

Wide habitat preference found in a rare, regional endemic species: *Iris brandzae* Prodán (Iridaceae Juss., subgenus Limniris, series Spuriae) in Romania

Simona Dumitrița Chirilă¹ 💿, Kiril Vassilev² 💿 & Alexandru Sabin Bădărău³ 回

Key words: Iris brandzae, subgenus Liminiris, series Spuriae, phytosociology of endangered species, ecology of rare plants, floristic composition, phytocoenological relevés.

Ključne besede: Iris brandzae, podrod Liminiris, serija Spuriae, fitosociologija ogroženih vrst, ekologija redkih rastlin, floristična sestava, fitocenološki popisi.

Corresponding author: Simona Dumitrița Chirilă E-mail: simonachirilasc@yahoo.com

Received: 24. 10. 2023 Accepted: 8. 2. 2024



Abstract

Iris brandzae is one of the most threatened plant species in Romania. The current distribution of this species is very fragmented, and the population size has been reduced in the last 30 years. Information on the habitat preferences of the species has not been summarized yet. In this context, this study aimed to identify the habitat preferences of the species I. brandzae in Romania. The study was carried out in the NE (Moldova) and SE (Muntenia) regions of Romania. For the vegetation analysis, a total of 46 relevés were used. To classify the vegetation, we applied the hierarchical agglomerative clustering method, using the ß-flexible algorithm with $\beta = -0.25$ and the Bray-Curtis dissimilarity. The data were represented as mean percentage values, according to the the Braun-Blanquet scale . Relationships between floristic composition and environmental variables were analyzed with Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA). The vegetation analysis showed that I. brandzae grows in xerophilous, mesophilous and halophilous grasslands and ash-alpine alluvial forests. The species occurs in the communities of the Stipion lessingianae alliance, of the Festucetalia valesiacae order, mainly. The results of the CCA analysis indicate that the variation of the floristic composition of *I. brandzae* is influenced by the annual mean temperature (BIO1).

Izvleček

Iris brandzae je ena najbolj ogroženih rastlinskih vrst v Romuniji. Trenutna razširjenost te vrste je zelo razdrobljena, velikost populacije pa se je v zadnjih 30 letih zmanjšala. Habitatne preference vrste še niso bili raziskane, zato v tej raziskavi ugotavljamo, v katerih habitatih se vrsta I. brandzae pojavlja. Raziskavo smo naredili v SV (Moldavija) in JV (Muntenia) regiji Romunije. Za analizo vegetacije smo uporablili skupaj 46 vegetacijskih popisov. Za klasifikacijo vegetacije smo uporabili metodo hierarhične klasifikacije z uporabo β -fleksibilnega algoritma (β = -0,25) in Bray-Curtisovim indeksom različnosti. Za mero abundance smo uporabili povprečne odstotne vrednosti v skladu s Braun-Blanquetovo skalo. Povezavo med floristično sestavo in okoljskimi spremenljivkami smo analizirali s korespondenčno analizo z odstranjenim trendom (DCA) in kanonično korespondenčno analizo (CCA). Analiza je pokazala, da *I. brandzae* uspeva na kserofilnih, mezofilnih in halofilnih traviščih ter jesenovih poplavnih gozdovih. Vrsta se pojavlja predvsem v združbah zveze Stipion lessingianae, reda Festucetalia valesiacae. Rezultati analize CCA kažejo, da na variabilnost floristične sestave I. brandzae vpliva povprečna letna temperatura (BIO1).

1 Danube Delta National Institute for Research and Development, Tulcea, Romania

² Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

³ Faculty of Environmental Sciences and Engineering, Babeş-Bolyai University, Cluj-Napoca, Romania

Hacquetia

Introduction

During the last three centuries, the habitats have undergone considerable changes as a result of anthropogenic activities and climate change, leading to a significant reduction of the geographical ranges of many plant species (O'Grady et al., 2004; Szczecińska et al., 2016). Thus, in the past, species such as Crambe tataria Sebeók (Chirilă et al., 2022), Pontechium maculatum (L.) Böhle & Hilger and Iris aphylla subsp. hungarica Hegi had a more extensive distribution in Romania at the beginning of the 20th century (Oprea, 2005; Dihoru & Negrean, 2009). However, these species are currently rare and endangered in the country (Mânzu et al., 2020; Chirilă et al., 2022). The steppe grasslands, where the previously mentioned species grow, are in danger due to the intensive growth of agriculture and the abandonment of traditional forms of land use (Hensen et al., 2005). Increased human activities have led to the expansion of pastures, which generally represent habitats that do not favor the development of these species (Bi et al., 2020). As a result, their populations in such habitats are numerically reduced. In this context, it is imperative to investigate the environmental and genetic factors influencing the performance of these species. Identifying whether habitat requirements are better indicators of rare and threatened species performance than population size and genetic diversity is critical. Conservation programs should be based on peripheral populations of the distribution range because peripheral populations have lower viability than central populations (Channell, 2004).

Iris brandzae Prodán (Iridaceae Juss.) is one of the rare and endangered species in Romania. The area of the species is restricted in Romania, the Republic of Moldova (Mânzu et al., 2020) and Ukraine (Derevenko, 2010; Volutsa, 2011). Although most populations were recorded in Romania (Oprea, 2005; Dihoru & Negrean, 2009), their number and size were significantly reduced in recent years (personal observations). In addition, the current geographical range of this species is very fragmented, being reported in different countries and regions, including Romania (Tutin et al., 1980; Dihoru & Negrean 2009), the Republic of Moldova (Tutin et al., 1980), Ukraine (Fedorov & TSvelev 2001), Asia Minor and the Carpathians (Fedorov & TSveley, 2001). In Romania, the species was reported in Moldova and Muntenia regions (Oprea, 2005). At Global and European level, the species has not yet been evaluated (EEA, 2023). In Romania, the status of the species varies, being considered vulnerable or rare (Oltean et al., 1994), vulnerable (Oprea, 2005), or with a low risk of extinction (Dihoru & Negrean, 2009). The range of the species is restricted due to factors such as

overgrazing, land use change, irrigation, etc. (Dihoru & Negrean, 2009).

Iris brandzae is a subendemic species (Oltean et al., 1994) that was first described by Iuliu Prodan in 1935 (British Iris Society, 1997). It was formed on the edge of the former Getic Gulf of the Sarmatian Sea. It later migrated to the plain after the retreat of the sea (Dihoru & Negrean, 2009). The species grows in hayfields, pastures, and grasslands (Grințescu et al., 1966), more or less saturated places, grasslands or sparse forests (Fedorov & TSvelev 2001; Dihoru & Negrean, 2009), salt marshes, wet grasslands (Roger & Martyn, 1991; British Iris Society, 1997; Tutin et al., 1980). It is a mesophytic-mesohygrophilous species that prefers well-drained, moist, humus-rich soils with a neutral pH, that grows on semi-shaded slopes (Dihoru & Negrean, 2009).

Information available on the habitat preferences of the species *I. brandzae* in Romania is incomplete. In this context, 46 phytocoenological relevés were analyzed: (1) to determine the floristic composition and phytogeographic characteristics of the identified groups; and (2) to determine the environmental variables that influence the variation of the floristic composition of the phytocoenoses of *I. brandzae*.

Materials and methods Study species

Iris brandzae (Figure 1) is a perennial species with a height of 15 cm to 25 cm (Dihoru & Negrean, 2009). The stem is compressed, scabrous (Dihoru & Negrean, 2009), thin and striated, and the leaves of the fascicles are thin and narrow, in number of 3 (Grințescu et al., 1966). The fruit is short and cylindrical (Grințescu et al., 1966; Dihoru & Negrean, 2009). Flowering occurs from April to May (British Iris Society, 1997). The flowers are blue-violet or deep blue (Cassidy & Linnegar, 1987).

It belongs to subgenus *Limniris* (Tausch) Spach, the nominate section *Limniris*, series *Spuriae* (Diels) Lawrence. This classification is sustained only by its very close resemblance to the other species in the series, especially *Iris graminea* L. and *I. sintenisii* Janka but the species was not included in any of the recent phylogenetic assessments of the subgenus or section (Wilson, 2004, 2006, 2009, 2011; Rodionenko, 2009; Wheeler & Wilson, 2014; Dorofeeva & Zhurbenko, 2020; Aukhadieva et al., 2021). The series *Spuriae* consists of 12–17 species (Rodionenko, 2007) which occur in various ecosystems from the western and central Palearctic forest-steppe, with the exception of *Iris graminea* and *I. spuria* L. which have their ranges extended across the forestry area of western and central





Figure 1: Iris brandzae Prodán: the habitat of the species (A), the flowering stage (B, C); Photos: S. D. Chirilä, May 2023). Slika 1: Iris brandzae Prodán: habitat vrste (A), faza cvetenja (B, C); Fotografije: S. D. Chirilă, maj 2023).

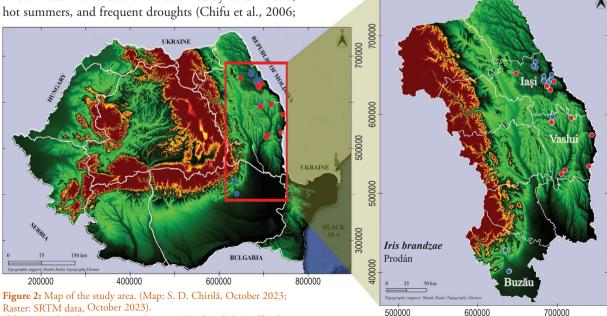
Europe (Meusel et al., 1965). Their ecology is very variate, from open xeric grasslands (*I. pontica*) up to closed canopy forests (*I. graminea*) and saline marshes (*I. spuria* L., *I. halophila* Pall.) but most of them occur in typical forest-steppe environments (open woodlands and meadow steppe grasslands; Meusel et al., 1965). However all individual species seems to have quite narrow, specific ecological requirements (Rodionenko, 2007). This contrasts with our findings in concerning the ecology of *Iris brandzae* Prodan, which seems to cover a large variety of habitats.

Study area

The study was carried out in localities in the northeastern regions of Romania – Moldova and Muntenia (Figure 2). The climate in this area is characterized by cold winters, hot summers, and frequent droughts (Chifu et al., 2006; Văduva, 2008). The elevation of the analyzed areas varies from 20 m to 268 m. The annual precipitation was from 384 to 569 mm, and the annual mean temperatures were from 9.1 $^{\circ}$ C to 10.8 $^{\circ}$ C.

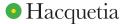
Vegetation sampling and classification

For the vegetation analysis, a total of 46 relevés (including 469 taxa) were used: 16 of these relevés were taken from the Romanian Grassland Database (RGD; Vassilev et al., 2018) made in the period 1964–2006, while the other 30 relevés were made between April and June 2014–2021 (personal data). The size of each personal record was 100 m², while the records retrieved from the Romanian



Slika 2: Zemljevid preučevanega območja. (Zemljevid: S. D. Chirilă, oktober 2023; Raster: podatki SRTM, oktober 2023).

Relevés–Personal data
Relevés–Romanian Grassland Database



Grassland Database ranged in size from 20 m² to 100 m². To facilitate further data analysis, we imported the relevés into JUICE 7.1 software (Tichý, 2002). Afterwards, we standardized the data set. This process involved: (i) the unification of taxonomy and nomenclature, and (ii) removal of taxa identified only to genus level. The final dataset included 46 relevés and 445 taxa.

To classify the vegetation, we applied the hierarchical agglomerative clustering method, using the ß-flexible algorithm with $\beta = -0.25$ and the Bray-Curtis dissimilarity, on the square-root transformed data. Quantitative data were represented as mean percentage values, according to the scale developed by the Braun-Blanquet (Braun-Blanquet, 1964) adapted by Borza & Boscaiu (1965). Also, the dendrogram was made using the dis-similarity matrix and the flexible beta algorithm in the GINKGO program, included in the VegAna package (Bouxin, 2005). The optimal number of clusters was determined by the corrected Rand index (Rand, 1971), and the mean Silhouette index (Rousseeuw, 1987). After identifying the optimal number of clusters, we exported a synoptic table of plant communities using the program JUICE version 7.1. The diagnostic species of each cluster were identified using the Phi coefficient in the JUICE program. Species with a phi coefficient value ≥ 0.5 were considered diagnostic. Later, they were validated by a permutation test (de Cáceres & Legendre, 2009) using the GINKGO software. The nomenclature and taxonomy of plant species follow the EURO+MED (2023), while the nomenclature of plant associations follow the Coldea et al. (2012, 2015). The cenotaxonomic affiliation of the plant associations follows Coldea et al. (2012, 2015). The nomenclature of the higher syntaxons units follows the Mucina et al. (2016). Habitat-level identification was performed using the EU-NIS-ESy Expert System (Chytrý et al., 2020).

Environmental variables

In the multivariate analysis, abiotic variables (elevation, aspect, slope, annual mean temperature, and annual precipitation) and edaphic variables (such as pH, P, and K content) were included. The elevation, aspect, and slope data were collected in the field, while information on annual mean temperature and annual precipitation was extracted from the WorldClim database (Fick & Hijmans, 2017). For personal data, soil samples were collected from each relevé. Afterwards, the chemical composition of the following elements was determined: phosphorus (P) and mobile potassium (K) according to ISO 11263 (1994); and soil pH according to SR EN ISO 10390 (2022). For the data obtained from the Romanian Grassland Database (RGD), the values for the chemical elements P, K, and soil pH (Ballabio et al., 2019) were extracted from the European Soil Database and Soil Properties (ESDAC).

Vegetation-environment relationship

To facilitate the interpretation of grouping and the exploration of floristic gradients, direct and indirect ordination analysis was performed. In this context, relationships between floristic composition and environmental variables were analyzed with Detrended Correspondence Analysis using CANOCO version 5.1 (ter Braak & Šmilauer, 2018). Detrended Correspondence Analysis (DCA) resulted in a gradient length greater than 3, allowing an unimodal method to be applied. Thus, Canonical Correspondence Analysis (CCA) was used to explore which explanatory variables explained a relatively large and significant proportion of the variation in species data. DCA analysis was performed to detect floristic gradients, and CCA (Canonical Correspondence Analysis) analysis was applied to quantify the effect of each environmental variable on floristic composition, using the Monte Carlo permutation test (999 permutations). We used the variance inflation factor (VIF) in CANOCO to assess collinearity between independent variables. The variables with the VIF value < 5 were taken into account, they are not multicollinear (Table 1). The relevés distribution map was made in QGIS version 3.28.3 (QGIS Development Team, 2022).

Table 1: VIF analysis between environmental variables.**Tabela 1:** VIF analiza okoljskih spremenljivk.

Variables	VIF
Elevation (m)	1.343
Bio1 – Annual temperature (°C)	2.253
Bio12 – Annual precipitation (mm)	1.257
рН	1.710
P – phosphorus (mg Kg ⁻¹)	1.318
K – potassium (mg Kg ⁻¹)	1.243

Results Syntaxonomic scheme of the obtained clusters

Iris brandzae was identified in four main habitat types EUNIS (Chytrý et al., 2020): R1 Dry grasslands, R3 Seasonally wet and wet grasslands, R6 Inland salt steppes, and T1 Broadleaved deciduous forests. In this context, 46 relevés were classified into five vegetation classes, six orders, seven alliances, and nine plant associations.

Hacquetia

Class: Festuco-Brometea Br.-Bl. et Tx. ex Soó 1947 Order: Festucetalia valesiacae Soó 1947

All.: Stipion lessingianae Soó 1947

Ass. *Taraxaco serotinae-Festucetum valesiacae* (Burduja et al. 1956, Răvăruț et al. 1956) Sârbu et al. 1999

Ass. Jurineo arachnoideae-Stipetum lessingianae (Dobrescu 1974) Chifu et al. 2006

All.: Festucion valesiacae Klika 1931 Ass. Medicagini minimae-Festucetum valesiacae Wagner 1941

Class: Festuco-Puccinellietea Soó ex Vicherek 1973

Order: Puccinellietalia Soó 1947

All.: Puccinellion limosae Soó 1933

Ass. Limonio gmelini-Artemisietum santonici (Soó 1927) Topa 1939

Ass. *Astero pannonici-Puccinellietum distantis* Gehu et al. 1994

Class: Molinio-Arrhenatheretea Tx. 1937

Order: Molinietalia caeruleae Koch 1926

All.: Deschampsion cespitosae Horvatić 1930

Ass. Poo trivialis-Alopecuretum pratensis Regel 1925

Order: *Potentillo-Polygonetalia avicularis* Tx. 1947 All.: *Potentillion anserinae* Tx. 1947

Ass. Rorippo austriacae-Agropyretum repentis (Timár 1947) R. Tx. 1950

Class: *Carpino-Fagetea sylvaticae* Jakucs ex Passarge 1968 Order: *Fagetalia sylvaticae* Pawłowski 1928

All.: Alnion incanae Pawłowski et al. 1928

Ass. Ulmeto campestris-Fraxinetum holotrichae Borza ex Sanda 1970

Class: *Salicornietea fruticosae* Br.-Bl. et Tx. ex A. Bolòs y Vayreda et O. de Bolòs in A. Bolòs y Vayreda 1950

Order: Halimionetalia verruciferae Golub et al. 2001 All.: Artemisio santonicae-Puccinellion fominii She-

lyag-Sosonko et al. 1989

Ass. *Nitrario schoberi-Artemisietum santonici* Mititelu 1982

Floristic composition and phytogeographic characteristics of the identified groups

The cluster analysis results are illustrated by a dendrogram and a synoptic table (Supplement E1). Based on the hierarchical classification of the analyzed relevés, the vegetation was classified into seven distinct clusters within the data set (Figure 3). Cluster sizes varied, with cluster 6 representing the lowest number of relevés (one relevé), while cluster 1 had the highest number (30 relevés).

Table 2: Values obtained for diversity indices, chemical and bio-climatic parameters. Values are means ± standard deviations (SD).Tabela 2: Vrednosti diverzitenih indeksov, kemijskih in biokli-matskih dejavnikov. Vrednosti so srednje vrednosti ± standardniodkloni (SD).

Cluster	рН	Elevation (m)	Slope (°)	$\Pr_{(mg~Kg^{-l})}$	K (mg Kg' ¹)	BIO1 (°C)	BIO12 (mm)
1	7.2±0.6	149±52	12±8	50±33	337±65	9.5±0.2	556±11
2	6.7±0.4	96±28	4.3±1.2	30±2	390±40	9.6±0.1	505±105
3	6.2	51±16		25	246	10±0.4	539±29
4	6.6	117		27	341	9.6	547
5	6.8±0.1	90±97	30±14	28±6	437±6.4	9.9 ± 0.7	532±32
6	7.3	175	4.4	42	327±27	9.5	563
7	6.7	268	8	27	360	9.9	537
8	5.8	97±5.5		21	204	10.8	518

Description of clusters

Cluster 1 – *Taraxaco serotinae-Festucetum valesiacae* association

The diagnostic species were *Iris graminea* (0.872, 0.001, ***), *Teucrium chamaedrys* (0.861, 0.001, ***), *Achillea pannonica* (0.816, 0.002, **), *Stipa pennata* (0.816,

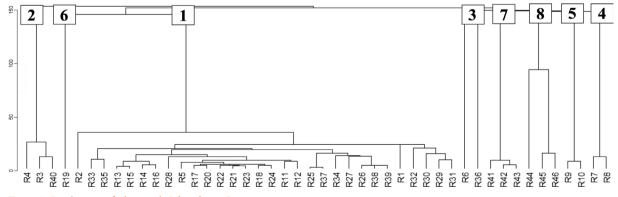


Figure 3: Dendrogram of relevés with *I. brandzae* in Romania. Slika 3: Dendrogram vegetacijskih popisov z *I. brandzae* v Romuniji. 0.004, **), Nonea pulla (0.786, 0.003, **), Inula germanica (0.762, 0.012, *), Adonis vernalis (0.755, 0.042, *), Schedonorus pratensis (0.732, 0.024, *), Polygala major (0.715, 0.016, *), Carex distans (0.693, 0.017, *), Leopoldia comosa (0.591, 0.035, *) and Festuca valesiaca (0.574, 0.001, ***).

The community is heterogeneous and is characterized by numerous species characteristic of the Festuco-Brometea class (Festuca valesiaca, Crambe tataria and Phlomis herba-venti subsp. pungens). This is the largest cluster, consists of 30 relevés (65%) in which 247 species were recorded. These communities occur at elevations from 20 m to 244 m, in the Iasi (66%) and Vaslui counties (27%). The sample area was 100 m², with 21 to 101 species recorded, and a mean of 44 species. Vegetation coverage was from 70% to 100%. The western and southwestern aspects and small slopes, from 3.1° to 10° (53%) predominate. The soils vary from moderately acidic, from 5.81 to 6.80 (33%), neutral, from 6.81 to 7.20 (17%) to slightly alkaline (50%). High concentrations of phosphorus (from 11 mg Kg⁻¹ to 138 mg Kg⁻¹) and potassium (from 240 mg Kg⁻¹ to 603 mg Kg⁻¹) were recorded. Also, the communities were identified in areas with annual mean temperatures from 9.1 °C to 10.2 °C, and annual precipitation from 521 mm to 569 mm.

Cluster 2 – *Limonio gmelini-Artemisietum santonici* association

The diagnostic species are Artemisia santonicum subsp. santonicum (1.000, 0.002, **), Galatella villosa (0.993, 0.001, ***), G. linosyris (0.986, 0.001, ***), Tripolium pannonicum (0.845, 0.014, *), Gypsophila muralis (0.816, 0.013, *), Trifolium fragiferum subsp. bonannii (0.816, 0.013, *), Veronica orchidea (0.813, 0.009, **), Asparagus officinalis (0.795, 0.037, *), Bassia prostrata (0.753, 0.016 *), Limonium gmelinii (0.749, 0.035, *), Agropyron cristatum subsp. pectinatum (0.747, 0.005 **), Atriplex littoralis (0.716, 0.027, *), A. tatarica (0.716, 0.027, *) and Plantago schwarzenbergiana (0.716, 0.035, *).

This cluster consists of three relevés (7%), distributed in Iaşi County. These communities were recorded at elevations from 71 m to 126 m, on small slopes (from 3.1° to 10°) with western and northwestern aspects. The annual mean temperature varied from 9.5 °C to 9.7 °C, and the annual precipitation varied from 384 mm to 566 mm. The sample area was 25 m², with the number of species from 13 to 23, with a mean of 19 species. Vegetation coverage was from 77% to 95%. The soils are moderately acidic, with medium concentrations of phosphorus and very high concentrations of potassium.

Cluster 3 – *Poo trivialis-Alopecuretum pratensis* **and** *Rorippo austriacae-Agropyretum repentis* associations.

The diagnostic species are *Phragmites australis* (0.885, 0.001, ***), *Alopecurus pratensis* (0.816, 0.010, **), *Bromus commutatus* (0.816, 0.010, **), *Carex acutiformis* (0.816, 0.010, **), *C. melanostachya* (0.816, 0.010, **), *Rumex crispus* (0.816, 0.010, **), *Lotus corniculatus* (0.769, 0.015, *) and *Vicia villosa* (0.741, 0.019, *).

This cluster consists of two relevés (4%), distributed in Vaslui and Iași counties, and is characterized by the presence of different species, such as *Beckmannia eruciformis, Carex acutiformis, Centaurea jacea, Rumex crispus, Trifolium hybridum, Agrostis stolonifera*, etc. The number of species varied from 45 to 48, with a mean of 47 species. Vegetation coverage was from 95% to 97%. The communities occur at elevation from 39 m to 62 m, where the annual precipitation were from 518 mm to 559 mm, and with annual mean temperatures were from 9.7 °C to 10.3 °C. The soils are moderately acidic, with medium concentrations of phosphorus and high concentrations of potassium.

Cluster 4 – Astero pannonici-Puccinellietum distantis **association.**

The diagnostic species is Puccinellia distans.

The cluster consists of two relevés (4%), distributed in Vaslui County. On the 30 m² sample area, 22 species were identified, with a mean vegetation coverage of 82%. Instead, on the sample area of 20 m², 20 species were identified, with a vegetation coverage from 77%. The communities were registered at elevation of 117 m, in areas with annual mean temperatures of 9.6 °C, and annual precipitation of 547 mm. The soils are moderately acidic, rich in potassium, and poor in phosphorus. The most common species were *Puccinellia distans*.

Cluster 5 – Medicagini minimae-Festucetum valesiacae association

The diagnostic species are *Anchusa ochroleuca* (1.000, 0.003, **), *Anisantha tectorum* (1.000, 0.003, **), *Astragalus exscapus* subsp. *pubiflorus* (1.000, 0.003, **), *A. ponticus* (1.000, 0.003, **), *Centaurea diffusa* (1.000, 0.003, **), *Cleistogenes serotina* (1.000, 0.003, **), *Hieracium virosum* (1.000, 0.003, **), *Xeranthemum annuum* (1.000, 0.003, **), *Linum hirsutum* (0.997, 0.002 **) and *Bothriochloa ischaemum* (0.991, 0.001, ***).

This cluster consists of two relevés (4%), distributed in Vaslui County, were included. These communities were recorded at elevations from 21 m to 158 m. Annual precipitation varied from 509 mm to 554 mm, and annual mean temperatures varied from 9.5 °C to 10.4 °C. The mean number of species per 100 m² was 69 (from 62 to 75), and the vegetation coverage was from 93% to 100%. The soils are moderately acidic, with excessive concentrations of potassium (437 mg Kg⁻¹), and medium concentrations of phosphorus.

Cluster 6 – Jurineo arachnoideae-Stipetum lessingianae association

The diagnostic species are *Muscari racemosum* (0.767, 0.001, ***), *Ferulago campestris* (0.728, 0.015, *), *Jurinea arachnoidea* (0.728, 0.025, *), *Veronica arvensis* (0.728, 0.027, *), *Viola arvensis* (0.728, 0.021, *), *Camelina microcarpa* (0.686, 0.039, *) and *Trinia kitaibelii* (0.642, 0.034, *).

This cluster consists of one relevé (3%), distributed in Iaşi County. The communities occur in areas with annual precipitation was 563 mm, and annual mean temperatures was 9.5 °C, at elevations 175 m. The aspects was southwest, and the slopes were small (from 3.1° to 10°). The mean number of species per 100 m² was 55, and the vegetation coverage was from 98%. The soils are weakly alkaline, rich in phosphorus and potassium.

Cluster 7 – *Nitrario schoberi-Artemisietum santonici* association

The diagnostic species are Artemisia maritima (0.894, 0.008, **), A. pontica (0.894, 0.006, **), Dianthus guttatus (0.894, 0.006, **), Lepidium ruderale (0.775, 0.016, *), Nitraria schoberi (0.775, 0.017, *), Puccinellia distans subsp. limosa (0.775, 0.016, *), Spergularia marina (0.775, 0.020, *), S. media (0.775, 0.017, *), Taraxacum besarabicum (0.775, 0.020, *), Podospermum canum (0.665, 0.040, *) and Camphorosma annua (0.632, 0.048, *).

This cluster consists of three relevés (7%), distributed in Buzău County. The number of species was from 15 to 22, with a mean of 19 species per 100 m². Vegetation coverage varied from 74% to 82%. Communities of *Nitrario schoberi* occur at elevation of 268 m, on small slopes (from 3.1° to 10°) with southwest aspect. The soils are moderately acidic, poor in phosphorus and rich in potassium. The annual mean temperature was 9.9 °C, and the annual precipitation was 537 mm.

Cluster 8 – Ulmeto campestris-Fraxinetum holotrichae association

The diagnostic species are Aegonychon purpurocaeruleum (1.000, 0.001, ***), Arctium lappa (1.000, 0.001, ***), Carex sylvatica (1.000, 0.001, ***), Colchicum autumnale (1.000, 0.001, ***), Ficaria verna (1.000, 0.001, ***), Fraxinus angustifolia (1.000, 0.001, ***), F. pallisae (1.000, 0.001, ***), Ligustrum vulgare (1.000, 0.001, ***), Lysimachia nummularia (1.000, 0., ***), Myosotis sylvatica (1.000, 0.001, ***), Oenanthe aquatica (1.000, 0.001, ***), Poa trivialis (1.000, 0.001, ***), Pulmonaria

officinalis (1.000, 0.001, ***), Quercus robur subsp. pedunculiflora (1.000, 0.001, ***), Ulmus minor subsp. minor (1.000, 0.001, ***), Valeriana officinalis (1.000, 0.001, ***) and Viola reichenbachiana (1.000, 0.001, ***).

This cluster consists of three relevés (7%), distributed in Buzău County. Communities with *Fraxinus pallisae* occur on flat terrain, with southwest aspect. The number of species was from 58 to 73, with a mean of 64 species per 100 m². Vegetation coverage was from 92% to 98%. Annual precipitation varied from 518 mm to 519 mm, and the annual mean temperature was 10.8 °C. The soils are moderately acidic, poor in potassium and phosphorus.

The relationship between floristic composition and environmental variables

The main factors influencing the variation of the floristic composition are highlighted by indirect gradient analysis (Figure 4). Given that the length of the gradients of floristic similarity was 6.59 (Table 3), it was decided to use the unimodal method, i.e. Canonical Correspondence Analysis (CCA).

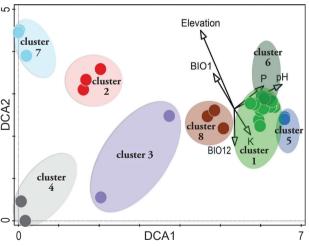


Figure 4: DCA ordination diagram of the 46 vegetation relevés. **Slika 4**: DCA ordinacijski diagram 46 vegetacijskih popisov.

Table 3: Summary of the DCA analysis.**Tabela 3:** Povzetek rezultatov analize DCA.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.8642	0.6683	0.3888	0.2819
Explained variation (cumulative)	11.66	20.68	25.93	29.74
Gradient length	6.59	4.5	2.52	2.6
Pseudo-canonical correlation (suppl.)	0.696	0.6758	0.4468	0.5444

Priimek et al. Skrajšan naslov članka

The DCA diagram highlights how various vegetation types are distributed on the first and second ordination axes. The main gradient of vegetation composition concerning the first axis can be explained by positive correlations with variables such as pH, phosphorus, and potassium. The second DCA axis was correlated with elevation and annual mean temperature (BIO1). This means that as elevation increases, the composition of the vegetation undergoes significant changes. Also, certain types of vegetation can be associated with more acidic or alkaline soils. Moreover, phosphorus and potassium concentrations can play an important role in the distribution of plant species (Figure 4).

BIO1 (annual mean temperature) and elevation are two key variables that were identified as having the greatest impact. BIO1 explains 7.1% of the total variation and contributes 33.3% to the first ordination axis of the DCA plot. The elevation, which explains 5.2% of the total variation and contributes 24.7% to the first axis, is also significant (Table 4).

Table 4: Results of the CCA ordination of the effect of variables on the floristic composition of communities with *I. brandzae.*

Tabela 4: Rezultati CCA ordinacije vpliva spremenljivk nafloristično sestavo združb z vrsto *I. brandzae*.

Variables	Contribution (%)	Explains (%)	pseudo- F	p- value	P (adj)
BIO1	33.3	7.1	3.3	0.001	0.006
Elevation	24.7	5.2	2.5	0.001	0.006
K-potassium	13.2	2.8	1.4	0.124	0.744
pН	13.9	2.9	1.4	0.055	0.33
BIO12	8.2	1.8	0.9	0.484	1
P-phosphorus	6.8	1.4	0.7	0.802	1

Discussion

The species *I. brandzae* occurs in Moldova and Muntenia regions (Mititelu et al., 1979–1980; Chifu et al., 2006, 2014; Popescu, 2013; Sîrbu et al., 2019; Mânzu et al., 2020). The results of our study, in which we identified eight distinct clusters associated with these communities, confirm the significant diversity present within these groups. All the other species in the section Limniris series Spuriae have quite limited ecological preferences and hence are rather specialized taxa.

Iris spuria L. (with all the five subspecies), I. xanthospuria B. Matthew & T. Baytop, I. orientalis Mill., I. pseudonotha Galushko and I. halophila Pall. are always related to halophile wet grasslands, an intrazonal type of habitat from the nemoral, forest-steppe, and steppe zones. Iris kerneriana Asch. & Sint. ex Baker, I. sintenisii Janka, I. ludwigii Maxim., *I. notha* M. Bieb. and *I. pontica* Zapal are present only in xeric grasslands, woodlands and thickets from the steppe and forest-steppe area (Fedchenko & Vvedenskii, 1935; Mathew, 1984). The habitat of *I. crocea* Jacq. is not well known, the species being reported mostly from cultivation and as a ruderal (Ali & Mathew, 2008). *I. graminea* L. lives in open forests and mesic grasslands from the nemoral zone and the forest-steppe (Meusel et al., 1965). In contrast with all the mentioned species our study indicates that *I. brandzae* Prodan is a species located solely in the forest-steppe, but in a much larger variety of different habitats, ranging from forests to mesic and xeric grasslands as the present study reveals for the first time.

Many species characteristic only for the forest-steppe areas are also adapted to a wide variety of habitats inside their ranges (Erdős et al., 2018). One of the most thoroughly analyzed cases is *Crambe tataria* Sebeók (Chirilă, 2024). The dimension of this phenomenon is not known nor are the eventual adaptative mechanisms behind it. The considerable ecological plasticity of *I. brandzae* is of a certain conservative importance. A species that is adapted to a large variety of habitats has better chances of survival than one that specializes in a single one. However, during our research we preliminary concluded that *I. brandzae* seems to be not so prone to resist diverse types of human impacts, like overgrazing associated with erosion and/or ruderalization – this being the theme of one of our future projects.

Conclusions

In Romania, the species *I. brandzae* is found in the *Festuco-Brometea*, *Festuco-Puccinellietea*, *Carpino-Fagetea sylvaticae* and *Molinio-Arrhenatheretea* vegetation classes. The species occurs most frequently in the *Taraxaco serotinae-Festucetum valesiacae* association. The floristic composition of the phytocoenoses with *I. brandzae* in the analyzed grasslands from Moldova is influenced by the annual mean temperature (BIO1).

In Muntenia region, it occurs in halophilous grasslands and in alluvial ash-anine forests, with a low frequency and in small groups in various areas. The species *I. brandzae* was identified in grasslands dominated by *Nitraria schoberi*, *Puccinelia distans*, *Fraxinus angustifolia* and *F. pallisae*. In Moldova region, the species can be found in halophilous, xerophilous and meso-xerophilous grasslands. In various localities in Iași and Vaslui counties, the species is rare or has a low frequency, in association with other dominant species such as Festuca valesiaca, *Alopecurus pratensis, Artemisia santonicum, Bothriochloa ischaemum, Limonium gmelinii, Elytrigia repens* or *Puccinellia distans*.

Hacquetia

ORCID iDs

Simona Dumitrița Chirilă 🕑 https://orcid.org/0000-0003-3397-1834

Kiril Vassilev D https://orcid.org/0000-0003-4376-5575 Alexandru Sabin Bădărău D https://orcid.org/0000-0001-

5113-2802

References

Ali, S.I. & Mathew, B. (2008). Iris in *Flora of Pakistan*, efloras, published on the Internet http://www.efloras.org [accessed 2024 January 05]. Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.

Aukhadieva, E., Kalashnik, N. & Ishbirdin, A. (2021). Discussion of some taxonomy issues of species of the genus *Iris* L. based on biomorphological and karyological characteristics. In *E3S Web of Conferences. EDP Sciences*, 254, 06008.

Ballabio, C., Lugato, E., Fernández-Ugalde, O.....& Panagos, P. (2019). Mapping LUCAS topsoil chemical properties at European scale using Gaussian process regression. *Geoderma*, 355, 113912.

Bi, X., Li, B., Zhang, L., Nan, B., Zhang, X. & Yang, Z. (2020). Response of grassland productivity to climate change and anthropogenic activities in arid regions of Central Asia. *Peer J*, 8, e9797.

Borza, A. & Boşcaiu, N. (1965). *Introducere în studiul covorului vegetal* (Introduction to the study of the vegetation carpet) [in Romanian]. Academia Republicii Populare Române.

Bouxin, G. (2005). Ginkgo, a multivariate analysis package. *Journal of Vegetation Science*, 16, 355–359.

Braun-Blanquet, J. (1964). Pflanzensoziologie. Grundzüge der Vegetationskunde. Springer.

British Iris Society (1997). A Guide to Species Irises: Their Identification and Cultivation. Cambridge University Press. p. 255.

Cassidy, G.E. & Linnegar, S. (1987). *Growing Irises*. Bromley: Christopher Helm. 68, 142.

Channell, R. (2004). The conservation value of peripheral populations: the supporting science. In Proceedings of the species at risk 2004 pathways to recovery conference. Victoria, British Columbia, Canada, pp. 1–17.

Chifu, T., Irimia, I. & Zamfirescu, O. (2014). *Festuco-Brometea*. In: Chifu, T. (Ed.). *Diversitatea fitosociologică a vegetației României*. Vol. II. Vegetația erbacee antropizată. Tom 1. Vegetația pajiștilor (Festuco-Brometea. In: Chifu T. The phytosociological diversity of Romania's vegetation. Vol. II. Anthropogenic herbaceous vegetation. Part 1. Meadow vegetation) [in Romanian]. Institutul European, Iași, pp. 113–397.

Chifu, T., Mânzu, C. & Zamfirescu, O. (2006). *Flora și vegetația Moldovei* (Flora and vegetation of Moldova) [in Romanian]. Universitatea Al. I. Cuza.

Chirilă, S. D., Cara, I.G. & Motrescu, I. (2022). Habitat preference of the endangered species *Crambe tataria* (Brassicaceae) from Romania. *Tuexenia*, *42*, 275–296.

Chirilă, S.D. (2024). Analiza eco-cenotică și structura genetică a populațiilor de *Crambe tataria* Sebeók din România (Eco-cenotic

analysis and genetic structure of *Crambe tataria* Sebeók populations in Romania) [in Romanian]. Presa Universitară Clujeană, pp. 272.

Chytrý, M., Tichý, L., Hennekens, S.M. & Schaminée, J.H.J. (2020). EUNIS Habitat Classification: expert system, characteristic species combinations and distribution maps of European habitats. *Applied Vegetation Science*, 23, 648–675.

Coldea, G., Indreica, A. & Oprea, A. (2015). Les associations végétales de Roumanie. 3-Les associations forestières et arbustives (Romanian plant associations. Forest and shrub associations) [in French]. Presa Universitară Clujeană, pp. 281.

Coldea, G., Oprea, A., Sârbu, I., Sîrbu, C. & Ștefan, N. (2012). *Les associations végétales de Roumanie. Tome 2: Les associations anthropogénes* (Romanian plant associations. Volume 2: Anthropogenic associations) [in French]. Presa Universitară Clujeană, pp. 482.

De Cáceres, M. & Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology*, *90*, 3566–3574.

Derevenko, T. (2010). Partes Horti botanici ChNU nuncupati. Yu Fedkovich in conservanda floristica diversitate Bukovina. In *Conservation of plant diversity*, pp. 310–315.

Dihoru, G. & Negrean, G. (2009). Cartea roșie a plantelor vasculare din România (Red book of vascular plants from Romania) [in Romanian]. Academia Română, p. 290.

Dorofeeva, M.M. & Zhurbenko, P.M. (2020). Comparative study of ovule structure and development in some species of *Iris* subgenus Limniris (Iridaceae). *Botanicheskij Zhurnal*, *105*(1), 15–31.

EEA (2023). Iris sintenisii subsp. brandzae (Prodàn) D.A.Webb & Chater. Data from: https://eunis.eea.europa.eu/species/186816. [accessed 2023 October 16].

Erdős, L., Ambarlı, D., Anenkhonov, O.A., Bátori, Z..... & Török, P. (2018). The edge of two worlds: A new review and synthesis on Eurasian forest-steppes. *Applied Vegetation Science*, *21*, 345–362.

EURO+MED (2023). Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. Data from: http://ww2.bgbm.org/EuroPlusMed [accessed 2023 October 16].

Fedchenko, B.A. & Vvedenskii, A.I. (1935). Iris in Komarov, V.L. (edit.) *Flora of the USSR* 4, Leningrad.

Fedorov, A. & Tsvelev, N.N. (2001). Flora of Russia 4. CRC Press.

Fick, S.E. & Hijmans, R.J. (2017). Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*, 14.

Grințescu, I., Nyarady, E.I., Paucă, A., Prodan, I., Şerbănescu, I. & Zahariadi, C. (1966). *Iris brandzae*, 500 p. In: Săvulescu, T. (1966). *Flora Republicii Socialiste România*. Academiei Republicii Socialiste România. p. 868.

Hensen, I., Oberprieler, C. & Wesche, K. (2005). Genetic structure, population size, and seed production of *Pulsatilla vulgaris* Mill. (Ranunculaceae) in Central Germany. *Flora-Morphology, Distribution, Functional Ecology of Plants, 200*(1), 3–14.

ISO 11263 (1994). Determination of phosphorus – Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution. Data from: https://www.iso.org/standard/19241.html. [accessed 2023 October 11].



Mathew, B. (1984). Iris in Davis, P.H. (edit.) *Flora of Turkey* 8, Edinburgh University Press.

Mânzu, C.C., Irimia, I., Cîşlariu, A.G. & Chinan, V.C. (2020). Chorological data for some rare plant species from ROSCI0222 Sărăturile Jijia Inferioară-Prut and ROSPA0042 Eleșteele Jijiei și Miletinului (Iași County). *Acta Horti Botanici Bucurestiensis*, 46, 35–54.

Meusel, H., Jäger, E. & Weinert, E. (1965). Vergleichende Chorologie der zentraleuropäischen Flora. Vol. 1. Fischer, Jena (Verbreitungskarte).

Mititelu, D., Ștefan, N. & Ciupercă, Gh. (1979–1980). Flora și vegetația rezervației "Pâclele" cu vulcani noroioși (Jud. Buzău) (Flora and vegetation of the reserve "Pâclele" with mud volcanoes) [in Romanian], pp. 99–120.

Mucina, L., Bültmann, H., Dierßen, K...... & Tichý, L. (2016). Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen and algal communities. *Applied Vegetation Science, 19*, 3–264.

O'Grady, J.J., Reed, D.H..... & Frankham, R. (2004). What are the best correlates of predicted extinction risk? *Biological Conservation*, *118*, 513–520.

Oltean, M., Negrean, G., Popescu, A.... & Mihăilescu, S. (1994). *Lista roșie a plantelor superioare din România* (Red list of higher plants from Romania) [in Romanian]: Studii, sinteze, documentații de ecologie. 1. Academia Română, Institutul de Biologie București, pp. 1–52.

Oprea, A. (2005). Lista critică a plantelor vasculare din România (Critical list of vascular plants from Romania) [in Romanian]. Univ. "Alexandru Ioan Cuza", Iași, pp. 668.

Popescu, I.E. (2013). Unicitatea patrimoniului natural din Rezervația de fânețe seculare de la Valea lui David Iași (The uniqueness of the natural heritage of the Secular Hay Reserve from Valea lui David Iași) [in Romanian]. *Mnemosyne*, *4*, 7–36.

QGIS Development Team (2022). QGIS version 3.28. Data from: https://qgis.org, [accessed 2023 October 14].

Rand, W.M. (1971). Objective criteria for the evaluation of clustering methods. *Journal of the American Statistical Association, 66*(336), 846–850.

Rodionenko, G.I. (2005). On the independence of the genus Limniris (Iridaceae). *Botanicheskij Zhurnal*, *92*(4), 547–554 [in Russian].

Rodionenko, G.I. (2009). A new system of the genus Iris (Iridaceae). Botanicheskij Zhurnal, 94(3), 423–435 [in Russian].

Roger, P. & Martyn, R. (1991). Perennials Vol. 1. Pan Books Ltd. p. 216.

Rousseeuw, P.J. (1987). Silhouettes: a Graphical Aid to the Interpretation and Validation of Cluster Analysis. *Computational and Applied Mathematics*, 20, 53–65. Sîrbu, C., Oprea, A. & Peregrym, M. (2019). New data about *Fritillaria meleagroides* in Romania. *Journal of Plant Development*, 26, 123–135.

SR EN ISO 10390 (2022). Soil, treated biowaste and sludge. Determination of pH. Data from: https://standards.iteh.ai/catalog/ standards/cen/35c1e689-fbc3-4bd2-a9e7-7162f61ae30b/eniso-10390-2022. [accessed 2023 October 14].

SRTM Data (2023). SRTM 90m Digital Elevation Data. Data from: https://srtm.csi.cgiar.org/srtmdata/. [accessed 2023 October 14].

Szczecińska, M., Sramko, G., Wołosz, K. & Sawicki, J. (2016). Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) Mill in East Central Europe. *PloS One*, *11*(3), e0151730.

ter Braak, C.J.F. & Šmilauer, P. (2018). *Canoco reference manual and user's guide: Software for ordination (version 5.10).* Biometris, Wageningen University & Research.

Tichý, L. (2002). JUICE, software for vegetation classification. *Journal of Vegetation Science*, 13, 451–453.

Tutin, T.G., Heywood, V.H., Burges, N.A.... & Richardson, O.I.B.K. (1980). *Flora Europaea*. Volume 5: Alismataceae to Orchidaceae (Monocotyledones).

Vassilev, K., Ruprecht, E., Alexiu, V...... & Dengler, J. (2018). The Romanian Grassland Database (RGD): historical background, current status and future perspectives. *Phytocoenologia*, *48*, 91–100.

Văduva, I. (2008). Clima României (Climate of Romania) [in Romanian]. Fundația România de Mâine.

Volutsa, O.D. (2011). *Iris brandzae* Prodán (Iridaceae) u flori Chernivec'koi' oblasti. Aktual'ni problemy botaniky ta ekologii'. (In Ukrainian).

Wheeler, A.S. & Wilson, C.A. (2014). Exploring phylogenetic relationships within a broadly distributed northern hemisphere group of semi-aquatic *Iris* species (Iridaceae). *Systematic Botany*, *34*(2), 277–284.

Wilson, C.A. (2004). Phylogeny of *Iris* based on chloroplast matK gene and trnK intron sequence data. *Molecular Phylogenetics and Evolution*, *33*, 402–412.

Wilson, C.A. (2006). Patterns of evolution in characters that define *Iris* subgenera and sections. *Aliso*, 22, 425–433.

Wilson, C.A. (2009). Phylogenetic relationships among the recognized series in *Iris* section Limniris. *Systematic Botany*, *34*, 277–284.

Wilson, C.A. (2011). Subgeneric classification in *Iris* re-examined using chloroplastsequence data. *Taxon*, 60, 27–35.