Ecology, biogeography, and distribution of the rare species Iris brandzae in Romania

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Abstract

Iris brandzae is a threatened plant species, distributed in Romania's North-East and South-East regions. The present study aims to examine the environmental conditions and update the species distribution in Romania. For environmental conditions, information about climatic, topographic, and soil chemical factors were collected and analyzed. For the biogeographic analysis, studies from the literature were consulted, and to update the species distribution, the populations mentioned in the literature were verified. *Iris brandzae* was recorded in 21 locations in Botoșani, Buzău, Iași and Vaslui counties, of which ten populations were recorded for the first time. The current trend of the area that the population occupies is stable (59%), while 41% of the population is decreasing. The conservation status of these populations, along with changes in their habitat distribution, is assessed as unfavourable-inadequate. The number of individuals per 100 m² in the studied habitats varied from 5 to 25.

The species grows on slightly acidic to neutral soils with moderate phosphorus and high potassium concentrations. The elevations were moderate, from 47 to 294 m a.s.l. on gentle and moderate slopes with northern, eastern aspects and frequently in communities with *Festuca valesiaca*. Moreover, *I. brandzae* has the most significant ecological adaptability in the southern aspects. The main threat is overgrazing. From a phytocoenological perspective, *I. brandzae* occurs in the more mixed landscape, found only in the nemoral forest-steppe region west and northwest of the Black Sea. In this context, these populations must be monitored over the long term to understand population dynamics. It is also necessary to carry out public awareness campaigns regarding the importance of the species, the expansion and designation of protected areas, and grassland management.

Highlights

In Romania, 21 populations of *Iris brandzae* have been recorded.

The current trend of the area occupied by the population is stable.

The conservation status of these populations, along with changes in their habitat distribution, are unfavourable-inadequate.

Iris brandzae is the only species in the *Spuriae* series that inhabits multiple habitats, ranging from mesohygrophilous-hygrophilous saline environments to weakly halophilous and xerophilous grasslands.The main threats to the species are overgrazing, climate variations, and changes in land use.

Introduction

Anthropogenic and natural factors threaten Biodiversity (Wamelink et al. 2014; Isbell et al. 2023). For example, expanding agricultural practices have transformed ecosystems such as wetlands, forests and grasslands into agricultural land, leading to a decline in biodiversity worldwide (Adebayo 2019). The main factor determining the reduction of biodiversity worldwide was habitat change, followed by overexploitation of natural resources and pollution (Jaureguiberry et al. 2022). Also, significant factors in species decline are climate change (IPCC 2022) and the expansion of invasive species (Jaureguiberry et al. 2022). Approximately 30% of all global species have disappeared or are on the verge of extinction since the 16th century (Humphreys et al. 2019; Isbell et al. 2023). In this context, biodiversity loss will negatively affect ecosystem functions and the benefits offered to people. At the same time, recent findings indicate that biodiversity loss will be even greater than previously thought. Among the partial causes of the decline of biodiversity is the underestimation of very little-known taxa as well as the limited involvement of experts in biodiversity. Thus, establishing conservation measures is essential. As such, until the year 2100, the disappearance of threatened species can be prevented through conservation and protection measures (Isbell et al. 2023).

Grasslands habitats are considered to be the richest in biodiversity on earth (Petermann and Buzhdygan 2021), but also among the most endangered habitats (Scholtz and Twidwell 2022). Within grasslands, many plant species are rare or endangered due to land use changes that have occurred due to infrastructure development, urbanization and agriculture (Foley et al. 2005; Condé et al. 2010; Wesche et al. 2010; Richardson et al. 2012). Thus, their ability to disperse but also habitat requirements limit their distribution (Bakker and Berendse 1999; Normand et al. 2011). Understanding the habitat requirements of rare plant species compared to common plant species is important for their protection (Bevill and Louda 1999).

At the habitat level, species are influenced by management practices, vegetation types, subtle variations in vegetation structure, soil characteristics, etc. (Richardson et al. 2012). In contrast, at the biogeographic scale, species are influenced by temperature and precipitation (Austin and Van Niel 2011).

One of the rare species in Romania is *Iris brandzae* Prodan, a species from the family *Iridaceae* Juss. (Ciocârlan 2000). This is a narrow endemic species of the forest-steppe of the low plains of the Eastern part of Romania and the Moldavian Plain. It occurs in Ukraine (Volutsa 2011), and in the low plains of the Republic of Moldova (Bădărău 2014). Also, *I. brandzae* originally appeared on the shores of the ancient Paratethys. This species has persisted in isolation at this location for over 7 million years (Negrean 2019). In Romania, the species has been recorded mainlyin dry grassland (*Festuco-Brometea* class), which mainly includes communities of *Festuca valesiaca,* and *Stipa lessingiana*. However, the species also occurs in mesophilic grasslands (*Molinio-Arrhenatheretea* class), in communities of *Elytrigia repens* (Sîrbu et al. 2019). The species was also observed in slightly halophilic grasslands (*Salicornietea fruticosae* class), in communities with *Artemisia santonicum* and *Nitraria schoberi* (Mititelu et al. 1979–1980; Chirilă et al. 2024). In addition, the presence of this species was also recorded in deciduous broad-leaved forests (*Carpino-Fagetea sylvaticae* class), in communities of *Fraxinus angustifolia* and *F. pallisae* (Chirilă et al. 2024). Based on the moisture index, *I. brandzae* is a meso-xerophilic species (Chirilă et al. 2024).

Based on field observations, we argue that *I. brandzae*, a species that exhibits great ecological adaptability, differs from *Iris sintenisii* Janka, a species characteristic of the forest-steppe areas of the

Balkans, northwestern Anatolia and the xeric intrazonal grasslands of the non-moral zone of Southern Italy. This obvious difference determined all Romanian botanists (Ciocârlan 2009; Dihoru and Negrean 2009; Sârbu et al. 2013) to consider *I. brandzae* as a distinct species. We consider that *I. brandzae* does not have subspecies status with *I. sintenisii*, morphologically being closer to *I. graminea*, *I. pontica* and even *I. spuria*, with which it can be confused relatively easily.

This study aimed to highlight the ecological characteristics and distribution of the *I. brandzae* species in Romania. The objectives of our study were: i) identification of the ecological conditions of the target species in Romania; and ii) updating the distribution of the species in the country. The research hypothesis was the following: the natural area of the populations of *I. brandzae* in Romania is restricted to some extent by habitat loss (intensification of grazing and transformation of grasslands into arable land).

Materials and methods

Study area

The study was conducted in 72 locations where *I. brandzae* was recorded in the regions of Moldova and Muntenia, during the period 2018-2024. These locations are in Botoșani, Buzău, Galați, Iasi, Vaslui, and Vrancea counties (Fig. 1). The climate in these regions is temperate-continental, with continental influences (Velea et al. 2023). The mean elevation of the regions where the species was confirmed varies from 112 (mean; in Moldova) to 201 m a.s.l. (mean; in Muntenia), while the annual mean temperatures and the amounts of mean annual precipitation vary from 9.6 °C and 559 mm (mean; in Moldova), to 10.4 °C, and 527 mm respectively (mean; in Muntenia) (based on Fick and Hijmans 2017).

Study species

Iris brandzae (Fig. 2) is a perennial species, with a height from 15 cm to 40 cm. The leaves are very narrow, up to 3.5 mm wide. Spathes herbaceous, strongly inflated, keeled, with conspicuous veins. Flowers 2(1) apical; perigon tube dilated at the tip, 18–25 mm long; outer tepals (falls) patent, glabrous, 9–10 mm wide, violet-blue with purple streaks; inner tepals (standards) erect, lanceolate to oblanceolate, narrower, purple-violet; stamen 3, below the stigma lobes; ovary of 12–15 mm long, 6-winged; capsule 6-winged with the upper sterile portion of 12–22 mm long. The seeds are wrinkled on the face, narrowly cartilaginous on the edges, and have a length of 4.5 mm (Grințescu et al. 1966; Dihoru and Negrean 2009). Flowering occurs between April and May (Sârbu et al. 2013).

Environmental conditions

Data collected for each found population of *I. brandzae* include location, plant association, aspect, slope (°), elevation (m a.s.l.), soil pH, annual mean temperature (°C), annual precipitation (mm), mean temperature – June (°C), mean precipitation – June (mm), vegetation cover (%), population density expressed as number of individuals per 100 m², identified threats, population conservation status, and EUNIS habitat type.

Values for mean temperature (June; °C), mean annual temperature (°C), mean precipitation (June; mm), annual precipitation (mm), and elevation (m a.s.l.) were obtained from the WorldClim database, at a resolution of 30 arc. sec. (Fick and Hijmans 2017). The assessment of the population status was carried out according to the criteria established in Article 17. The values for slope (°) and aspect (°) were extracted based on the raster obtained from SRTM. Values for soil pH, but also for phosphorus (P) and potassium (K) concentrations (*Ballabio* et al. 2019) were extracted from the European Soil Database & soil properties, at a resolution of 500 m (http://esdac.jrc.ec.europa.eu/).

Distribution analysis

Various data sources were used to update information on the distribution of *I. brandzae* species in Romania, including management plans, scientific articles, identification keys, herbaria, researchers' observations, and other field notes. In 2018–2024, investigations were carried out in different locations in the Moldova and Muntenia regions to verify the presence of the species *I. brandzae*. The information collected, both from the field and from literature, was integrated and presented in the form of a distribution map using the software QGIS version 3.34.3 (QGIS Development Team 2024). This map illustrates both the populations of *I. brandzae* recorded in the literature, some confirmed by us in the field, and some population first reported in this paper.

Population status

The population status was assessed according to the criteria of Article 17 of the Habitats Directive (2019–2024): (i) Current trend of the area occupied by the population (- = decreasing; 0 = stable; + = increasing); (ii) Population conservation status (FV = favourable; U1 = unfavourable-inadequate; U2 = unfavourable-bad); (iii) Changes in the distribution pattern of habitat types (FV = favourable; U1 = unfavourable-inadequate; U2 = unfavourable-bad). In each location, flowering stems of *I. brandzae* were counted (Supplementary Material 1).

Plant association and nomenclature

For each population found of *I. brandzae*, the plant association was mentioned. The nomenclature of plant species followed Euro+Med (2025), except for *I. brandzae*, and the nomenclature of plant associations followed Chifu et al. (2014). Habitat identification used the EUNIS-ESy expert system (Chytrý et al. 2020).

Statistical analysis

To determine significant differences between groups (regions) regarding the means of environmental variables, ANOVA was applied. Subsequently, after significant differences were identified ($p < 0.05$), the Tukey post-hoc test was applied for multiple comparisons. The analyses were performed in the R

Statistical Software (v4.1.4; R Core Team 2024), via the 'ggplot2' v3.5.1. (Wickham, 2016) and 'dplyr' v1.1.4 packages (Wickham et al., 2023). Violin plot type graphs were made in the R Statistical Software, which has the following interpretation: the middle point represents the median; the thick grey bar in the centre represents the interquartile range; the thin gray line represents the rest of the data distribution. To convert barplot graphs to a polar graph, ggplot2::coord_polar() was used.

Results

Distribution of the species *Iris brandzae* in Romania

According to our data and those from the literature, 72 populations of *I. brandzae* have been recorded in Romania till now (Supplementary Material 1). Following field trips, 21 populations have been recorded (Fig. 3; Table 1), of which 10 were confirmed and 11 are new observations. According to the total number of confirmed populations and newly recorded populations in 2018–2024, we consider the species to be rare in Moldova (16 records – 76%) and Muntenia (five records – 24%).

Distribution of the species in Moldova

In the Moldova region, 63 populations (55 data from the literature and eight new data) were recorded (Supplementary Material 1) distributed in some locations in Botoșani, Galați, Iaşi, Vaslui, and Vrancea counties (Fig. 4). This data is based on several studies and herbarium collections over time, starting in the 20th century.

Most of the populations were recorded outside protected areas. Eight populations were confirmed in the field, and another eight new populations were found (Table 1). In the locations analysed, the predominant EUNIS habitat is grassland: R1B Continental dry grassland (true steppe); and R36 Moist or wet mesotrophic to eutrophic pasture. The plant associations in which *I. brandzae* occurs are *Rorippo austriacae-Agropyretum repentis* (Timar 1947) R. Tx. 1950, *Taraxaco serotinae-Festucetum valesiacae* (Burduja et al. 1956, Răvăruț et al. 1956) Sârbu, Coldea et Chifu 1999, and *Jurineo arachnoidea-Stipetum lessingianae* (Dobrescu 1974) Chifu, Manzu et Zamfirescu 2006. The most frequent plant association where *I. brandzae* occurs is *Taraxaco serotinae-Festucetum valesiacae*.

The number of individuals / 100 m^2 varies from 5 to 25. In most of the locations investigated the current trend of the area occupied by *I. brandzae* populations from Moldova is stable (69%). In some populations, the population trend is decreasing (31%; Table 1). This decline suggests ongoing ecological stress, potentially due to changes in land use and overgrazing, climate variations, or other types of environmental degradation of habitats. The conservation status of the population varies from favourable (19%) to unfavourable (81%). Changes in the distribution pattern of habitat type areas ranged from unfavourable-inadequate (69%) to unfavourable-bad (13%). This information shows the high risk of continued degradation and underlines the vulnerability of grassland ecosystems in the region, thus requiring appropriate conservation and restoration measures to be established.

Distribution of the species in Muntenia

According to the literature (Supplementary Material 1), in the south-eastern region of Romania (Muntenia), *I. brandzae* was recorded in nine locations (six records from the literature and three new data) from Buzău County. At present, the species has been recorded in five locations in Buzău County (Table 1): Berca, Pâclele Mici, Pâclele Mari, Spătaru, and Tintești-Frasinu Forest (Fig. 5). At Spătaru and in Tintești-Frasinu Forest, *I. brandzae* was recorded in a deciduous forest-type habitat, in the *Ulmeto campestris-Fraxinetum holotrichae* association, characteristic of lowland areas with medium humidity. In contrast, at Pâclele Mici, *I. brandzae* is found in association *Taraxaco serotinae-Festucetum valesiacae*, and at Pâclele Mari, *I. brandzae* occurs in associations *Taraxaco serotinae-Festucetum valesiacae* and *Jurineo arachnoidea-Stipetum lessingianae*. The species also occurs in grasslands with *Syringa* and *Cotinus* shrubs, under the downy oak forest, at the edge of the grasslands, exactly where it meets the halophilous association *Nitrario schoberi-Artemisietum santonici*. At Berca, the species was found in the *Nitrario schoberi-Artemisietum santonici* association. The number of individuals per 100 m² is low in these locations, estimated at 5–10 individuals per 100 m² in Spătaru and 10–15 individuals per 100 m² in Berca, Pâclele Mici, Pâclele Mari, and Tintești-Frasinu Forest. The conservation status of the population and the changes in the habitat's surface distribution pattern are classified as unfavourable-inadequate (100%). The current trend in the area occupied by the population of *I. brandzae* is decreasing (60%; Pâclele Mici, Spătaru, and Tintești-Frasinu Forest) and stable (40%; Berca and Pâclele Mari).

Environmental conditions

Iris brandzae predominantly occurs on gentle slopes (3 to 10), with 52%, with southwest aspect (62%). In terms of elevation, the species is found at elevation from 100 to 200 m a.s.l. (Fig. 6).

The ANOVA results indicated a statistically significant difference in elevation between regions. Thus, following the application of the Tukey test, it was observed that the Moldova region had a significantly lower elevation compared to the Muntenia region. For slope and aspect, the ANOVA results indicated no statistically significant differences between regions (Fig. 7).

Regarding bioclimatic factors (Fig. 8), *I. brandzae* occurs in locations with mean annual precipitation from 517 to 569 mm/year and mean annual temperatures from 9.1 to 10.8 °C. For mean precipitation in June ranged from 74 to 91 mm, and mean temperatures in June ranged from 18.9 to 20.5 °C.

For mean annual precipitation (mm) – BIO12, the ANOVA showed significant differences between regions. Since $p < 0.05$, the null hypothesis of no differences between region means was rejected. When applying the Tukey test, it was shown that the Muntenia region has significantly lower values for BIO12 compared to the Moldova region. For mean annual temperature (°C) – BIO1, the ANOVA results indicated significant differences between regions. Since $p < 0.05$, the null hypothesis was rejected. Thus, the Tukey test showed that the Muntenia region has significantly higher values for BIO1 compared to the Moldova region. Regarding the mean annual temperature in June, the results of the ANOVA analysis showed that there are no statistically significant differences between regions. According to the Tukey test, no significant differences were identified between regions. For mean precipitation (mm) in June, the ANOVA results indicated significant differences between regions. According to the Tukey test, the Muntenia region had significantly lower mean precipitation in June compared to the Moldova region (Fig. 9).

In locations where *I. brandzae* occurs, soils ranged from slightly acidic to neutral, with moderate phosphorus concentrations and high potassium (Fig. 10).

Regarding soil pH, the ANOVA indicated no statistically significant differences between regions, as the p-value is higher than the significance threshold of 0.05. According to the Tukey test, no significant differences were found between regions. Regarding phosphorus, the ANOVA results indicated statistically significant differences between regions. According to the Tukey test, the Muntenia region recorded significantly lower phosphorus levels compared to the Moldova region. For potassium, the ANOVA results showed statistically significant differences between counties, with $p > 0.05$. Following the application of the Tukey test, it was confirmed that there are significant differences between Muntenia and Moldova regions. This is consistent with the ANOVA result (Fig. 11).

Populations status

Most of the populations of *I. brandzae* (62%) have a stable trend. This shows that the analysed species can maintain the area occupied under certain conditions. However, 38% of the populations are decreasing, which indicates habitat degradation or climatic variations.

The conservation status of *I. brandzae* populations and changes in the distribution pattern of habitat type areas showed that most populations are in an unfavourable-inadequate status (76%), and 10% of the populations are in an unfavourable-bad status. Only 14% of the analysed populations are in a favourable status.

Discussion

Environmental conditions

Iris brandzae is a forest-steppe grassland species, typical of open and semi-arid habitats. This study confirms that the species occurs in slightly acidic to neutral soils with varying potassium and phosphorus concentrations (Chirilă et al. 2024). Topographic analysis indicates that *I. brandzae* currently occurs at elevations ranging from 47 to 294 m a.s.l. without significantly extending the known range (Fick and Hijmans 2017) compared to previously recorded data (20–341 m a.s.l.).

From a phytocoenological perspective, *I. brandzae* occurs most frequently in the *Taraxaco serotinae-Festucetum valesiacae* association. This has been reported both in older studies (Dobrescu 1971; Mititelu 1973; Sîrbu 2003) and in more recent studies (Chirilă et al. 2024). Also, *I. brandzae* was recorded in other associations, such as *Ulmeto campestris-Fraxinetum holotrichae*, *Rorippo austriacae-Agropyretum repentis*, *Jurineo arachnoidea-Stipetum lessingianae,* and *Nitrario schoberi-Artemisietum* *santonici* Mititelu 1982, which were reported in the 2024 study (Chirilă et al. 2024). In the literature (Dobrescu 1970; Mititelu 1973; Mititelu et al. 1979–1980; Sanda et al. 1995–1996; Ștefan et al. 2009), *I. brandzae* was also reported in the associations *Poo trivialis-Alopecuretum pratensis* Regel 1925, *Astero pannonici-Puccinellietum distantis* Gehu et al. 1994, *Limonio gmelini-Artemisietum santonici* (Soó 1927) Țopa 1939, *Taraxaco serotinae-Bothriochloetum ischaemi* (Burduja et al. 1956) Sârbu, Coldea et Chifu 1999, but we did not report the presence of the species in these associations during the study period.

Human activities such as overgrazing, conversion of grasslands into arable land, and roads for agricultural exploitation negatively affect the *I. brandzae* species and its habitat. Thus, the increase in the number of animals on the grasslands causes soil compaction and natural vegetation degradation. Soil compaction can prevent root growth and reduce the ability of the analysed plant to obtain essential nutrients. In addition, overgrazing can reduce biodiversity, affecting other species that contribute to the ecological balance of the habitat. The conversion of grasslands to agricultural land means the habitat loss of *I. brandzae*. This conversion not only destroys established plants but also changes soil structure, which affects habitat. Soil erosion and chemical pollution caused by construction activities and road traffic can also pose a threat to *I. brandzae*. There are also issues related to road construction and use as an agricultural practice, which can fragment habitats and act as a barrier to the spread or natural reproduction of *I. brandzae*. Another threat to *I. brandzae* is the collection of individuals, which can further reduce the size of already affected populations.

Updated distribution

Through field investigations carried out, 21 populations were found, 11 of which were recorded for the first time. In comparison, the literature has recorded 61 populations (Supplementary Material 1). The current trend of the area occupied by populations is stable. However, the conservation status of the populations is classified as unfavourable-inadequate mainly due to anthropogenic impact.

The early information on the distribution of the *I. brandzae* species in Romania includes data collected from herbaria and scientific articles, in the period 1895–1960. Thus, the first mentions of the species dates back to 1895, from Cetățuia (Iași County). Later, in 1897–1916, the species was also recorded in some grasslands in Iași County, by Emil Țopa and Constantin Petrescu (Supplementary Material 1). In the first half of the 20th century, the species was also recorded in other locations in the regions of Moldova and Muntenia. Starting from the second half of the 20th century (1960–1990), an expansion of the species *I. brandzae* was recorded, showing a wider distribution compared to the data in the literature. The largest number of investigations was carried out by Constantin Dobrescu, conducting studies during the period 1951–1974, in the Moldavian region. In the last two decades, fewer populations of *I. brandzae* have been confirmed, which means a narrowing of the distribution area. Recent information (2018–2024) indicates that the species is present in some locations in Romania, but has a fragmented distribution. Most confirmations (80%) come from Iași County.

The biogeographic, ecological and evolutionary context of the species

Iris brandzae belongs to the subgenus *Limniris* (Tausch) Spach series *Spuriae* (Diels) Lawrence (Troitskyi et al. 2021). Data from the literature (Tillie et al. 2000; Wilson 2004, 2006, 2009; Wheeler and Wilson 2014) has proven that the subgenus is polyphyletic. However, the species from series *Spuriae* analyzed in these papers appeared as a monophyletic group, though no one approached the entirety of the species within it. The number of species in the series varies between 12 and 17, the difference resulting from the different statuses accorded by different authors to some taxa - species, subspecies or varieties (Lenz and Day 1963; Meusel et al. 1965; Grubov 1977; Rodionenko 1987; Mathew 1989; Species Group of the British Iris Society 1997; Wilson 2006; Euro+Med 2025; POWO 2025).

The analysis of the available literature for chorological and ecological data (Fedtschenko 1935; Wendelbo and Mathew 1975; Baytop and Mathew 1984 Mathew 1984, 1989; Zhao et al. 2000; Czerepanov 2007; Alexeeva 2008; Pyak et al. 2008; Ali and Mathew 2011; Akhter et al. 2012; Güner 2012; Pils 2022; Euro+Med 2025; POWO 2025) revealed to us that from a biogeographic point of view most of the species in the series *Spuriae* are linked to the forest-steppe and steppe biomes in the western and central Palearctic. Only a few taxa are located in the nemoral and subtropical mediterranean forestry areas in the westernmost Atlantic part of the Palearctic. Still, these are always situated in ecosystems that are similar or resemble the ones populated by their relatives in the steppe or forest-steppe areas. It also became obvious that, except for two taxa, all the species in the series *Spuriae* fall into two ecologically very contrasting categories.

One group of hygrophilous-slightly halophilous species is linked to marshy, slightly saline meadows: *I. spuria* L., 2n = 22, western Palearctic (in the nemoral zone, with a very disjunct range); *I. spuria* subsp. *carthaliniae* (Fomin) B.Mathew, 2n = 44, Caucasian steppes and forest-steppes; *I. spuria* subsp. *musulmanica* (Fomin) Takhtadjan, 2n = 44, Iranic steppes and forest-steppes; *I. halophila* Pall. (with var. *sogdiana* (Bunge) Skills), 2n = 44, western and central Palearctic steppe and forest-steppe; *I. notha* Bieberstein, 2n = 38, 42, 44, Ciscaucasian forest-steppe; *I. pseudonotha* Galushko, 2n=unknown, eastern Transcaucasian forest-steppe; *I. orientalis* Mill., 2n = 39, 40, Anatolian forest-steppe with littoral intrazonal populations in the Aegean area; *I. crocea* Jacquemont, 2n = 40, forest-steppe like areas in the western Himalayas; *I. xanthospuria* B. Mathew et T. Baytop, 2n = 40, Anatolian southern forest-steppe, and *I. reichenbachiana* Klatt, 2n=36, westernmost Palearctic, intrazonal littoral ecosystems.

A second group of species is adapted to xeric and mesoxeric grasslands: *I. pontica* Zapal, 2n = 72, with disjunct areas in the forest-steppe and steppe from the Pontic, northern Caucasian, and Transylvanian areas; *I. sintenisii* Janka, 2n = 16, 32 the forest-steppe areas in the Balkans, north-western Anatolia, and xeric intrazonal grasslands from the nemoral area in southern Italy; *I. demetrii* Achv. et Mirzoeva, 2n = 38, Transcaucasian forest-steppe; *I. haussknechtii* Bornm., 2n = 18, northern Anatolian forest-steppe; *I. ludwigii* Maxim., 2n = 38, western Altai Mountains steppe and forest-steppe and *I. foetidissima* L., 2n = 40, westernmost Palearctic, subtropical forest-steppe areas in northwestern Africa and intrazonal mesoxeric chalcophile-saxicolous ecosystems in westernmost Europe.

The species *I. graminea* L., 2n = 34 and *I. brandzae* Prodan, 2n = 20, stand apart from these two distinct ecological groups of species. The first one populates the mesic, rarely mesoxeric grasslands from the nemoral and nemoral forest-steppe areas in the western Palearctic. Still, the habitats it occupies are always a mix of meadows and grassy woodlands with dense patches of thickets and are very similar to the forest-steppe landscape. *Iris brandzae* Prodan is a species that occurs only in the nemoral forest-steppe west and north-west of the Black Sea (southeastern Romania, southern Moldavia, southwestern Ukraine), and is the single species in the series *Spuriae* which manifest a large ecological adaptability in a mixed landscape: it can occur in xeric and mesoxeric grasslands, mesic and mesohygrophile (sometimes slightly halophile) grasslands, open forests and also mesosaline grasslands (Chirilă et al. 2024). Regarding *I. colchica* Kem.-Nath., its taxonomic status is still unclear and it replaces *I. graminea* in the Caucasus, in the nemoral forests and has an absolutely identical ecology.

This contrasts the narrow ecological niches of all the other species in the series *Spuriae* and raises interesting questions about the place of this species in this group in an evolutionary context. We mentioned above the chromosome number of all species after the Lenz and Day (1963), Mathews (1989), and Species Group of the British Iris Society (1997) to reveal the extreme heterogeneity concerning this aspect in the series *Spuriae*. Moreover, the morphology of the chromosomes in various species is highly variable (Lenz and Day 1963). This makes clear that this series' main driving evolutionary mechanisms are related to complex chromosomal mutations/evolution, a relatively rare case for a monophyletic species group. This makes series *Spuriae* and its ecologically peculiar member *I. brandzae* an ongoing interesting research subject for the future.

Conclusions

Iris brandzae has a more restricted distribution currently, compared to data in the literature, and can be considered a rare species in Romania. This species shows great ecological adaptability, being found in mesoxeric, mesic and mesohygrophile, slightly halophile grasslands, and open forests.

In Moldova, *I. brandzae* occurs in steppe and forest-steppe grasslands habitats, in the *Taraxaco serotinae-Festucetum valesiacae* and *Jurineo arachnoidea-Stipetum lessingianae* associations, and occasionally in meso-xerophilous communities of *Rorippo austriacae-Agropyretum repentis* association. In Muntenia, the species also occurs in forest-steppe areas, but in azonal vegetation communities, from alluvial deciduous forests, slightly halophilic grasslands to dry grasslands, in the *Ulmeto campestris-Fraxinetum holotrichae*, *Nitrario schoberi-Artemisietum santonici*, *Taraxaco serotinae-Festucetum valesiacae* and *Jurineo arachnoidea-Stipetum lessingianae* associations.

The influence of climate and soil conditions is reflected in the state of habitats and plant communities in the Moldova and Muntenia regions. The annual mean temperatures and precipitation varied in the locations analysed, with different nutrient concentrations and soil pH. The populations of *I. brandzae* in the analysed locations have an unfavourable conservation status with habitat degradation tendencies, which indicates that urgent restoration and protection measures are needed.

Declarations

Funding

No funding was received for conducting this study.

Conflict of Interest

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

Ethical approval

No approval of research ethics committees was required to accomplish the goals of this study

Informed consent

Author contributions

Simona Dumitrița Chirilă: Conceptualization, Methodology, Investigation, Software, Data curation, Writing- Original draft preparation. **Ciprian Claudiu Mânzu**: Data curation, Visualization, Investigation, Writing - review and editing, Supervision. **Alexandru Sabin Bădărău**: Data curation, Visualization, Investigation, Writing - review and editing, Supervision. **Culiță Sîrbu**: Data curation, Visualization, Investigation, Writing - review and editing, Supervision.

Mátis Attila: Data curation, Visualization, Investigation, Writing - review and editing, Supervision.

Data availability

The data that support the findings of this study are included within this paper and its supplementary information. Any other data are available from the corresponding author upon request.

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Table

Table 1 is available in the Supplementary Files section.

Figures

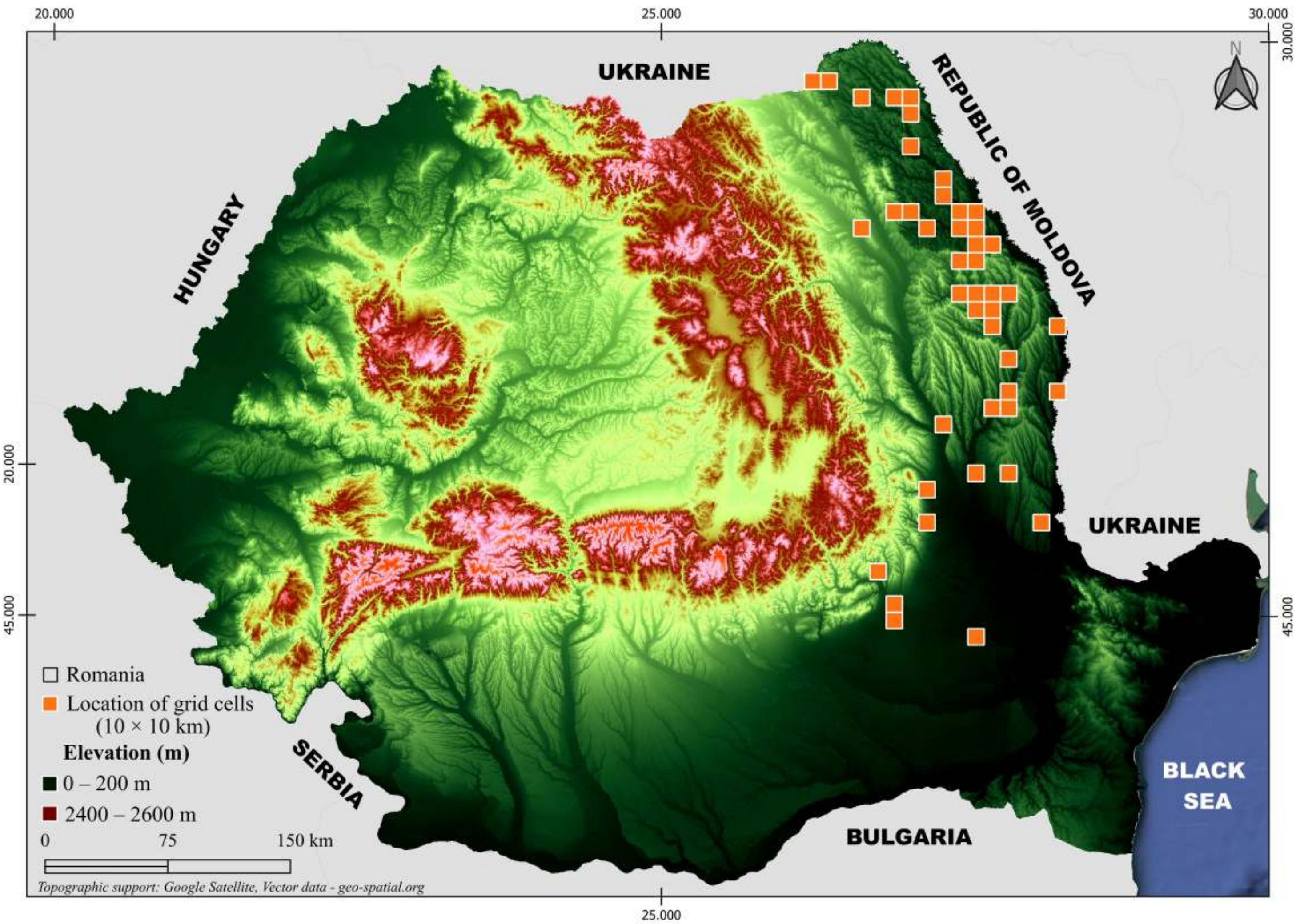

Figure 1

Map of the study area and the location of grid cells (10 × 10 km) with the locations where *I. brandzae* was recorded (Map: Simona Chirilă)

Figure 2

Iris brandzae. a) habitat, b, c) flowers (Photographs: Simona Chirilă, Iași, Aprilie 2024)

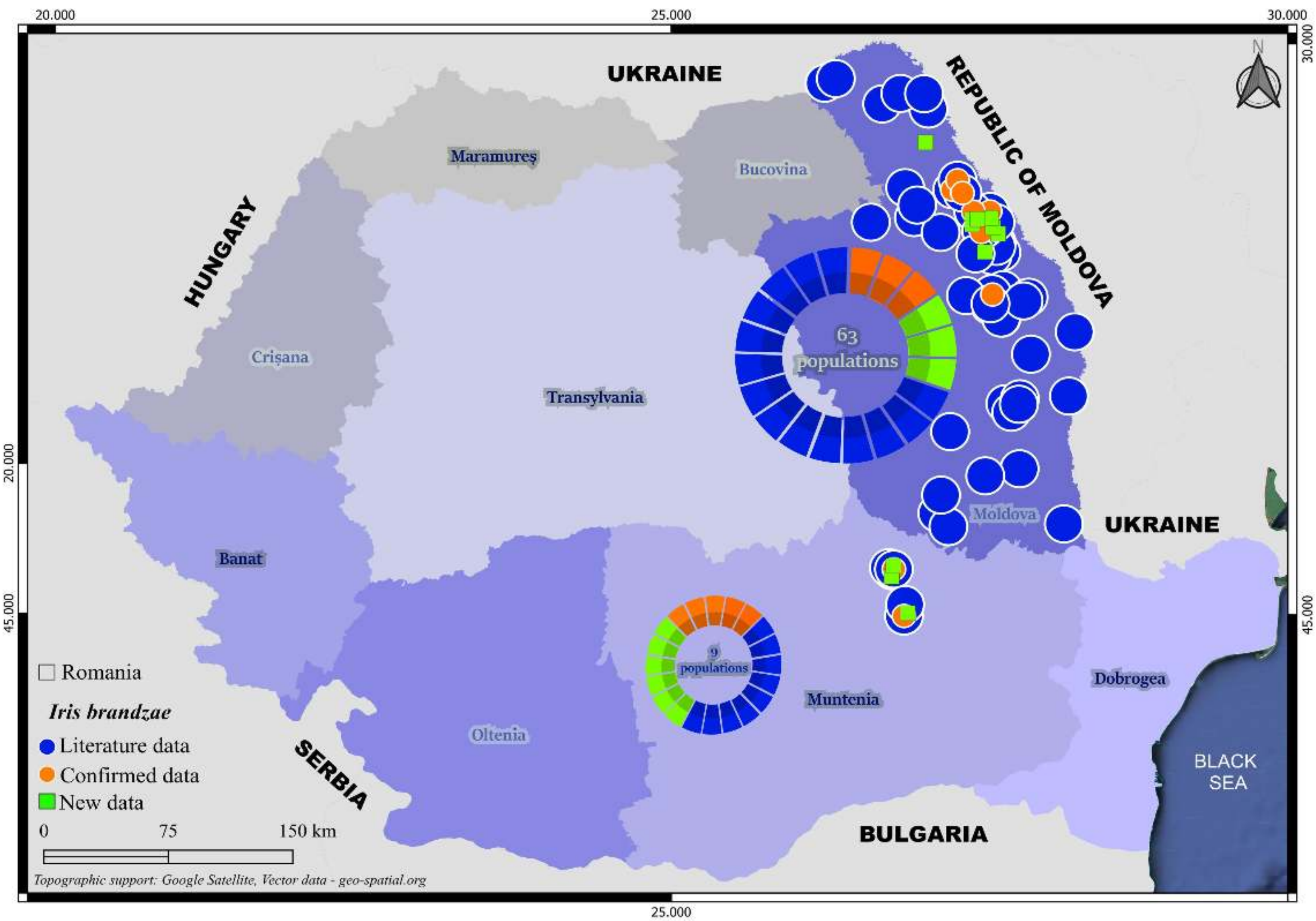

Figure 3

Distribution of *Iris brandzae* in Romania (Map: Simona Chirilă)

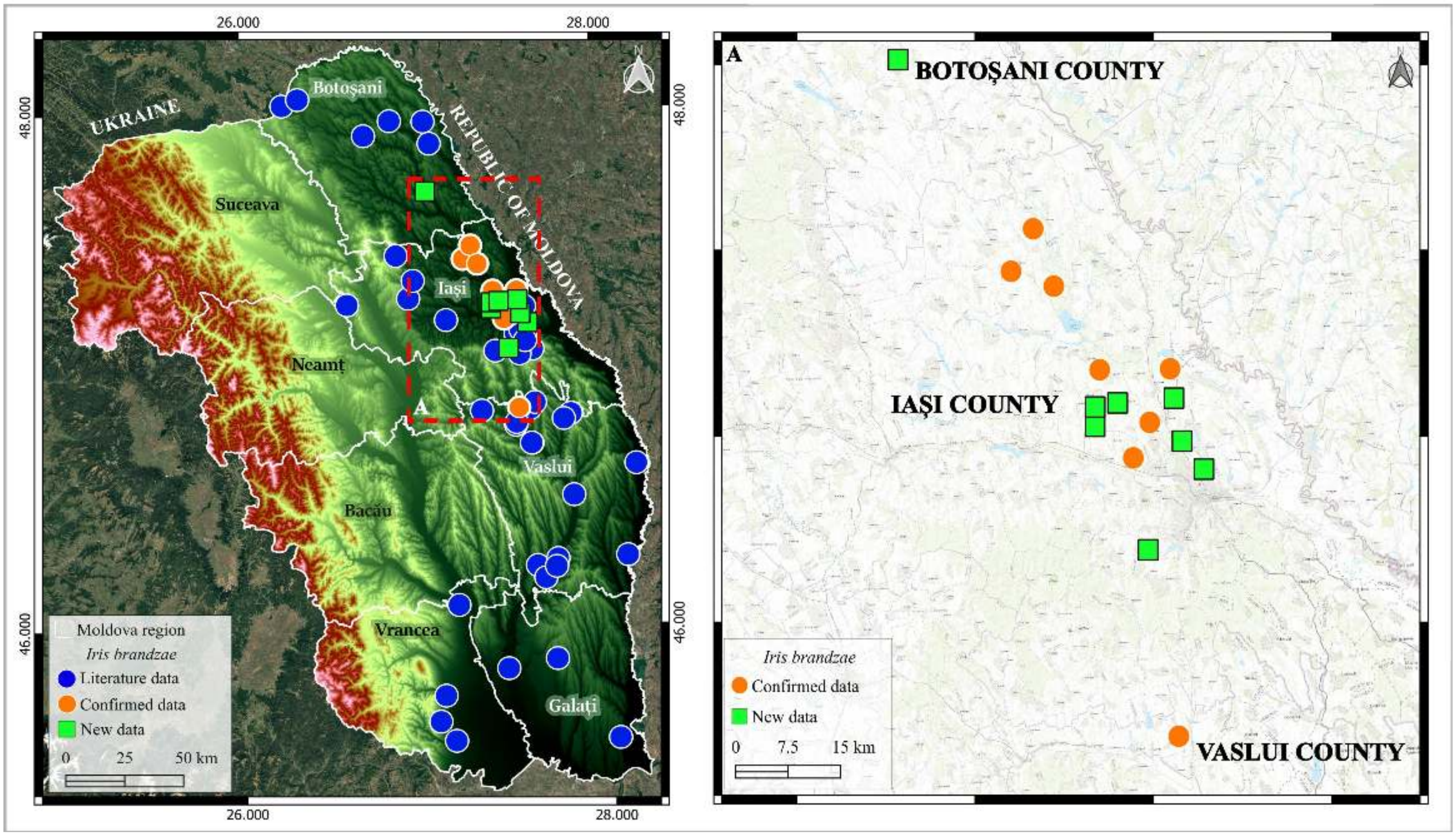

Figure 4

The distribution of *I. brandzae* in Moldova

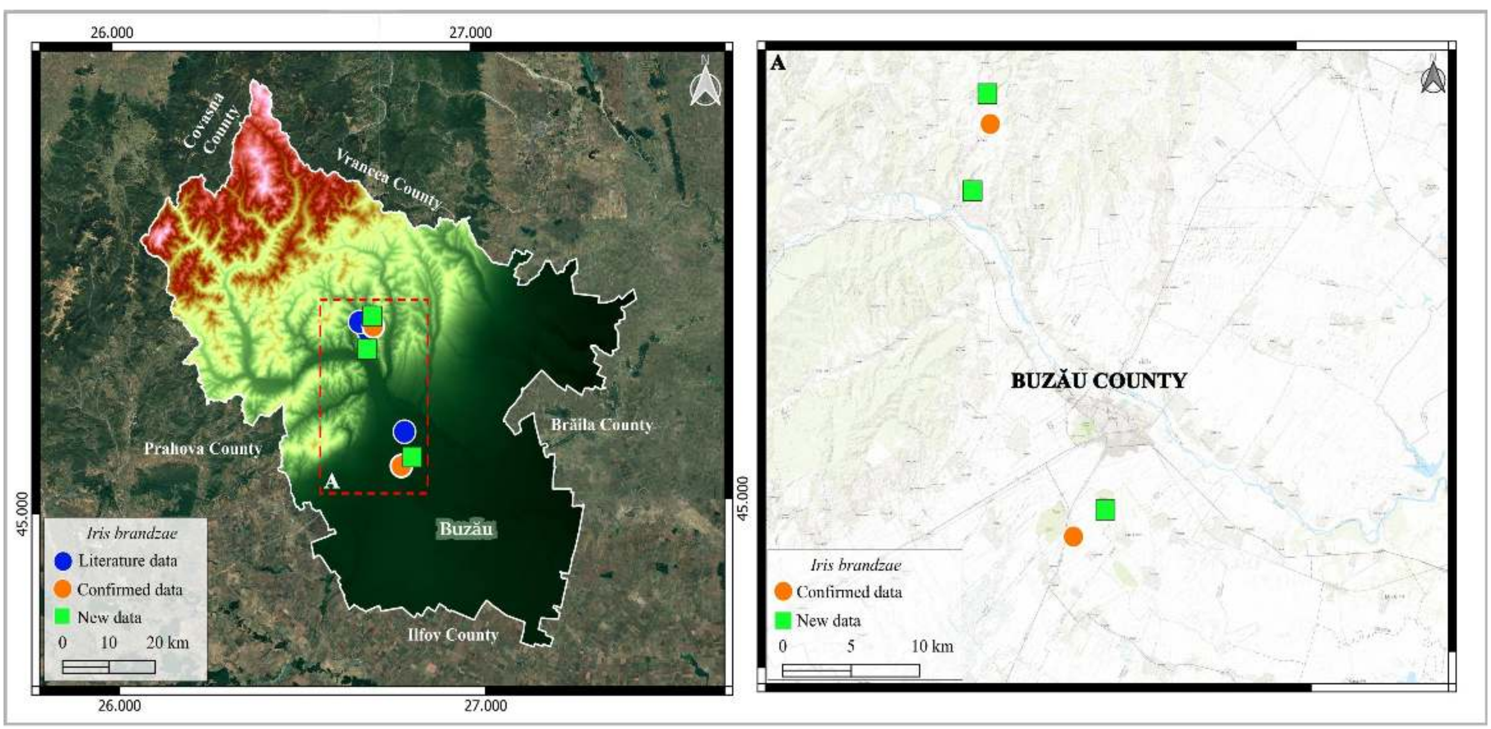

Figure 5

The distribution of *I. brandzae* species in Muntenia

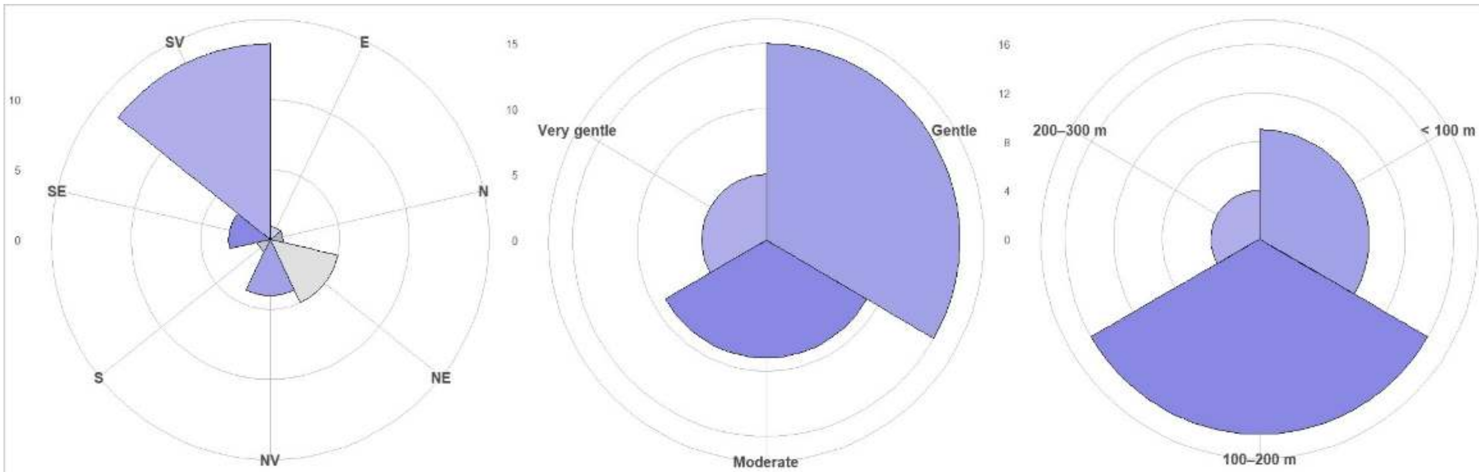

Figure 6

Topographic preferences of *Iris brandzae*

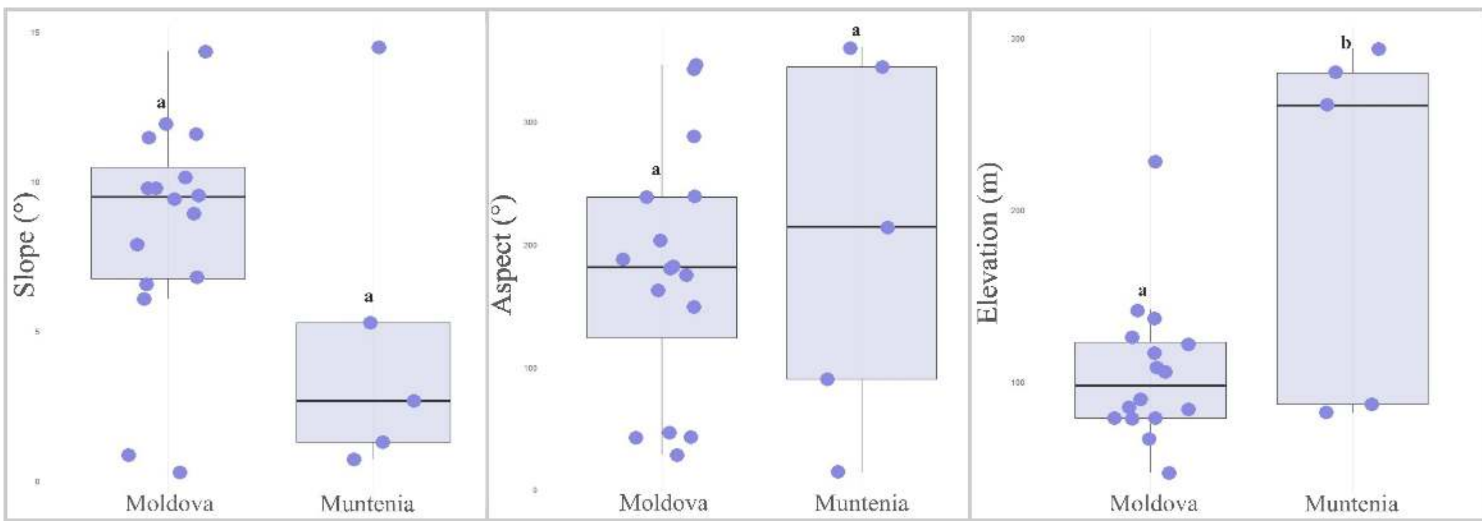

Figure 7

Differences between counties for topographic factors

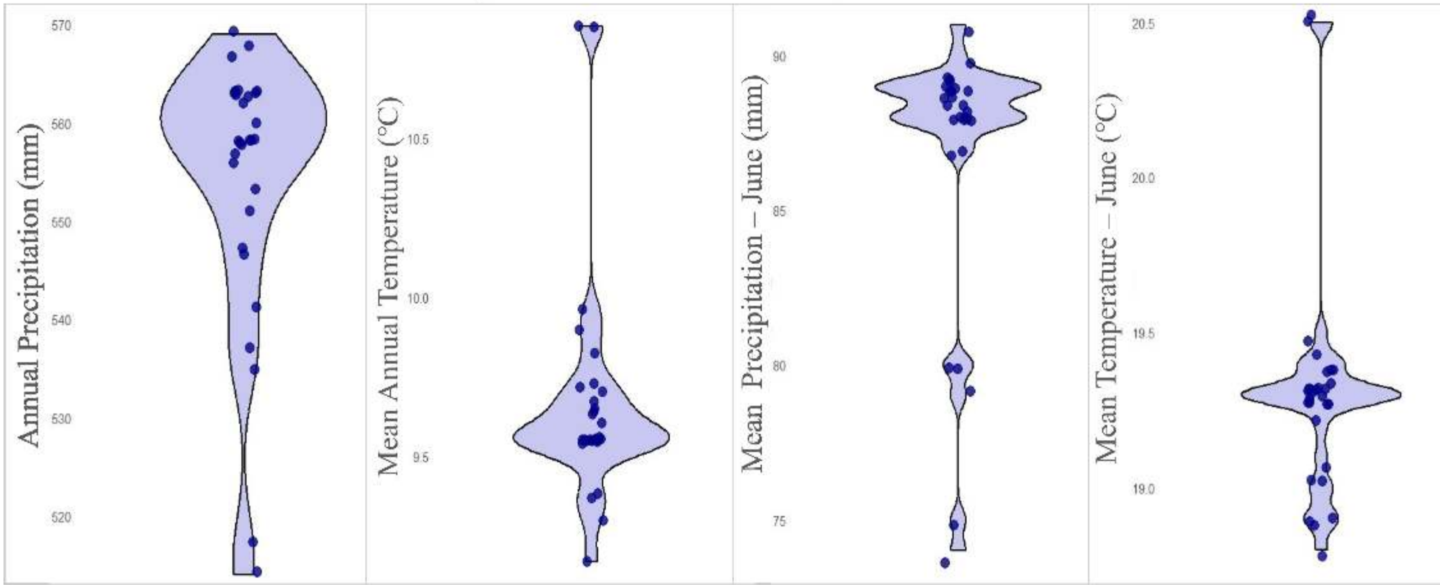

Figure 8

Bioclimatic preferences of *Iris brandzae*

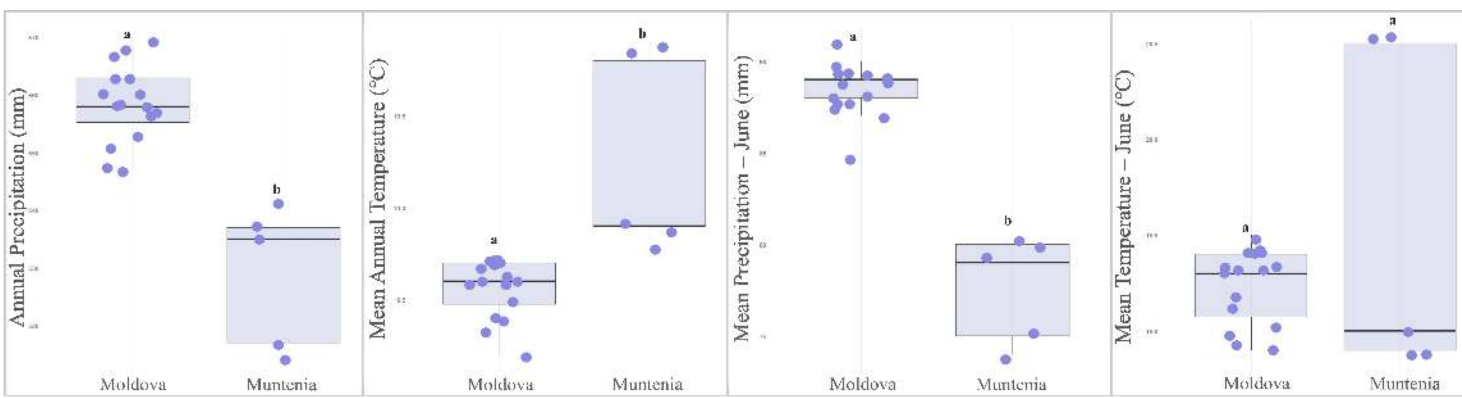

Figure 9

Differences between counties for bioclimatic factors

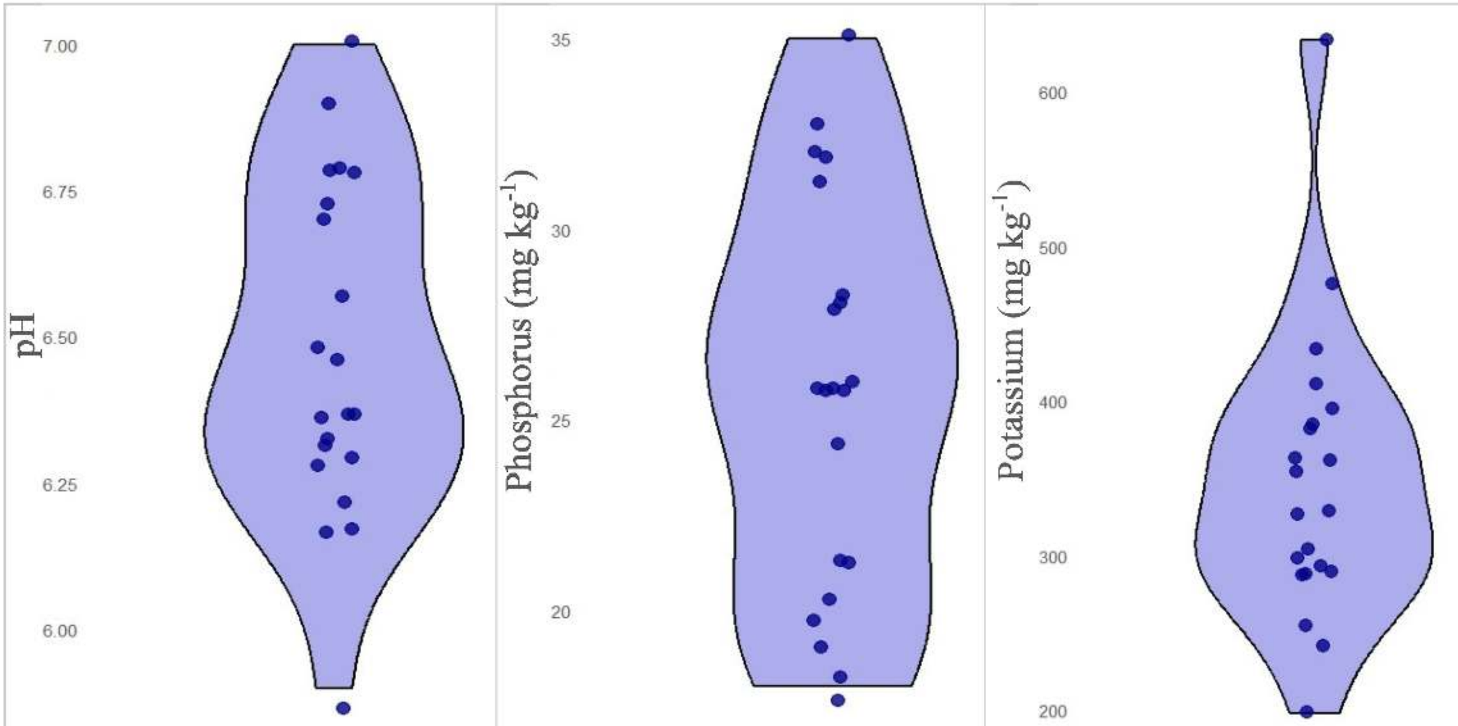

Figure 10

Soil chemical preferences of *Iris brandzae*

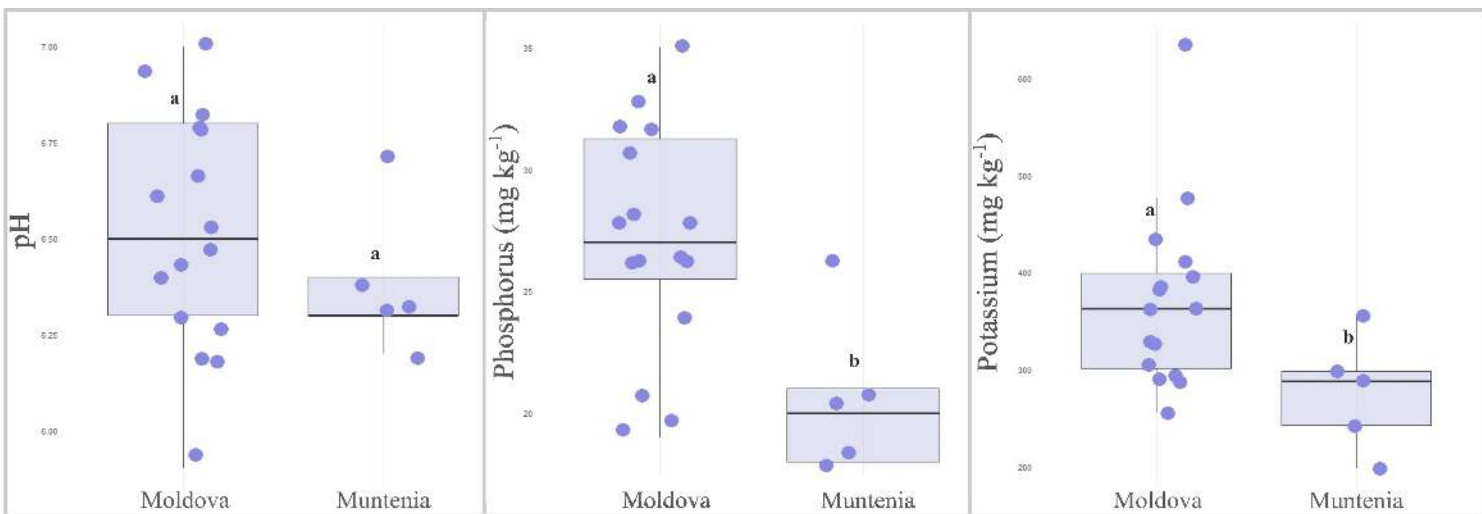

Figure 11

Differences between counties for soil chemical factors

Supplementary Files

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- ReferencessupplementaryIrisbrandzaeRevision1.docx
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