature of Warmest Quarter (℃)	BIO10
14	Mean Temperature of Coldest Quarter (℃)	BIO11
15	Annual Precipitation (mm)	BIO12
16	Precipitation of Wettest Month	BIO13
17	Precipitation of Driest Month	BIO14
18	Precipitation Seasonality	BIO15
19	Precipitation of Wettest Quarter	BIO16
20	Precipitation of Driest Quarter	BIO17
21	Precipitation of Warmest Quarter	BIO18
22	Precipitation of Coldest Quarter	BIO19
B.3	**Aridity**	
23	De Martonne Aridity Index - annual (mm / ℃)	IADM
B.4	**Chemical variables**	
24	Total aluminum (%)	Al
25	Total arsenic (mg Kg^{-1})	As
26	Total nitrogen (%)	N
27	Total calcium (%)	Ca
28	Total organic carbon (%)	C_{org}
29	Total iron (%)	Fe
30	Mobile phosphorus (mg Kg^{-1})	P
31	Total magnesium (%)	Mg
32	Total sodium (%)	Na

No. crt.	Variable	Abbreviation
33	Total oxygen (%)	O
34	pH	pH
35	Total lead (mg Kg^{-1})	Pb
36	Mobile potassium (mg Kg^{-1})	K
37	Total silicon (%)	Si
C	**Anthropic variables**	
1	The distance between the nearest locality and the plot (km)	DAL
2	Distance between agricultural land and plot (m)	DTP
3	Type of management	TM

The Pearson correlation matrix was created in the PAST program to select the independent variables. In this context, strongly correlated variables (correlation coefficient > 0.7 or <- 0.7) were identified and removed from the data set (which initially included 24 variables).

1.3.3.2 Realization and selection of generalized linear models

To identify the most important variables that explain the variation of the characteristics of *C. tataria* populations, a method developed by BURNHAM and ANDERSON (2002) was applied, by which, based on AICc (corrected Akaike Information Criterion), the generalized linear models are identified better in describing the relationships between the characteristics of *C. tataria* populations and the biotic and abiotic variables studied.

1.3.3.3 Identifying important variables and testing the relationship with them

For a variable to be considered "important", it must be included in at least half of the models. Subsequently, the relationship between each important environmental variable and the characteristics of *C. tataria* populations was described using simple, linear, or unimodal generalized linear models. The graphs were made in the Minitab Statistical Software version 21.1.0 (Minitab, 2021).

1.4 Investigations on the micromorphology of leaf areas from C. tataria

The plant material was fixed in FEA for the analysis of scanning electron microscopy (SEM; BOZZOLA and RUSSELL, 1999). This analysis consisted of making cross-sections of the leaf, under a stereomicroscope, with a razor blade. Subsequently, the small leaf fragments were dried at a critical point with CO_2 (EMS 850 Critical Point) and then coated with a thin layer of gold particles (30 nm). Finally, the samples were examined by scanning electron microscopy.

1.5 Coenotic analysis of C. tataria populations

1.5.1 Vegetation sampling

For the establishment of phytocoenological relevés, the method of selective sampling was chosen. The sample area size was 100 m2, respecting the recommendations for the study of grasslands in Romania (CRISTEA *et al*., 2004). At this stage, 47 phytocoenological relevés were carried out (including 213 species). Scientific names were standardized according to the Euro + Med PlantBase database (http://ww2.bgbm.org/EuroPlusMed/). A quantitative assessment of abundance - dominance (AD) was made using the Braun - Blanquet scale (CRISTEA *et al*., 2004), and the constancy (K) of the species was also calculated.

1.5.2 Determination of phytocoenoses

The relevés were imported into the JUICE program version 7.0 (TICHÝ, 2002) to analyze the floristic composition. This analysis includes the following steps:

- establishing the matrix associated with the average values of the abundance-dominance intervals transformed by extracting the square root, corresponding to the abundance-dominance (AD) notes given in the field;
- elimination of species that were present only in 1-2 relevés;
- creating the dissimilarity matrix using the Bray-Curtis metric;

- creating the dendrogram using the GINKGO program (BOUXIN, 2005);
- determining the optimal number of clusters, using two indices: Silhouette (ROUSSEEUW, 1987) and Rand corrected (RAND, 1971);
- determination of diagnostic species using the Indicator Value Index (IndVal; DUFRÊNE and LEGENDRE, 1997);
- habitat classification. The habitat code was taken from the expert classification system for habitats (CHYTRÝ *et al*., 2020).

1.5.3 Analysis of the relationships between floristic composition and environmental variables

The identification of the variables that influence the floristic composition of the phytocoenoses of *C. tataria* involved the following steps: (i) data transformation (by extracting the square root); (ii) performing the Detrended Correspondence Analysis - DCA; (iii) performing the Canonical Correspondence Analysis - CCA. The ordinograms were made in CANOCO version 5 (TER BRAAK and ŠMILAUER, 2012). The abiotic, biotic, and anthropogenic variables mentioned in **Table 1.1** were studied in this analysis.

1.5.4 Description of the type of plant community

Phytocoenological relevés were carried out to describe the type of plant community. The plant associations were identified based on the diagnostic species and the specialized literature (COLDEA *et al*., 2012; CHIFU *et al*., 2014).

1.6 Analysis of the genetic structure of the species C. tataria

1.6.1 Plant material collection

The plant material was collected from 15 localities and consisted of basal leaves, young and healthy of *C. tataria*. The plant material was collected in plastic tubes, filled with silica gel, and stored in the refrigerator at a temperature of - 22 ° C. The number of samples collected varied between 1 and 5.

1.6.2 Highlighting the genetic variability by the ISSR technique

1.6.2.1 DNA extraction

DNA extraction from samples collected by *C. tataria* was performed using the GenElute Plant Genomic DNA Miniprep kit from Sigma Aldrich (Sigma-Aldrich, G2N70), with 70 reactions. All operations performed for this activity complied with the actual DNA extraction protocol: prepare plant tissue, release DNA, filter lysate, prepare column, bind DNA, wash column, and elute DNA.

1.6.2.2 DNA amplification

The GoTaq G2 Green master Mix kit from Promega (Promega, 2014 - 2018) was used to amplify DNA samples. This activity included four stages: testing and selection of primers, DNA quantification, the composition of the PCR reaction, and the DNA sample amplification protocol.

Regarding the composition of the Polymerase Chain Reaction (PCR), it had a total volume of 15 μL. The amplification conditions of the samples were performed in the Eppendorf Mastercycler gradient device, according to the following program: initial denaturation of ten minutes at 95 °C; 35 cycles of denaturation of 30 s at 95 °C, annealing of the primer 45 seconds at 50 °C, extension 2 minutes at 72 °C; and the final extension of 10 minutes at 72 °C.

1.6.2.3 Agarose gel electrophoresis

Separation and highlighting of polymorphic bands of PCR amplified DNA was performed by agarose gel electrophoresis. In this context, the following operations were performed: agarose gel preparation, preparation of amplified samples, loading of samples on gel, and electrophoresis.

1.6.2.4 Image capture and analysis

The visualization and photography of the electrophoresis gels were made at the Geni transilluminator with the 2 Mpx camera from SynGene. The photos with the gels were introduced in the BioNumerics 8 software platform, with the help of which the 1D images were processed. Data on the calculated genetic distances were exported to the MEGA X (Molecular Evolutionary Genetics Analysis) program version 10 (KUMAR *et al.*, 2015). Within this program, the dendrogram was constructed based on genetic distances.

1.6.2.5 Quantification of genetic diversity

The values of the generated densitometric curves were exported from BioNumerics version 8 and converted into genetic distances in the extension for Microsoft Excel: GenAlEx 6.51b2 (PEAKALL and SMOUSE, 2006, 2012). They were calculated using a template for dominant markers in which the number of individuals, the number of harvest localities, and the number of loci were entered: the genetic distances between the samples of the 15 harvest localities; the Shannon index for each locus, and the average for the 15 harvest localities; expected heterozygosity (He); allelic frequencies for each locus; genetic distance Nei (D), between groups.

PARTIAL CONCLUSIONS

The selective sampling method was applied to update the data on the distribution of *C. tataria* species in Romania and vegetation analysis.

For the eco-coenotic characterization of the study species, the phytocoenological relevés method was used. The data obtained were analyzed both classically and by multivariate statistical analysis techniques.

To identify and describe the relationships between the characteristics of *C. tataria* populations and some environmental variables, data were collected in the same sample areas (relevés - used to characterize vegetation), and regression analyses were used.

The evaluation of the genetic variability at intra and inter-population levels of some populations belonging to the species *C. tataria* was performed with the help of the ISSR-PCR technique. The criteria for selecting this molecular technique are accessibility, a high degree of reproducibility, and automation.

The extraction of DNA from *C. tataria* samples was performed according to a commercial kit, GenElute Plant Genomic DNA Miniprep Kit, of 70 reactions. DNA separation and identification were performed by agarose gel electrophoresis.

For the investigations on the micromorphology of the leaf areas from *C. tataria*, scanning electron microscopy (SEM) was used.

CHAPTER 2 RESULTS AND DISCUSSIONS

INTRODUCTION

Assessment of eco-coenotic requirements, knowledge of diversity, and genetic structure are prerequisites for developing measures to protect the species *C. tataria*. These conditions are essential in managing *ex situ* and *in situ* conservation actions of *C. tataria* populations.

This study extended the knowledge on the distribution, eco-coenotic conditions, and genetic structure of *C. tataria* populations. As the present study consisted in collecting data at a relatively fast and non-exhaustive rate, being analyzed only some populations, we mention that the data on the species *C. tataria* will be completed through future research. The objectives of the study were the following: (a) analysis of the distribution and identification of the causes that determine the restriction of the populations in Romania; (b) analysis of habitat characteristics, populations, vegetation, and the relationship between them and environmental variables; (c) biometric analysis of some morphological parameters; (d) investigations on the micromorphology of foliar areas from *C. tataria* (SEM); (e) diversity and genetic structure of the species *C. tataria*; and (f) elaboration of measures for the conservation of *C. tataria* populations.

2.1 Species distribution analysis

Analysis of old and recent data on the presence of *C. tataria* in Romania (CHIRILĂ, 2021) includes 168 localities (**Fig. 2.1**). According to the total number of populations mentioned in the literature, it was found that the species is common in Transylvania (64.66%), less common in Moldova (26.93%), rare in Muntenia (5.98%), very rare in Dobrogea (1.79%) and Banat (0.59%).

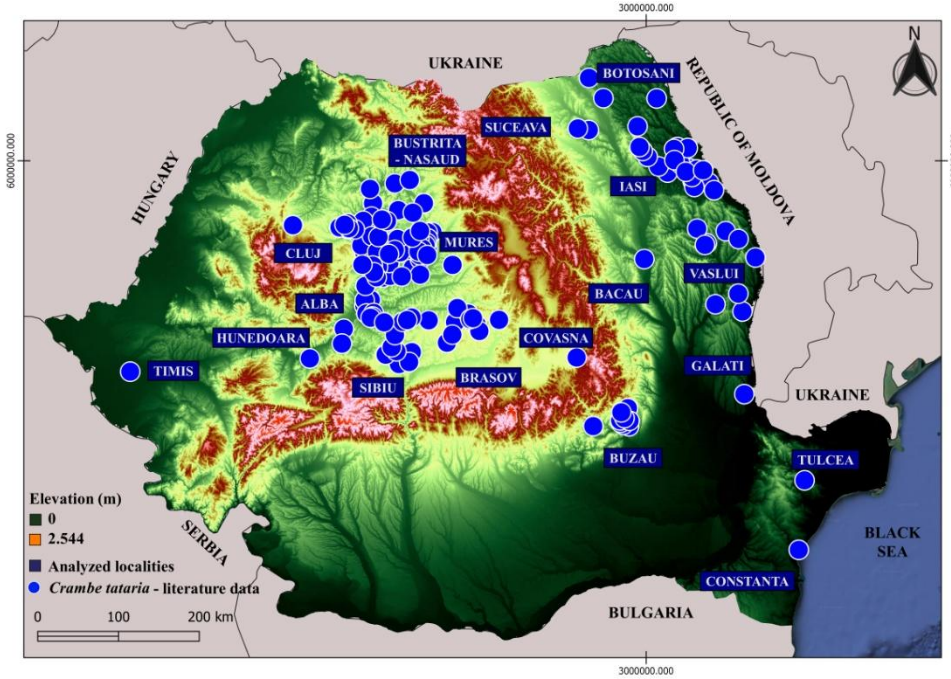

Fig. 2.1 - Distribution of *C. tataria* populations mentioned in the literature.

In 2019 - 2021, 81 populations were checked (CHIRILĂ, 2021). Although all verified localities had potentially favorable conditions for *C. tataria*, it was identified in only 21 localities (**Fig. 2.2**).

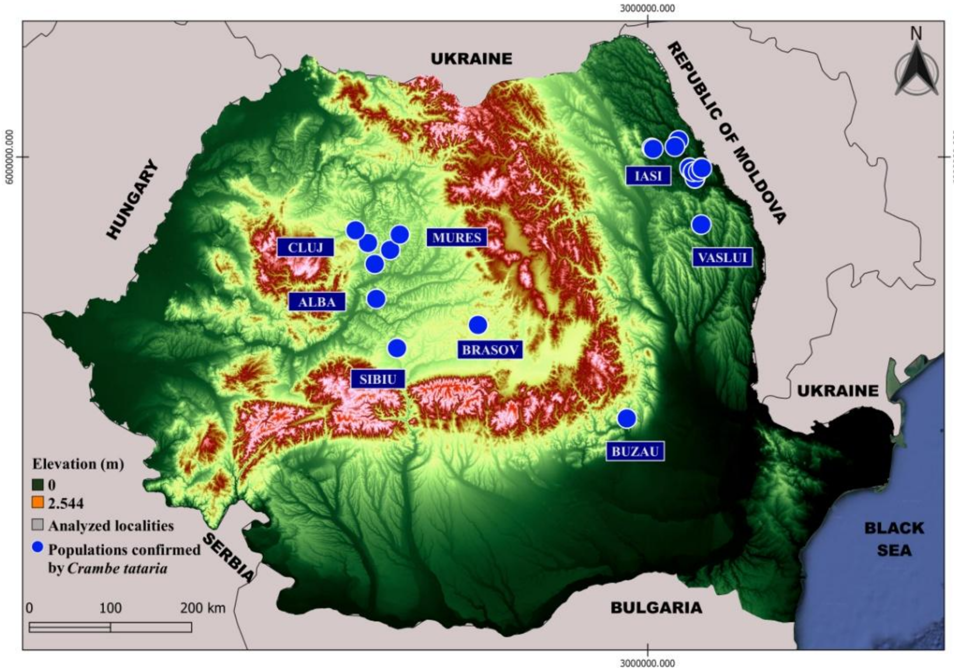

Fig. 2.2 - Distribution of confirmed populations of *C. tataria* in the field.

2.1.1 Discussions on the distribution and causes of restriction of the population

2.1.1.1 Distribution of the species in Transylvania

In Transylvania, most populations are outside the protected areas (75.00%), and 25.00% are inside the protected areas.

2.1.1.2 Distribution of the species in Moldova

In Moldova, most populations are outside protected areas (62.23%), and 37.77% are inside protected areas.

2.1.1.3 Distribution of the species in Muntenia

In Muntenia, *C. tataria* was identified in Pâclele (Buzău County), at an elevation of 271 m. Shrubs limit the spread of the species in the grasslands of this site.

2.2 Habitat characteristics analysis

2.2.1 Soil type characterization

In the analyzed localities, the following genetic soil types were identified: luvic phaeozem in Muntenia and Transylvania; calcium chernozem in Moldova; gleic luvisol in Moldova and Transylvania; and orthic luvisol in Moldova.

2.2.2 Soil analysis: chemical parameters

2.2.2.1 Variation in pH and total organic carbon

The soil reaction was weakly alkaline, and the total organic carbon concentration in the soil was low (**Fig. 2.3**).

2.2.2.2 Variation of secondary macronutrients (*Ca and Mg*)

The calcium (Ca) and magnesium concentrations determined for each sample surface are low in the analyzed soils (**Fig. 2.3**).

2.2.2.3 Variation of primary macronutrients (*N, P, and K*)

Analysis of primary macronutrients showed that 86.95% of the values fall into the category of "good" soils in the case of nitrogen, 43.47% of the values fall into the category of "very good" soils in the case of phosphorus, and 80.85% of the values fall into the category of "very good" soils in the case of potassium (**Fig. 2.3**).

2.2.2.4 Heavy metal variation (*Al, As, Pb and Fe*)

In the analyzed soils, the aluminum and iron concentrations were low, and the concentrations of arsenic and lead were high (**Fig. 2.3**).

2.2.2.5 Variation of silicon, oxygen, and sodium

In the analyzed plots, the soils are rich in silicon and poor in oxygen and sodium (**Fig. 2.3**).

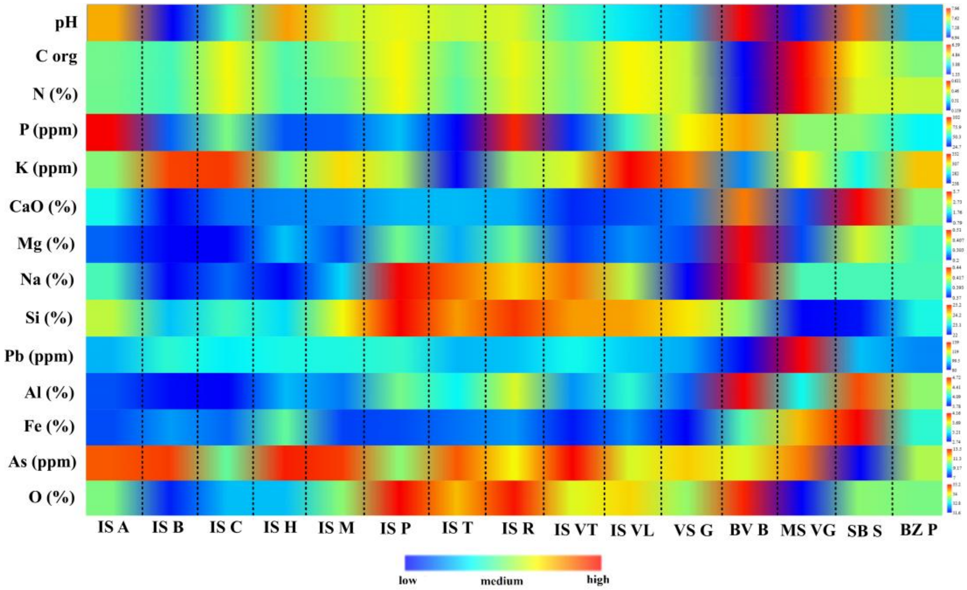

Fig. 2.3 - Variation of soil chemical properties. Localities: IS A (Iași Alexandru cel Bun), IS B (Iași Bădeni), IS C (Iași Ceplenița), IS H (Iași Horlești), IS M (Iași Miroslava), IS P (Iași Popricani), IS T (Iași Tăutești), IS R (Iași Rediu), IS VT (Iași Vânători), IS VL (Iași Vulturi), VS G (Vaslui Glodeni), BV B (Brașov Bunești), MS VG (Mureș Valea Glodului), SB S (Sibiu Slimnic), BZ P (Buzău Pâclele).

2.2.3 Discussions on habitat characteristics

The investigated areas are characterized by zonal soils and soils that have a subzonal character. The low concentration of organic carbon is due to precipitation and temperatures (ZHAO *et al*., 2017). Regarding arsenic, its growth in the soil is due to the use of pesticides in controlling plant pests (MISSIMER *et al*., 2018), and the cause of high concentrations of lead is due to human activities (FAHR *et al*., 2013). The silicon concentration in the soil increases due to pH, organic matter, etc. (ANGGRIA *et al*., 2020).

The increase of primary (nitrogen, phosphorus, and potassium) and secondary (calcium) macronutrients in the analyzed soils may be due to several factors: humus and water content, temperature, soil pH, etc. (JACKSON, 2014). The decrease in the concentration of magnesium and iron in the soil is due to the pH of the soil (JONES, 2020). The decrease in aluminum is due to the increase in pH (SZURMAN-ZUBRZYCKA *et al*., 2021). Also, soil's oxygen presence is limited by soil compaction and precipitation (NEIRA *et al*., 2015).

2.3 Analysis of population characteristics

2.3.1 Analysis of demographic parameters

The sample size selected at the national level and analyzed in this paper was 1.863 individuals, with an average of 88.71 individuals per population. Compared to elevation, it was found that with the increase of this parameter, there was a decrease in the number of individuals (**Fig. 2.4**). The population density was estimated at 5.64 individuals per sample area. Within the analyzed populations, the identified individuals were grouped in the following categories: vegetative individuals (51.26%), flowering individuals (43.10%), and individuals with leaves and flowers (5.63%).

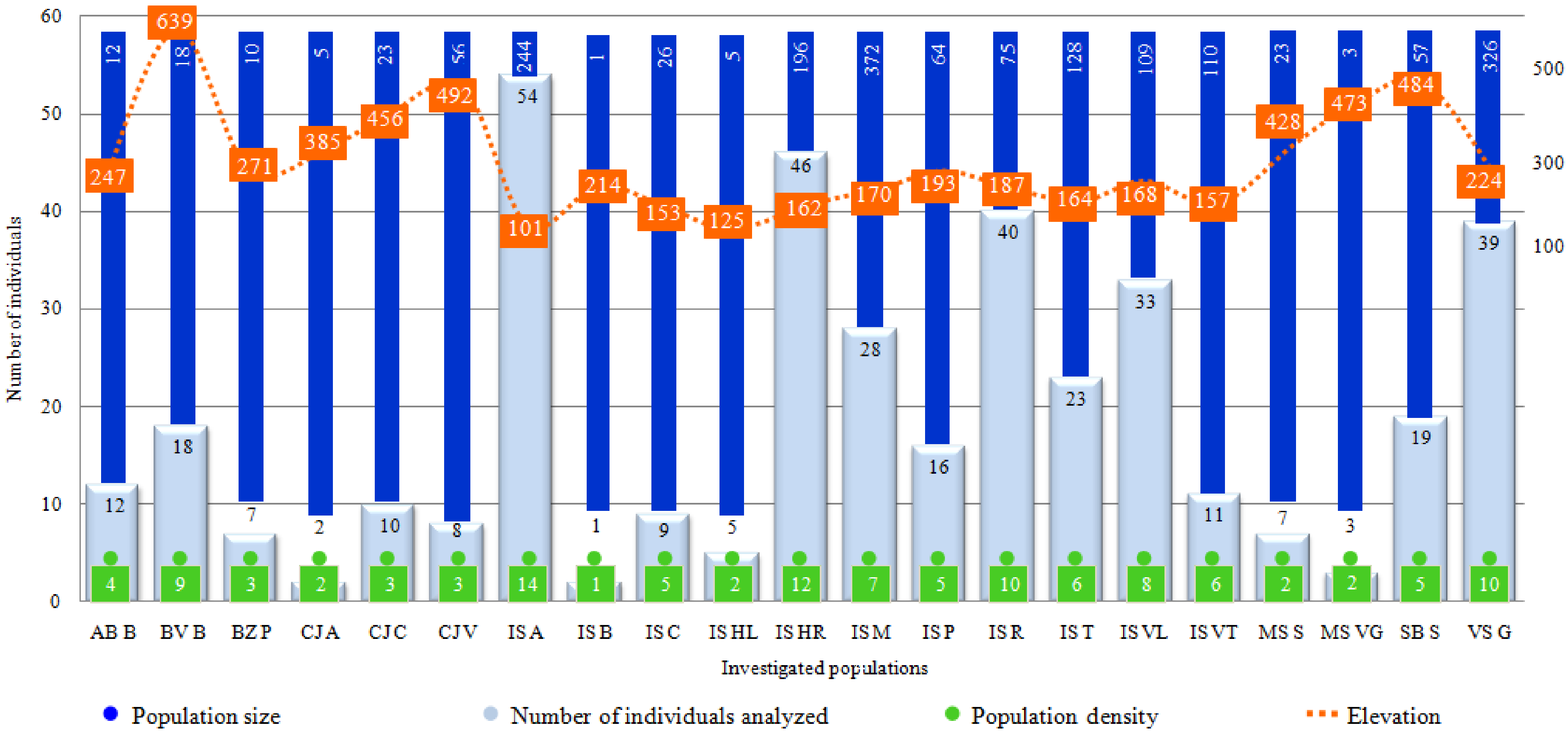

Fig. 2.4 - The number of individuals analyzed and the elevation.

2.3.2 Biometric analysis of some morphological parameters

2.3.2.1 Plant height

The height of the plant recorded values between 39.00 cm and 67.31 cm. Most values regarding the height of the plant are in the range of 40 cm - 60 cm (**Fig. 2.5**).

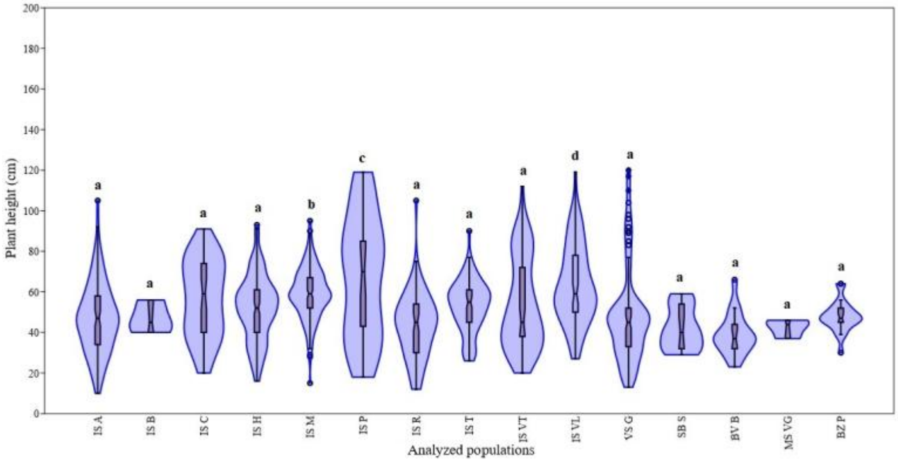

Fig. 2.5 - The concentration of plant height values: point in the center of the box - median; cassette length - interquartile range; the length of the line extending out of the box - the interval.

2.3.2.2 Number of leaves per plant

The lowest average value was measured in the population from Horlești (2.03), and the highest was measured in the individuals from Valea Glodului (10.33). Most values regarding the number of leaves are in the range of 1 - 2.5 (**Fig. 2.6**).

2.3.2.3 Leaf size

The lowest average value was measured in the population from Horlești (2.03), and the highest was measured in the individuals from Valea Glodului (10.33). Most values regarding the number of leaves are 1 - 2.5 (**Fig. 2.7**). Regarding the width of the leaf, it varies between 10.5 cm (population from Bădeni) and 23.27 cm (population from Bunești). Most values regarding the leaf width are in the range of 14 cm - 23 cm (**Fig**. **2**.**8**).

2.3.2.4 Inflorescence circumference

The highest average was recorded in the population from Glodeni (233 cm), and the lowest was measured in the individuals of the population from Bădeni (62 cm). Most of the recorded values are in the range of 150 cm - 200 cm (**Fig**. **2**.**9**).

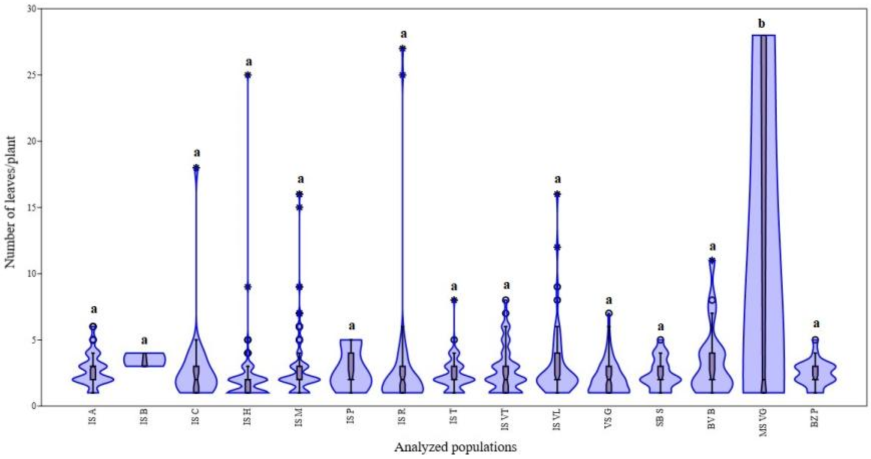

Fig. **2**.**6** - **The concentration of values on the number of leaves/plant**: **point in the center of the box - median; cassette length - interquartile range**; **the length of the line extending out of the box - the interval**.

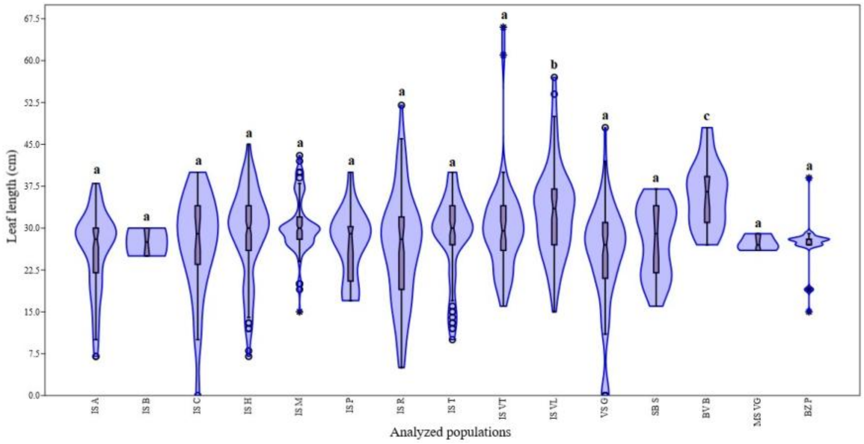

Fig. **2**.**7** - **The concentration of values on leaf length**: **point in the center of the box - median**; **cassette length - interquartile range**; **the length of the line extending out of the box - the interval**.

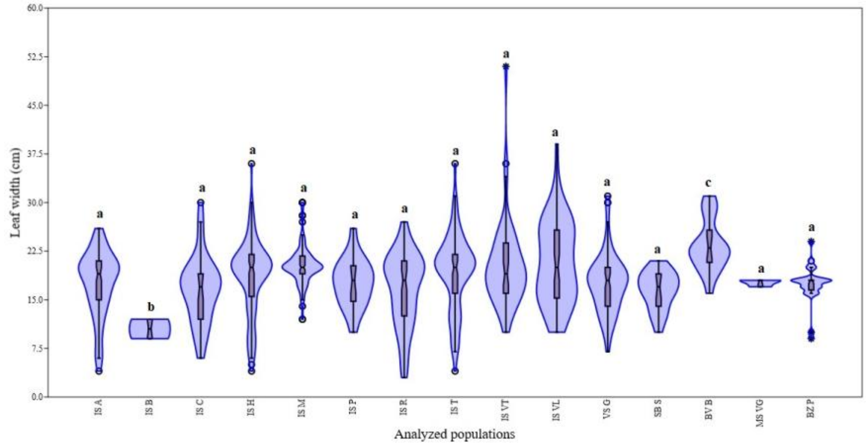

Fig. 2.8 - The concentration of leaf width values: point in the center of the box - median; cassette length - interquartile range; the length of the line extending out of the box - the interval.

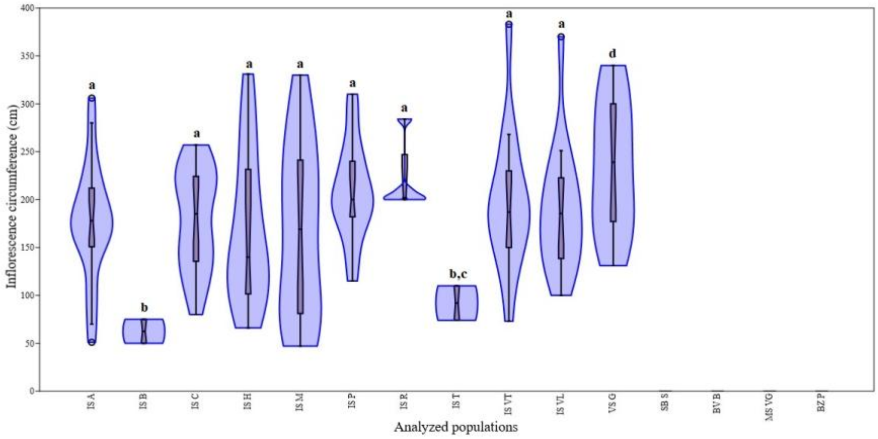

Fig. 2.9 - The concentration of values on the inflorescence circumference: point in the center of the box - median; cassette length - interquartile range; the length of the line extending out of the box - the interval.

2.3.3 Discussions on population characteristics

According to the literature (BALL, 1964), the height of *C. tataria* plants varies from 60 cm to 150 cm, depending on the season and population density. The study by HEWITSON (2020) showed that plants that grow in drought conditions have low values regarding the number of leaves and leaf size. According to RIAZ *et al*. (2015), a higher number of leaves per plant may be due to good environmental adaptation and the availability of nitrogen concentration in the growing substrate.

2.4 Analysis of the relationships between population characteristics and environmental variables

2.4.1 The relationship between plant height and environmental variables

The height of the plant (**Fig. 2.10**) decreases simultaneously with the increase of vegetation cover and calcium concentration and increases with the rise of lead concentration. The relationship between plant height and type of management is positive in grazed grasslands and negative in non-grazed grasslands.

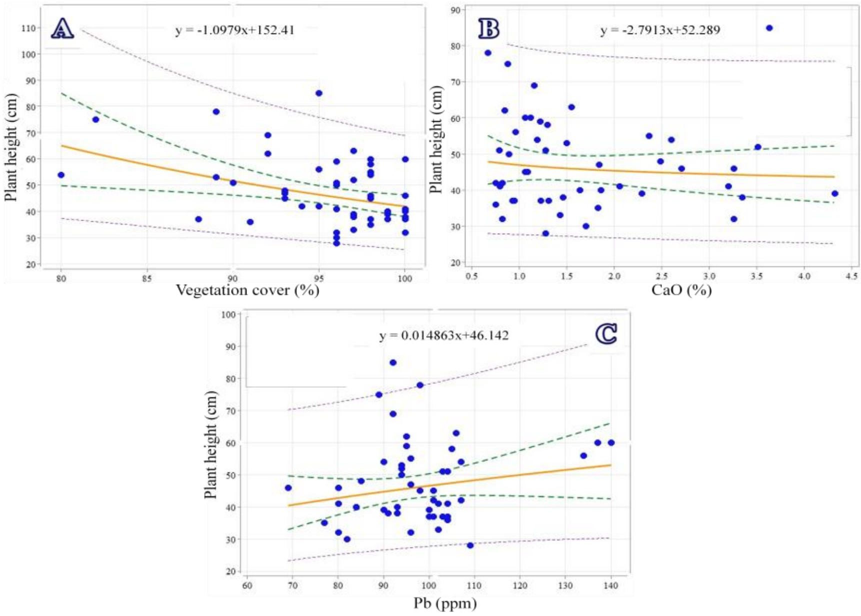

Fig. 2.10 - The relationship between plant height and vegetation cover (A), calcium - Ca (B) and lead - Pb (C).

2.4.2 The relationship between the number of individuals per sample area and the environmental variables

The relationship between the number of individuals and silicon is of the polynomial type of degree II. Thus, the increase of the silicon concentration determines the rise in the number of individuals up to the value of 24.10 %. Increasing the concentration of silicon above this value causes a decrease in the number of individuals. The relationships between the number of individuals and pH, vegetation cover, and slope are linearly positive (**Fig. 2.11**).

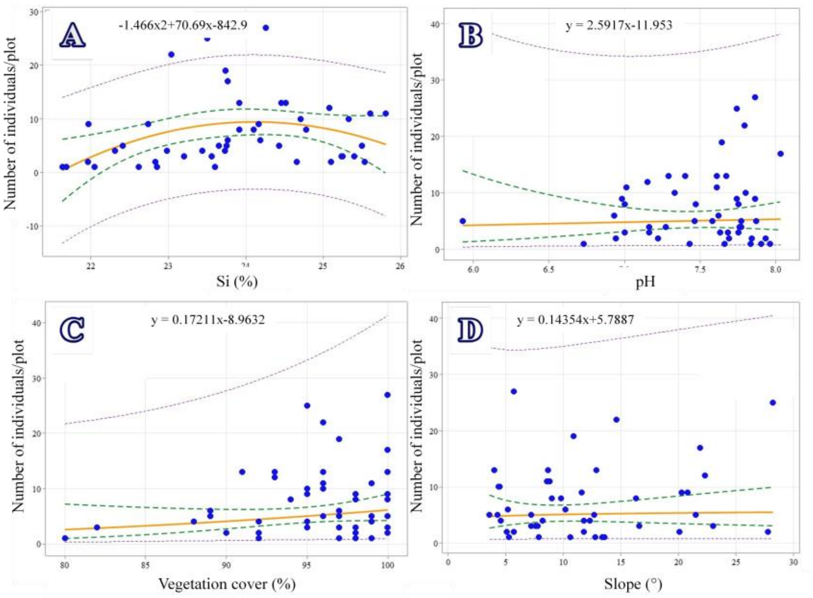

Fig. 2.11 - Relationship between the number of individuals and silicon - Si (A), pH (B), vegetation cover (C), and slope (D).

2.4.3 The relationship between inflorescence circumference and environmental variables

The relationships between the inflorescence circumference and phosphorus, the average annual temperature, pH, and the distance between the nearest locality and the sample area are linearly positive, and the relationship between the circumference of the inflorescence and the height of vegetation is linearly negative (**Fig. 2.12**).

2.4.4 The relationship between the number of leaves per plant and environmental variables

The relationship between the number of leaves and silicon is of the second-degree polynomial type. The increase of the silicon concentration determines the decrease of the number of leaves per plant up to the value of 24.70%. Regarding the relationship between the number of leaves and calcium, it is linearly negative (**Fig. 2.13**).

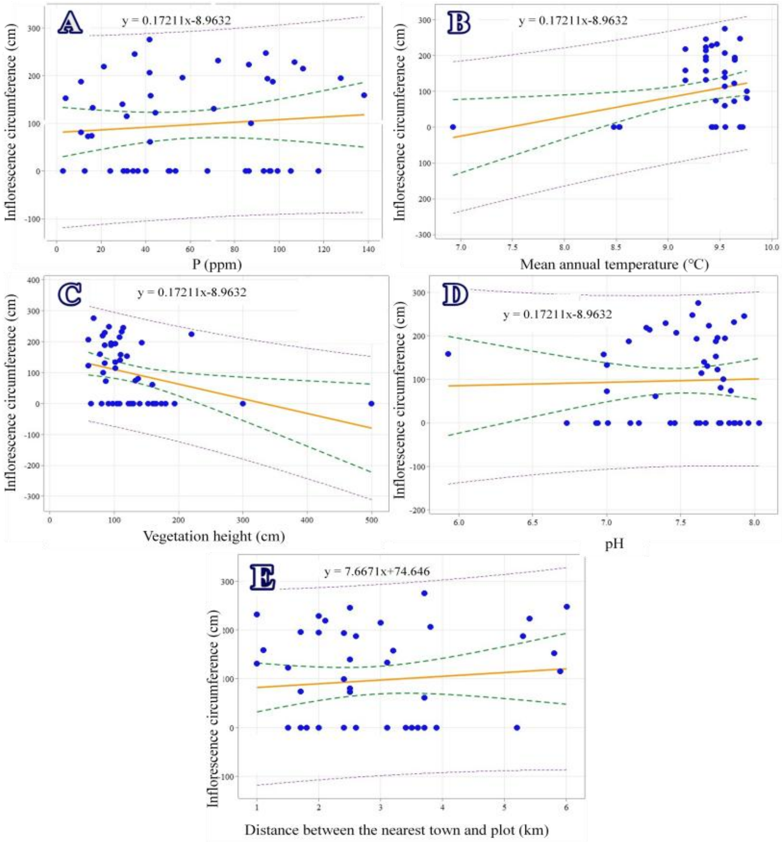

Fig. 2.12 - Relationship between inflorescence circumference and phosphorus - P (A), average annual temperature (B), vegetation height (C), pH (D), and distance between nearest locality (E).

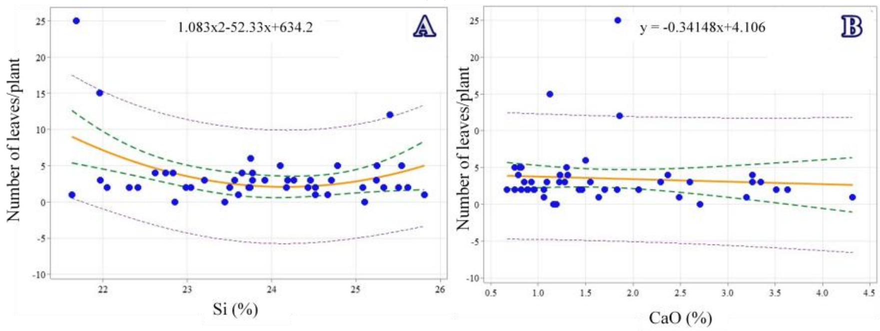

Fig. 2.13 - The relationship between the number of leaves and silicon - Si (A) and calcium - Ca (B).

2.4.5 The relationship between the proportion of flowering individuals and environmental variables

The variation in the proportion of flowering individuals was explained negatively by the cover and height of the vegetation and positively by the mean annual temperature (**Fig. 2.14**).

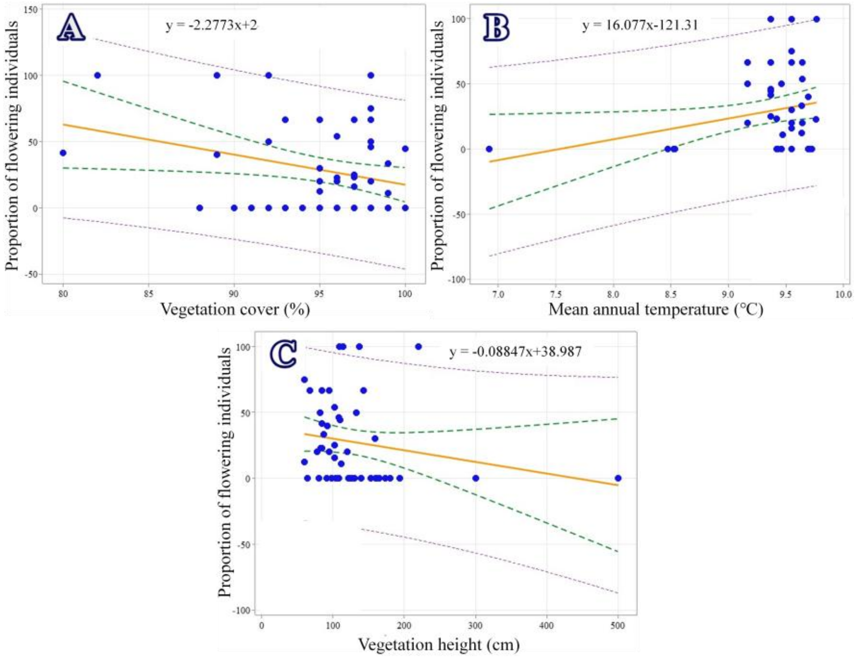

Fig. 2.14 - The relationship between the proportion of flowering individuals and vegetation cover (A), the mean annual temperature (B), and the height of vegetation (C).

2.4.6 The relationship between leaf size and environmental variables

The relationship between leaf size and phosphorus is linearly positive. The relationship between leaf size and management type is positive in non-grazed grasslands and negative in grazed grasslands (**Fig. 2.15**).

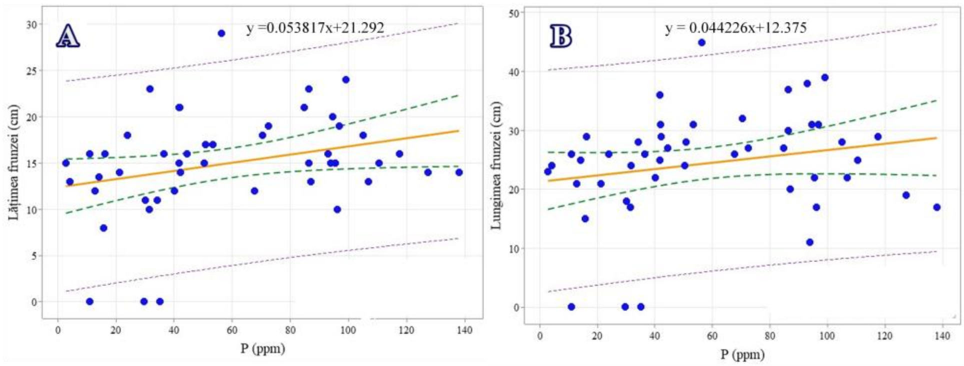

Fig. 2.15 - The relationship between leaf length (A), leaf width (B), and phosphorus.

2.4.7 Discussions on the relationship between population characteristics and environmental variables

2.4.7.1 The relationship between population characteristics and soil chemical variables

Although in the literature (HORVÁTH, 2005; PUSHKAROVA *et al*., 2016) it is mentioned that the species *C. tataria* prefers soils with a high calcium content, it has been shown that the presence of a high concentration of calcium in the soil (> 6%), had a negative influence on plant height and the number of leaves per plant. This may be due to interactions with other nutrients and/or chemicals dissolved in the soil solution and to a decrease in mean annual precipitation (WHITE și BROADLEY, 2003).

Regarding the lead concentration in the soil, its increase has led to an increase in plant height, but not in the case of population size and the proportion of flowering individuals, in which lead has a negative effect on them. SHU *et al*. (2014) showed that lead had a stimulating effect on the height of seedlings of *Jatropha curcas* L. This stimulating effect is related to the hormetic impact, which shows a positive response to exposure to a low level of a toxic substance (JALAL *et al*., 2021).

The presence of silicon in a high concentration in the soils of the analyzed grasslands is due to the smoke from the rubber burns, and the fact that most of the grasslands are grazed. According to MCNAUGHTON and TARRANTS (1983), in soils in grazed areas, silicon is found in high concentrations. In the case of phosphorus, we can indicate that a lower concentration of phosphorus in the soil would affect the increase in leaf size and inflorescence. This effect can be explained by cell division and expansion (KIM and LI, 2016). The importance of phosphorus was also demonstrated in the study by SUGIER *et al*. (2019), which showed a positive relationship between the size of *Arnica montana* L. leaves and phosphorus.

2.4.7.2 The relationship between population characteristics and the structural characteristics of plant communities

The negative correlation between plant height and vegetation cover is explained by the competition of plant species and the shading of grasslands with woody species. The results obtained correspond to previous studies (NAGASHIMA and HIKOSAKA, 2011), in which it was shown that shaded plants provide fewer resources to plant height and more resources to leaves. The high nitrogen content explains the negative correlation between the proportion of flowering individuals and vegetation cover in the soil.

2.4.7.3 The relationship between population characteristics and anthropogenic variables

The grazed grasslands had a positive influence on the height of the plant. Although mechanized mowing has rarely been observed in the grasslands analyzed, it could have a negative impact on *C. tataria* species. The increase in leaf size and plant height also depends on the concentration of nutrients in the soil.

2.4.7.4 The relationship between population characteristics and abiotic variables

The positive correlation between the number of individuals and the slope could be explained by aridity (YANG *et al*., 2020). Regarding bioclimatic variables, it was shown that the variation of the proportion of flowering individuals and the circumference of the inflorescence was best explained by the mean annual temperature.

2.5 Coenotic analysis

2.5.1 Syntaxonomic analysis

The phytocoenoses of the identified associations are framed from the phytocoenological point of view in the classes *Festuco-Brometea* and *Molinio-Arrhenatheretea*.

2.5.2 Interpretation of numerical analyzes of the floristic composition

After making the dendrogram, which resulted from applying the hierarchical agglomerative clustering algorithm, it was cut into nine partitions with ten clusters (**Fig. 2.16**), which were subsequently analyzed using the corrected Rand and Silhouette indices.

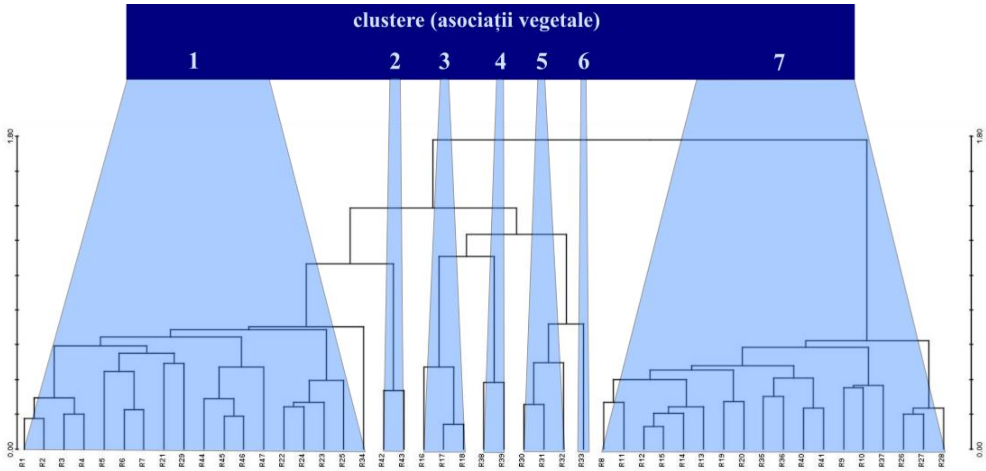

Fig. 2.16 - Cluster dendrogram by applying the flexible beta algorithm of phytocoenological releves from the 15 investigated localities.

Following the corrected Rand index application, it was found that partitions 7 with 8 clusters have the highest value (0.961). After calculating the Silhouette index, it was found that the partition with 7 clusters has the maximum value (0.31403). Therefore, with the help of these indices, the partition with 7 clusters was taken into account for the syntaxonomic level of association.

2.5.3 Habitat characterization

C. tataria (**Photo. 2.1**) was identified in two classes of vegetation: the class *Festuco-Brometea* (xerophilous grasslands) and the class *Molinio-Arrhenatheretea* (mesophilous grasslands). Of these two habitat types, it has been observed that the analyzed species prefers xerophilous grasslands. These meadows are characterized by moderate mean annual temperatures, higher silicon, arsenic, lead concentrations, lower elevation, and mean annual precipitation compared to mesophilic grasslands. Vegetation coverage was, on average, 95.00%, and the richness of vascular plant species was, on average, 47 species per 100 m^2.

Photo. 2.1 - Flowering (A) and vegetative (B) specimens of *C. tataria* (original photo).

The alliances in which the identified plant associations were included were the following: *Festucion valesiacae* Klika 1931, *Stipion lessingianae* Soó 1947, *Cirsio - Brachypodion pinnati* Hadač et Klika in Klika et Hadač 1944 and *Arrhenatherion* Koch 1926.

2.5.4 Description of plant associations

Cluster 1: Association *Taraxaco serotinae - Festucetum valesiacae* (Burduja *et al*. 1956, Răvăruț *et al*. 1956) Sârbu, Coldea et Chifu 1999.

Location: This association is spread in the meadows from Alexandru cel Bun, Bădeni, Ceplenița, Popricani, Rediu, Vulturi, Tăutești (Iași County) and Glodeni (Vaslui County; **Photo. 2.2**).

Stationary conditions: Phytocoenoses of *Festuca valesiaca* Gaudin have been identified on arid coasts, with an elevation between 90 m and 244 m, with variable inclinations, from 4 ° to 22.3 °. The mean annual precipitation was between 545 mm and 569 mm, and the mean annual temperature was 9.16 ° C to 9.75 ° C.

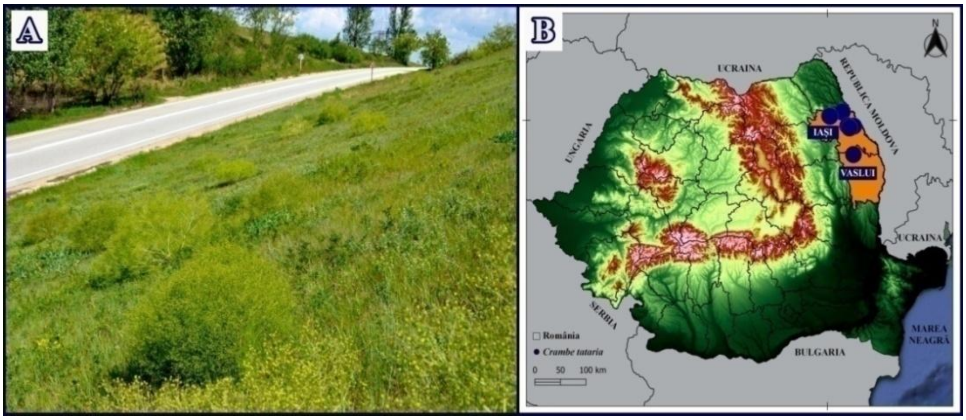

Photo. **2.2** - ***Taraxaco serotinae*** **-** ***Festucetum valesiacae*** (**A**) **and association's location** (**B**).

Vegetation structure: The upper layer of vegetation was represented by the species *Achillea pannonica* Scheele, *Centaurea orientalis* L., etc., and the middle layer was formed by plant species: *Adonis vernalis* L., *Ajuga laxmannii* (Murray) Benth. etc. The species of small plants (20 cm - 25 cm) which form the lower layer are represented by *Plantago lanceolata* L., *Thymus pannonicus* L., etc.

Floristic and phytocoenological composition: The xerophilous character is illustrated both by the edifying species *Festuca valesiaca* and by species phytocoenologically included in the alliance *Jurineo arachnoideae - Euphorbion stepposae* and the order *Festucetalia valesiacae*.

Ecological conditions: According to ecological indices, xeromesophilic (68.46%), mesothermal (44.54%), and weakly alkaline (39.63%) species predominate. Bioform analysis revealed the presence of hemicryptophyte species (68.69%), and the spectrum of geoelements is dominated by Eurasian elements (46.95%).

Cluster 2: Association *Medicagini minimae - Festucetum valesiacae* Wagner 1941

Location: Phytocoenoses of *Festuca valesiaca* were identified on the sunny hills between Bunești and Viscri (Brașov County; **Photo**. **2.3**).

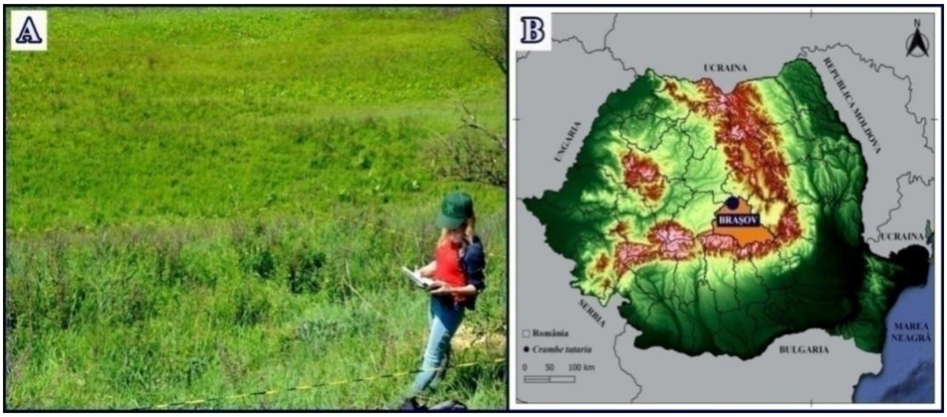

Photo. 2.3 - *Medicagini minimae - Festucetum valesiacae* (A) and association's location (B).

Stationary conditions: The analyzed association is spread at high elevation (640 m), where the mean annual precipitation is 606 mm, and the mean annual temperature is 6.92 °C. Phytocoenoses of *Festuca valesiaca* grow on sunny coasts, with a south-eastern aspect, where the slope has values between 13.6 ° and 21.9 °.

Vegetation structure: The upper layer of vegetation was represented by *Allium scorodoprasum* L. etc., and the middle layer was represented by the species *Cytisus albus* Hacq., *Festuca valesiaca*, etc. In the lower layer, fewer plant species were observed (*Convolvulus arvensis* L., etc.).

Floristic and phytocoenological composition: The general coverage achieved by the species within the phytocoenoses is between 98.00% and 100%. From the phytocoenological point of view, in the floristic composition, besides the edifying species (*Festuca valesiaca*), the species belonging to the classes *Festuco-Brometea*, *Quercetea pubescentis*, and *Rhamno-Prunetea.*

Ecological conditions: The diversity of the floristic composition is also reflected in the spectrum of the main ecological indices in which the meso-xerophilic (62.16%), mesothermal (54.05%), and weakly alkaline (43.24%) species are highlighted. Bioform analysis revealed the presence of hemicryptophyte species (52.63%), and the spectrum of geoelements is dominated by European elements (45.94%).

Cluster 3: Association *Thymo pannonici - Chrysopogonetum grylli* Doniță *et al*. 1992

Location: Phytocoenoses of *Chrysopogon gryllus* have been identified in the grasslands in the northern part of Pâclele (Buzău County; **Photo**. **2.4**).

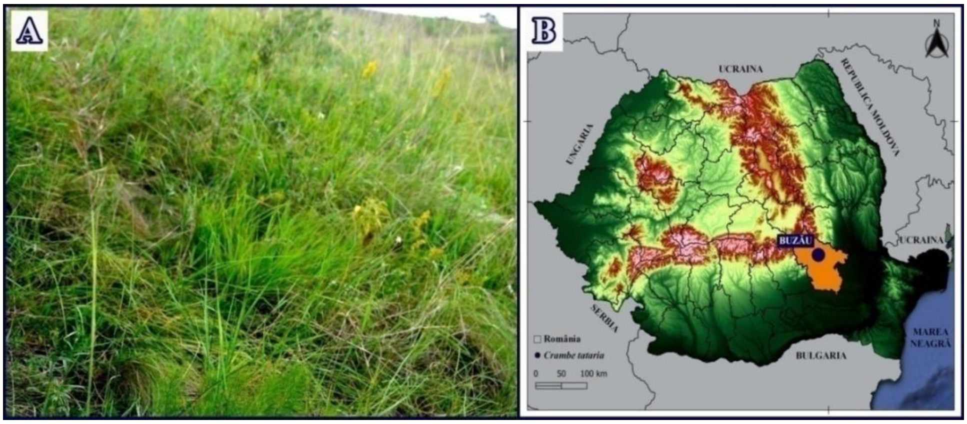

Photo. **2.4** - ***Thymo pannonici* - *Chrysopogonetum grylli*** (**A**) **and association's location** (**B**).

Stationary conditions: The phytocoenoses of the association are found at elevations with values between 205 m and 337 m. The mean annual precipitations showed values between 540 mm and 543 mm, and the mean annual temperature was between 9.69 ° C and 9.72 ° C. Regarding the relief, the analyzed phytocoenoses were identified on sunny slopes with moderate inclination.

Vegetation structure: Species *Chrysopogon gryllus*, *Bothriochloa ischaemum* (L.) Keng, *Brachypodium pinnatum* (L.) P. Beauv. etc. forms the upper layer of vegetation, and the lower layer of vegetation is represented by *Adonis vernalis*, *Carex humilis*, etc.

Floristic and phytocoenological composition: The general vegetation cover was, on average, 95.66%. The quantitatively asserted species are *Chrysopogon gryllus* and *Stipa tirsa*. Also, the species *Achillea nobilis* L., *Adonis vernalis*, etc., are highlighted by the constant character in the relevés. By the presence of bushes in the vicinity of the relevés carried out in Pâclele locality, it is possible to explain the presence of some species characteristic of the classes *Rhamno* - *Prunetea* and *Trifolio* - *Geranietea*.

Ecological conditions: The analysis of ecological indices revealed the predominance of meso-xerophilous (42.3%), thermophilic (35.06%), and weak-alkaline (41.02%) species. The spectrum of bioforms highlights the dominance of hemicryptophytes (70.37%), and the analysis of geoelements revealed the dominance of Eurasian elements (45.67%).

Cluster 4: Association *Festuco rupicolae* - *Brachypodietum pinnati* Mahn 1965

Location: The association was identified only in the grasslands of Valea Glodului (Mureș County; **Photo**. **2.5**).

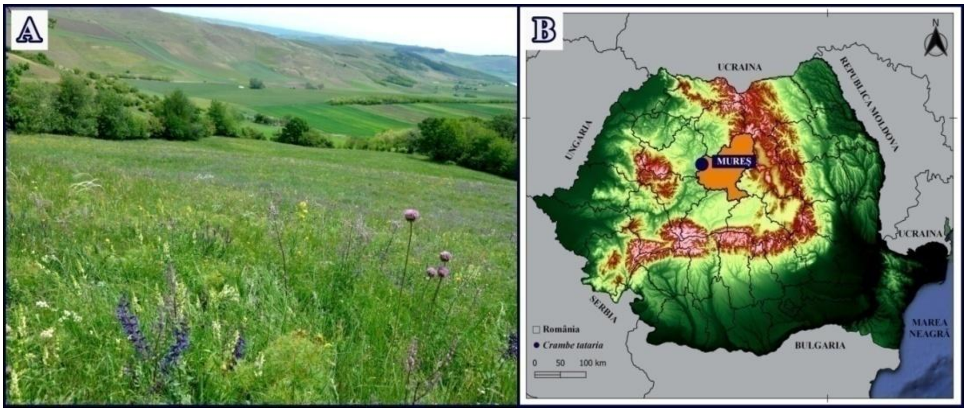

Photo. **2.5** - ***Festuco rupicolae* - *Brachypodietum pinnati* (A) and association's location (B)**.

Stationary conditions: In the researched area, the altitudinal gap was between 472 m and 473 m. The mean annual precipitation was 615 mm, and the mean annual temperature was 8.50 °C. From the point of view of relief, the analyzed association prefers slopes with a north-eastern orientation and a slope inclination between 5.3 ° and 27.8 °.

Vegetation structure: The upper layer of the analyzed phytocoenoses was represented by *Arrhenatherum elatius*, *Brachypodium pinnatum*, etc., and the lower layer was represented by *Ajuga laxmannii*, *Arabis hirsuta* (L.) Scop. etc.

Floristic and phytocoenological composition: Two relevés were included in this association, and 70 species were identified to achieve 100% coverage. The floristic composition is very varied and rich, in which an important percentage has the species characteristic of the alliance *Festucion valesiacae*.

Ecological conditions: The analysis of ecological indices showed the predominance of meso-xerophilous (61.76%), mesothermal (52.94%), and weakly alkaline (42.64%) species. The spectrum of bioforms highlights the predominance of hemicryptophyte species (69.56%), and the spectrum of geoelements revealed the predominance of Eurasian species (54.28%)

Cluster 5: Association *Elytrigietum hispidi* (Dihoru 1970). Popescu et Sanda 1988

Location: Representative phytocoenoses of this association have been identified in some grasslands in Slimnic (Sibiu County; **Photo. 2.6**).

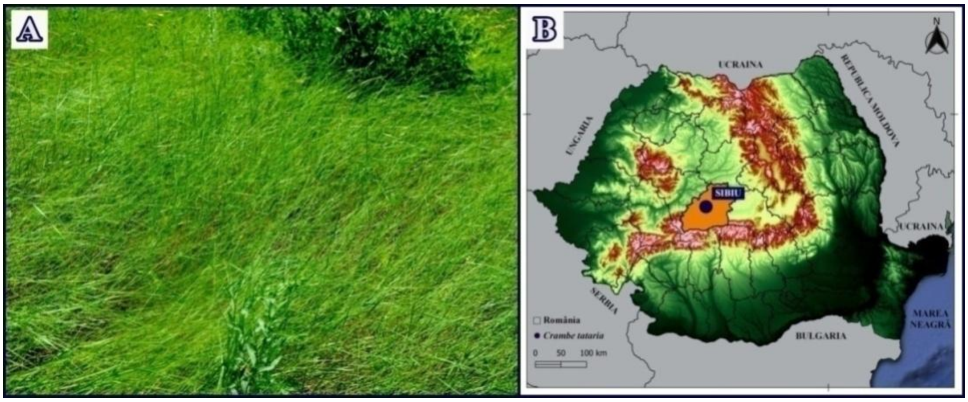

Photo. 2.6 - *Elytrigietum hispidi* (A) and association's location (B).

Stationary conditions: The phytocoenoses of the association were found at an elevation between 481 m and 485 m in areas with moderate mean annual precipitation, which varies between 561 mm and 616 mm, and with moderate mean annual temperatures between 8.53 °C and 9.45 °C.

Vegetation structure: The species in the upper layer showed heights between 30 m and 65 m. The most representative species are: *Achillea collina*, *Centaurea scabiosa* L. etc. The lower layer is well individualized by *Carex humilis*, *Convolvulus arvensis*, *Galium verum* L., etc.

Floristic and phytocoenological composition: The physiognomy of the association is illustrated by *Elytrigia intermedia*, which is distinguished by a high cover, along with which grow many species typical of the alliance *Festucion valesiacae* and the order *Festucetalia valesiacae*.

Ecological conditions: Phytocoenoses of *Elytrigia intermedia* are characterized by the presence, with considerable frequencies of meso-xerophilous (66.99%), mesothermal (43.00%), and weakly alkaline (38.23%) species. The analysis of bioforms revealed the predominance of hemicryptophyte species (60.56%), and the study of geoelements revealed the predominance of Eurasian species (56.33%).

Cluster 6: Association *Arrhenatheretum elatioris* Br.-Bl. ex Scherrer 1925

Location: The phytocoenoses of the analyzed association were identified on Zackel hill, in Slimnic locality (Sibiu County; **Photo**. **2.7**).

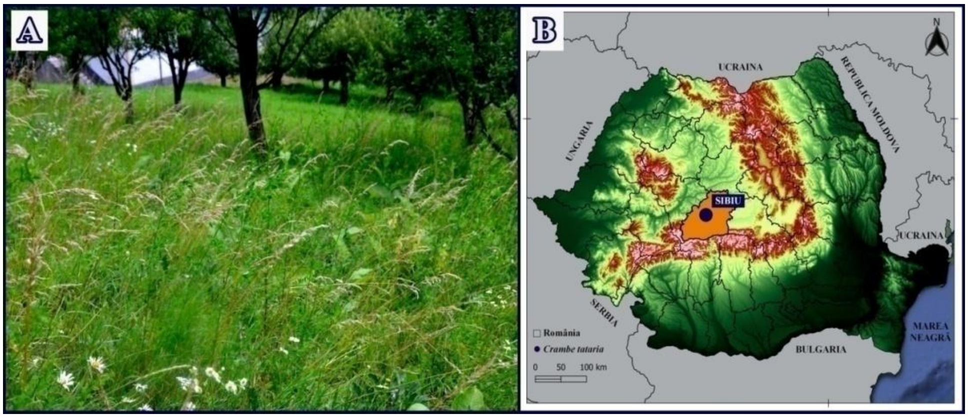

Photo. **2.7** - ***Arrhenatheretum elatioris*** **(A) and association's location (B)**.

Stationary conditions: Within the analyzed vegetal association, the north-eastern aspect predominates, in which the slope is slightly inclined (11.8 °). This association was also identified at an elevation of 485 m.

Vegetation structure: The upper layer of the analyzed vegetation is represented by *Brachypodium pinnatum* etc., and the lower layer is represented by the species *Carex humilis, Convolvulus arvensis*, etc.

Floristic and phytocoenological composition: The floristic composition is rich in species, among which there is a significant nucleus of species characteristic of the alliance *Festucion valesiacae*, the order *Festucetalia valesiacae*, and the class *Festuco-Brometea*.

Ecological conditions: The ecological analysis demonstrated the predominance of meso-xerophilous (65.51%), mesothermal (58.62%), and euriacidophilous (31.03%) species. The spectrum of bioforms is dominated by hemicryptophyte species (58.62%), and the spectrum of geoelements revealed the predominance of Eurasian species (62.06%).

Cluster 7: Association *Jurineo arachnoideae - Stipetum lessingianae* (Dobrescu 1974) Chifu, Mânzu et Zamfirescu 2006.

Location: The analyzed association was identified in the grasslands from Horlești, Miroslava, Popricani, Rediu, Tăutești and Vânători localities (Iași County; **Photo**. **2.8**).

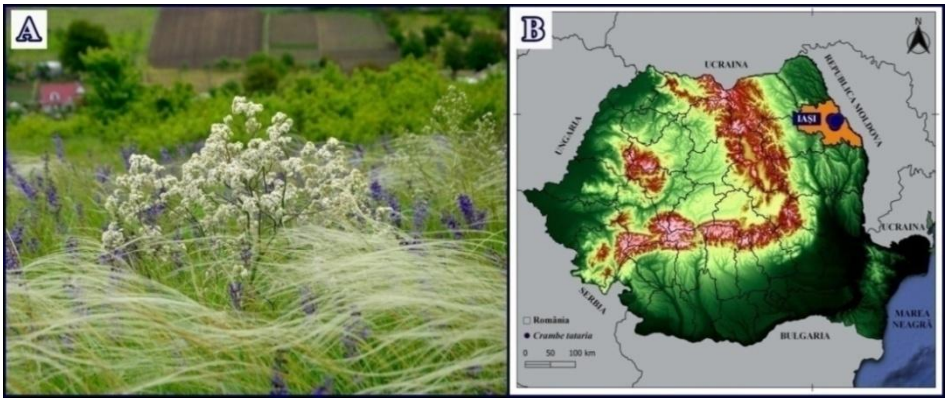

Photo. **2.8 -** ***Jurineo arachnoideae - Stipetum lessingianae*** **(A) and association's location (B).**

Vegetation structure: The upper layer of vegetation was represented by *Phragmites australis* etc., and the lower layer included the species *Fragaria viridis* Weston, *Polygala major* Jacq. etc

Floristic and phytocoenological composition: The floristic composition is rich, with a coverage of 95.44%. Numerous species characteristic of the alliance *Stipion lessingianae* and the order *Festucetalia valesiacae* have been identified, which form the main coenotic background of the analyzed association.

Ecological conditions: The analysis of ecological indices showed the predominance of meso-xerophilous (70.00%), thermophilic (43.92%), and weakly alkaline (43.51%) species. The analysis of bioforms highlights the dominance of hemicryptophytes (60.57%), and regarding geoelements, Eurasian elements predominate (41.34%).

2.6 Analysis of the relationships between the floristic composition and the environmental variables

2.6.1 Detrended Correspondence Analysis (DCA)

In the Detrended correspondence analysis (DCA), the position of the 47 relevés can be observed spatially, ordered according to the similarity of the floristic composition along the axes of the ordinogram (**Fig. 2.17**).

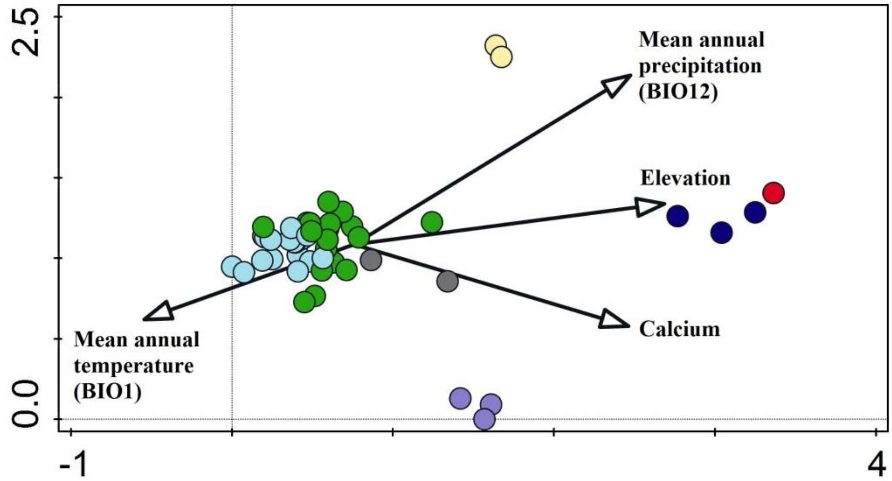

Fig. 2.17 - Ordonation the DCA of the 47 relevés: light blue = *Taraxaco serotinae - Festucetum valesiacae* association, yellow = *Medicagini minimae - Festucetum valesiacae* association, purple = *Thymo pannonici - Chrysopogonetum grylli* association, gray = *Festuco rupicolae - Brachypodietum pinnati* association, light green = *Elytrigietum hispidi* association, red = *Arrhenatheretum elatioris* association, dark blue = *Jurineo arachnoideae - Stipetum lessingianae* association.

Following the Detrended correspondence analysis (DCA), it was found that DCA 1 was considered the most important axis (**Table 2.1**).

Table 2.1

Summary of the Detrended correspondence analysis on the 47 relevés.

	DCA 1	DCA 2	DCA 3	DCA 4
Eigenvalue	0.4658	0.216	0.1576	0.092
Explained variation (cumulative)	17.05	24.95	30.72	34.08
Gradient length	3.36	2.32	2.87	1.97
Pseudo-canonical correlation	0.9685	0.9484	0.6903	0.9103

2.6.2 Canonical Correspondence Analysis (CCA)

Canonical Correspondence Analysis showed that the most important variables that significantly explained the variation of the floristic composition were the mean annual precipitation (BIO12) and the elevation (**Table 2.2**).

Table 2.2

CCA results highlighting the effect of abiotic/biotic/anthropogenic variables on the floristic composition of communities with *C. tataria* investigated.

Variables	Explain %	Contribution %	pseudo-F	p value	Adjusted p value
Mean annual precipitation	10.5	15.2	5.3	0.0001	0.0026
Elevation	6.5	9.4	3.4	0.0002	0.0052
Mean annual temperature	6.9	10.1	3.9	0.0002	0.0052
Calcium	4.5	6.5	2.6	0.0002	0.0052
Type of management	3.3	4.9	2	0.0317	0.8242

2.6.3 The relationship between the floristic composition and the environmental variables

The first DCA axis was positively correlated with the mean annual precipitation (BIO12), elevation and calcium concentration and negatively with the mean annual temperature (BIO1). In this context, a separation can be observed between the xerophilous communities of *Taraxaco serotinae - Festucetum valesiacae*, which are characterized by elevation (169 m) and low mean annual precipitation (558 mm), moderate slopes (11 °), high mean annual temperatures (9.4 °C) and rich in the number of species (39 species per 100 m^2), compared to the mesophilic communities of *Arrhenatheretum elatioris*.

- *Mean annual precipitation*

The mean annual precipitation (BIO12) was the most important variable that explained 10.5% of the variation of the floristic composition. The increase in precipitation quantity determines the change of the floristic composition of the analyzed phytocoenoses, from *Stipa lessingiana* grasslands, etc., that grow on arid lands in *Arrhenatherum elatius* grasslands on higher humidity lands.

- *Elevation*

The second variable was elevation, which explained 6.5% of the variation of the floristic composition. The altitudinal gap varied from 90 m in the xerophilous communities of *Taraxaco serotinae - Festucetum valesiacae,* at an elevation of 485 m, in mesophilic communities of *Arrhenatheretum elatioris*.

- *Mean annual temperature*

The mean annual temperature (BIO1) explained 6.9% of the variation of the floristic composition. The highest temperature values were recorded in the communities of *Thymo pannonici - Chrysopogonetum grylli* (9.70 °C), and the lowest values were recorded in the communities of *Medicagini minimae - Festucetum valesiacae* (6.92 °C).

- *Calcium*

The calcium concentration explained 4.5% of the variation of the floristic composition. In this context, the lowest values were observed in the association *Festuco rupicolae - Brachypodietum pinnati* (1.09%), and the highest values were recorded in the association *Elytrigietum hispidi* (3.76%)

2.6.4 Habitat discussions

C. tataria grows in a limited variety of plant communities. Thus, in the investigated regions (Moldova, Muntenia, and Transylvania), the habitat types in which the species *C. tataria* was observed are similar to those described in the literature (BĂDĂRĂU, 2001; DONIȚĂ *et al*., 2005; GAFTA and MOUNTFORD, 2008; KELL, 2011).

In this study, it was shown that the species *C. tataria* grows in two types of habitats: xerophilous grasslands and mesophilic grasslands. Of these habitats, the study's results indicated that xerophilous grasslands are the preferred habitat of the analyzed species.

2.7 Investigations on the micromorphology of leaf areas

2.7.1 The lower epidermis of the leaf

The presence of rare unicellular multicellular or unicellular tectors hairs is observed. The tectors hairs and epicuticular wax coexist; tectors hairs are very rare.

2.7.2 The upper epidermis of the leaf

The presence of rare, long, single-celled tectors hairs was observed. The presence of secretory hairs with bicellular glands exposed by cuticle damage was identified.

2.8 Diversity and genetic structure of the species C. tataria

2.8.1 DNA extraction

The concentrations of the DNA extracts showed values between 14 µg / mL and 144.9 µg / mL, and the purity of the DNA samples, measured as A260 / A280, was between 1.52 and 2.07.

2.8.2 DNA amplification

Following the testing of the 43 primers, the amplification and reproducibility of the polymorphic fragments of *C. tataria* were performed by only five ISSR primers (UBC no. 814, 823, 828, 854, and 891). These primers generated 67 polymorphic bands, 100%.

2.8.3 Parameters of genetic diversity

The average share of polymorphic bands was 45.67%. The average value for the expected heterozygosity (H_e) was 0.169, which reveals a homozygous tendency, the highest probability for heterozygous loci being highlighted for Vulturi with a value of H_e = 0.283. The Shannon index (H) was calculated to be 0.251, indicating a moderate degree of diversity, and the genetic distance of Nei (d) amounted to 0.196, suggesting a trend towards uniformity and a low degree of genetic differentiation.

2.8.4 Dendrogram analysis

Based on the genetic distances, a UPGMA dendrogram was generated, which highlights distinct groups of samples from different collection locations:

Cluster 1 included samples from the North-East region of Romania: Miroslava (P1, P2, P3, and P4), Rediu (P5, P6, P7, and P8), and Vulturi (P9, P10, P11, and P12). In this group, there are two large subgroups, which highlight an exchange of genetic material between the populations of Rediu and Vulturi and an earlier differentiation of the samples from Miroslava (**Fig. 2.18**).

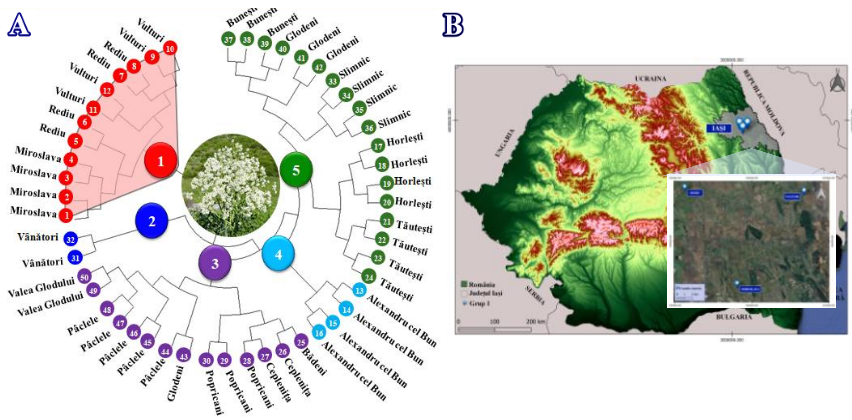

Fig. 2.18 - Cluster 1: dendrogram (A) and harvesting locations (B).

Cluster 2 included the samples from Vânători (P31 and P32), which indicates a high degree of differentiation from the rest of the samples in Iași County. This group was characterized by the lowest values of genetic diversity indices (**Fig. 2.19**).

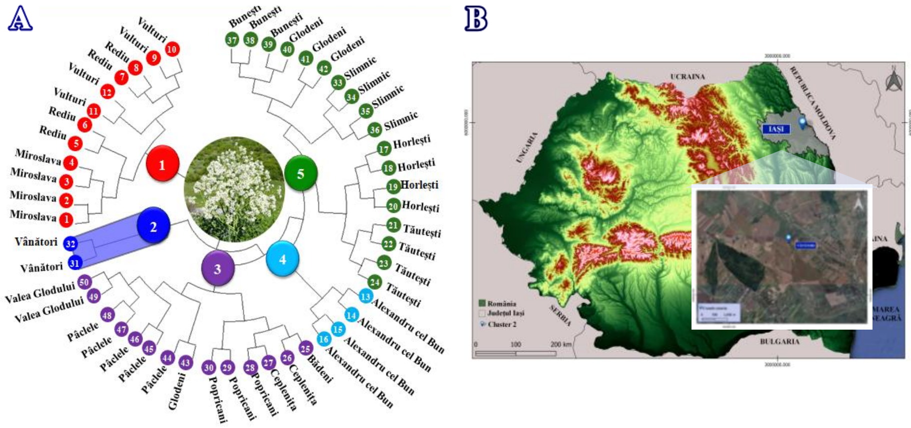

Fig. 2.19 - Cluster 2: dendrogram (A) and location of harvest (B).

Cluster 3 (**Fig. 2.20**) includes associated samples in its subgroup from Bădeni (P25), Ceplenița (P26 and P27), and Popricani (P28, P29, and P30) from Iași County; the second subgroup includes samples from Pâclele locality (P44, P45, P46, P47, and P48) from Buzău County, samples from Valea Glodului locality (P49 and P50) from Mureș County and a sample from Glodeni locality (P43), from Vaslui County.

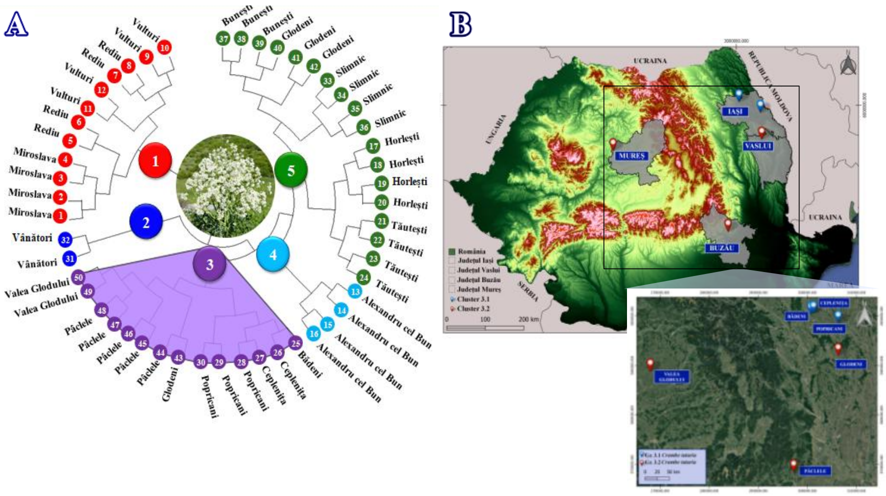

Fig. 2.20 - Cluster 3: dendrogram (A) and harvesting locations (B).

Cluster 4 includes samples only from the North-East region of Romania - Moldova: Alexandru cel Bun locality from Iași County (P15, P16, P17, and P18), grouped individually but with similarity to the samples from cluster 5 (**Fig. 2.21**).

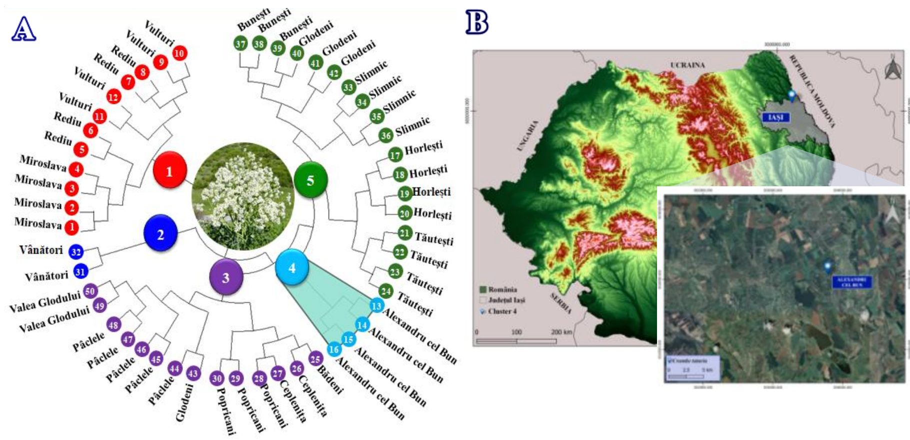

Fig. 2.21 - Cluster 4: dendrogram (A) and location of harvest (B).

Cluster 5 was separated into two groups: in group 5.1 are included the samples from the region of Moldova - the localities Horlești (P17, P18, P19, and P20) and Tăutești (P21, P22, P23, and P24). Group 5.2 includes the samples from the Transylvania region - Slimnic locality (P33, P34, P35, and P36), from Sibiu county, Bunești locality (P37, P38, and P39), from Brașov County and from Moldova region - Glodeni locality (P40, P41, P42, and P43), from Vaslui County (**Fig. 2.22**).

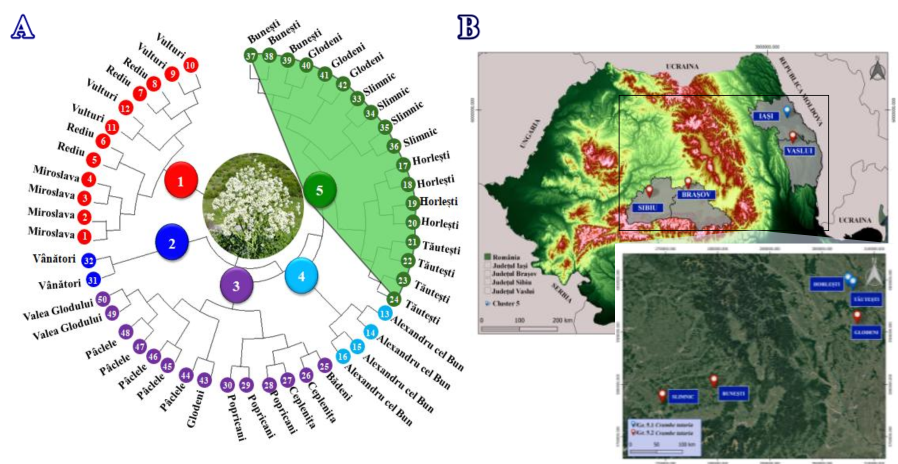

Fig. 2.22 - Cluster 5: dendrogram (A) and harvesting locations (B).

2.8.5 Discussions on diversity and genetic structure

2.8.5.1 Genetic diversity

This study evaluated the inter and intra-population variation of *C. tataria* species in Romania using ISSR markers (repetitive inter-simple sequences). Thus, the ISSR-PCR technique used to know the diversity, structure, and genetic differentiation of *C. tataria* populations, has proven reliable.

Compared to large populations, small populations have less genetic diversity due to genetic drift and inbreeding (WU *et al*., 2015). This study showed that, although *C. tataria* is considered an endangered species, the level of genetic diversity was moderate (PBP = 45.67 %; He = 0.169; I = 0.251). Moderate genetic polymorphism is due to primers that generated 100 %, polymorphic bands. Compared to other species, the percentage of polymorphic bands in the case of samples of *C. tataria* was lower than in *Brassica napus* L. - 87 % (ABDELMIGID, 2012) or *Draba dorneri* Heuff. - 78.94 % (CATANĂ *et al*., 2013).

2.8.5.2 Colonization events

The distribution of plant species in Europe has been limited to glacial refuges, as well as to the subsequent recolonization of high latitudes in warmer periods, due to repeated cycles of glaciations that have occurred since the late Pleistocene (THOMPSON, 1999). Currently, the origin of many species of the genus *Crambe* is of the post-Pliocene type (FRANCISCO-ORTEGA *et al*., 1999).

2.8.5.3 Estimation of conservation status

The evaluation of *C. tataria* populations in Romania was performed according to ecological characteristics, such as habitat types, soil preferences, and population characteristics: size, density, and population structure.

Population size is the most widely used indicator for estimating conservation status.

2.9 Elaboration of conservation strategies

2.9.1 *In situ* conservation measures

Populations of *C. tataria* showed moderate genetic diversity and partially favorable environmental conditions. Thus, based on the ecological and genetic data of the populations investigated by *C. tataria*, it can be admitted that the main causes of endangerment of the analyzed species are habitat degradation and fragmentation. Therefore, populations of *C. tataria* could be stabilized through some in situ conservation measures, such as rotational grazing, control or elimination of shrub species, the establishment of a buffer zone, manual mowing, control of invasive species, informing the authorities, informing and promoting the protection of habitats, and species, etc.

2.9.2 *Ex situ* conservation measures

Some *ex situ* conservation measures (storage protocol, seed banks, botanical gardens) will be established to facilitate quasi-*in situ* measures (field collections). *Ex situ* conservation is also important to support the reestablishment of wild populations.

2.9.3 Legislative measures

In the case of legislative measures, we mention:

- extension of some protected areas;
- designation of an area as a protected natural area.

PARTIAL CONCLUSIONS

The field research to update the distribution of *C. tataria* species led to verifying 81 populations, of which only 21 were confirmed. They are distributed near the localities of Alba, Brașov, Buzău, Cluj, Iași, Mureș, Sibiu and Vaslui counties.

The main limiting factors that determine the degradation of the species' habitat, as well as the decrease of the size and number of *C. tataria* populations in grasslands at a national level, are the destruction of the breeding place, intensification of agriculture, overgrazing, and fires.

Scanning electron microscopy analysis showed that the leaf blade is amphistomatic, and the stomata are of the anisocytic type, being present in both epidermis. Internervural has observed the presence of rare unicellular, long tectors hairs and the presence of epicuticular wax.

The recorded values of the chemical elements showed that the species *C. tataria* prefers soils with a low alkaline pH, with a rich content of primary (nitrogen, phosphorus, and potassium) and secondary (calcium) macronutrients, as well as a low organic carbon total. At the same time, the evaluation of the content of heavy metals in the soil (arsenic and lead) varies between the analyzed populations, depending on the positioning of the studied areas. In this case, exceedances of the normal values for both arsenic and lead were recorded. Similarly, the values of the proportion of silicon in the soil are also very high. Low values were recorded regarding aluminium, iron, magnesium, oxygen, and sodium content.

Currently, the size of *C. tataria* populations is decreasing compared to data from the last two decades presented in the literature. Instead, comparing the data from 2021 with those from 2019 shows that the size of the analyzed populations and subpopulations registered an increase in the number of individuals.

The percentage of individuals in a vegetative state exceeded the percentage of flowering individuals. The population's conservation status can be considered unfavorable in most of the identified populations. The anthropogenic influences (grazing with sheep) are high due to the presence of sheepfolds and animal husbandry in these areas.

The analyzed characteristics of the investigated populations indicate a good situation for the populations from Moldova and some from Transylvania, based on the type of management. However, three populations have been identified that are currently declining. The habitats in which these populations grow are rich in nitrogen and potassium.

From a phytocoenological point of view, seven plant associations have been identified in which the species *C. tataria* grows: *Arrhenatheretum elatioris*, *Elytrigietum hispidi*, *Festuco rupicolae* - *Brachypodietum pinnati*, *Jurineo arachnoideae* - *Stipetum lessingianae*, *Medicagini minimae* - *Festucetum valesiaca* and *Thymo pannonici* - *Chrysopogonetum grylli*..

C. tataria grows in a limited variety of habitats, consisting of plant species characteristic of the vegetation classes *Festuco-Brometea* and *Molinio-Arrhenatheretea* and the alliance *Festucion valesiacae* or transitions between the communities between the alliances *Cirsio-Brachypodion pinnati* and *Festucion valesiacae*.

The detrended correspondence analysis (DCA) showed that the floristic composition changes along with precipitation, elevation, and calcium gradients. The canonical correspondence analysis (CCA) indicated that the variation in floristic composition is controlled by climate, especially mean annual precipitation (BIO12).

The evaluation of the genetic variability at intra and inter-population levels of some populations belonging to the *C. tataria* showed that the analyzed populations maintain a relatively moderate genetic diversity.

GENERAL CONCLUSIONS

This thesis aimed to identify the eco-coenotic conditions and genetic structure of the species *C. tataria*, as well as to establish measures to protect it. Thus, through the theoretical and practical debate on the achieved objectives, the following conclusions were highlighted:

Updating the data on the distribution of *C. tataria* species in Romania led to identifying 21 populations spread in some localities in Moldova, Muntenia, and Transylvania. The results indicated that the distribution of *C. tataria* populations is uneven, and their distribution by localities is limited by the current state of phytocoenoses, climatic characteristics, and the impact of anthropogenic factors.

Most populations of *C. tataria* grow in relatively fragmented habitats. In this context, anthropogenic factors that negatively affect both the habitat and population size of *C. tataria* are overgrazing and conversion of grasslands to agricultural land.

Chemical analysis of soil samples showed low concentrations of total organic carbon and rich in nutrients and heavy metals. Thus, the values obtained for the 14 chemical analyzes show slightly significant differences in the case of samples taken from the 15 localities. Regarding soil pH, *C. tataria* species prefers alkaline soils.

In the individuals of the populations from Glodeni, Bădeni, Tăutești, and Slimnic, the highest values were registered, and the specimens from the populations from Vulturi and Popricani presented the lowest values.

The proportion of flowering individuals was lower than that of vegetative individuals. Thus, most flowering individuals were observed only in the populations of Miroslava and Horlești. In terms of population size, it showed a slight increase over the three years of study. Most individuals were registered in the populations of Miroslava (Iași County) and Glodeni (Vaslui County).

Demographic parameters (population size and proportion of flowering individuals) are mainly influenced by the structural characteristics of plant communities (height and vegetation cover). The results showed that soil chemical variables are the best predictors of morphological parameters.

The eco-coenotic analysis showed that the *C. tataria* vegetates in a limited variety of plant communities, characteristic of xerophilous grasslands (class *Festuco* - *Brometea*), together with many transgressive species present in mesophilic grasslands (class *Molinio* - *Arrhenatheretea*). Of these two types of grasslands, the optimal habitat of the species is xerophilous grasslands.

The results obtained showed in the case of the lower and upper epidermis of the leaf areas of *C. tataria*, the presence of unicellular, long, and multicellular hairs, with massive multicellular base; syntopism is present or absent; stomata are present, anisocytic type; tectors hairs and epicuticular wax coexist; and the leaf blade is amphistomatic. In the case of the upper epidermis, the tectors hairs are absent or are extremely rare and present internervurally. The samples from Rediu, Tăutești, Vânători and Vulturi (Iași County) lack tectors hairs, and the epicuticular wax is poorly represented.

Genetic analysis has shown that populations of *C. tataria* maintain moderate levels of genetic variability at the intra-population level and low levels of genetic differentiation at the interpopulation level. At the same time, we consider great adaptability within the investigated populations. In this case, we admit that the main measure in the case of this species is *in situ* conservation.

OWN CONTRIBUTIONS

In this doctoral thesis, the importance of the results obtained is supported by the following main contributions:

- critical analysis of the literature on the species *C. tataria*;
- characterization of the soil type;
- analysis of the distribution of the species in Romania;
- identification of the causes that determined the restriction of the populations of the analyzed species;
- chemical analysis of soil samples;
- analysis of population and habitat characteristics;
- biometric analysis of morphological parameters;
- identification and description of plant associations;
- analysis of the relationship between the floristic composition, the characteristics of the populations, and the variables studied;
- analysis of genetic variability at inter and intrapopulation levels of *C. tataria* populations;
- investigations on the micromorphology of foliar areas from *C. tataria* (SEM);
- elaboration of measures regarding the protection of the analyzed species.

RESEARCH PERSPECTIVES

The decline of *C. tataria* species, as well as the development of strategies for the protection of the species using ecological and genetic methods, still require a concentrated deepening of the prospects for further research in several research directions:

- deepening some aspects of phylogeny and phylogeography.
- conducting studies on the spatial modeling of *C. tataria* populations in Romania;
- expanding research on the influence of the number of ruminants and the areas occupied by pastures on the species *C. tataria*.

SCIENTIFIC ACTIVITY

As the first author, the results obtained in the doctoral thesis were capitalized through the presentation of symposia and the publication of scientific articles in specialized journals.

- **Articles published in ISI-rated journals**

Simona Dumitrița CHIRILĂ (2022) - Analysis of the characteristics of some populations of *Crambe tataria* Sebeók from Romania. *Acta Oecologica*, **114** (5): 103810. doi: 10.1016/j.actao.2021.103810. **IF**: 1,674.

- **Articles published in BDI-rated journals**

Simona Dumitrița CHIRILĂ (2021) - Ecological and chorological studies of the species *Crambe tataria* Sebeók from Romania. *Romanian Journal of Biology - Plant Biology*, **66** (1-2): 39 - 54.

- **Presentations at national and international symposia**

Simona Dumitrița CHIRILĂ (2021) - Characteristics of plant communities of *Crambe tataria* Sebeók in Romania. International symposium: "CURRENT TRENDS IN NATURAL SCIENCES", University of Pitesti, May 28 - 31, 2021. **Poster**. doi: 10.13140/RG.2.2.32674.56003/1

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