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Predictive distribution modelling of newly described endemic Dinaric species *Cirsium greimleri* Bureš

Prediktivno modeliranje distribucije novoopisane endemične dinarske vrste *Cirsium greimleri* Bureš

Ajla Berberović[1,2,*], Fatima Pustahija[2,3], Samir Đug[2], Sonja Šiljak-Yakovlev[2,4], Edina Muratović[2]

1 I The National Museum of Bosnia and Herzegovina, Zmaja od Bosne 3, 71000 Sarajevo, Bosnia and Herzegovina
2 Laboratory for Research and Protection of Endemic Resources, Department of Biology, Faculty of Sciences, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
3 Faculty of Forestry, University of Sarajevo, Zagrebačka 20, 71000 Sarajevo, Bosnia and Herzegovina
4 Ecologie, Systématique et Evolution, Université Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, bâtiment 680–12, route 128, 91190 Gif-sur-Yvette, France

ABSTRACT

Cirsium (thistle) is one of the most taxonomically demanding genera within the Compositae. These taxonomic difficulties are hypothesized to result from limited morphological differentiation, incipient speciation and/or hybridisation among taxa, and misinterpretations of faded and incomplete herbarium specimens. According to the latest data, the Dinarides and the Eastern Alps are inhabited by the endemic, diploid, newly described species *Cirsium greimleri*, which often occurs in sympatry with *C. rivulare* and *C. erisithales*. The area of the related, vicarious, tetraploid, and endemic species *C. waldsteinii* is limited to the southeastern Carpathians. Considering that the new knowledge refutes the existence of *C. waldsteinii* in Bosnia and Herzegovina, it was necessary to review all *C. waldsteinii* data discovered so far in Bosnia and Herzegovina. The results obtained from the distribution of the species, the predictive modelling of its ecological niche, and the analysis of the genome size confirmed the existence of the species *C. greimleri* in Bosnia and Herzegovina and its potential hybridisation. This study indicates the need for further research into the sympatry of *C. greimleri* and its relatives, as well as the essential taxonomic revision of this complex.

Keywords: Endemic, Greimler's thistle, predictive modelling, taxonomic complexities

INTRODUCTION – *Uvod*

The genus *Cirsium* Mill., known as thistle, is one of the largest genera in Compositae, containing approximately 250 taxa (Segarra-Moragues et al., 2007; Ackerfield et al., 2020) or roughly 400–450 species (Michálková et al., 2018; Bureš et al., 2018) widely distributed across the Northern Hemisphere. *Cirsium* species are either perennial or biennial, with good adaptability to various environments such as wetlands, meadows, steppes and even desert landscapes (Charadze, 1963; Davis & Paris, 1975; Petrak, 1979; Keil, 2006; Kadereit & Jeffrey, 2007; Segarra-Moragues et al., 2007; Yildiz et al., 2016). During the 17th and 18th centuries, early references to the

* Corresponding author: Ajla Berberović, berberovica3@gmail.com

genus *Cirsium* were often inconsistent, and diverse taxa were understood as representatives of different genera (Tournefort, 1694; Linnaeus, 1753; Miller, 1754; Adanson, 1763; Necker, 1790; Sweet, 1825; Cassini, 1826; Candolle & Duby, 1828; Lessing, 1832; Candolle, 1837; Koch, 1851; Fourreau, 1869; Gray, 1874; Greene, 1892). According to the Flora Europaea (Werner, 1996), *Cirsium* species belong into three *Cirsium* sections: *Eriolepis*, *Cirsium* and *Cephalonoplos*.

It was considered that *Cirsium waldsteinii* Rouy is naturally present in Eastern Alps, Carpathians and Dinarides, where its presence in the western Balkans region is confirmed by the local floras (Domac, 1967; Josifović, 1970; Bjelčić et al., 1983). However, botanists have encountered challenges in identifying this species due to discrepancies in its descriptions across different Florae. Molecular investigations have revealed taxonomic complexities within the genus *Cirsium* due to the frequent hybridisation of its representatives (Bureš et al., 2004). As a result of a comprehensive study by Bureš et al. (2018) it was described that endemic diploid species *C. greimleri* Bureš (Greimler's thistle), inhabited the Dinaric and Eastern Alpine regions. The range of its closely relative tetraploid vicar species *C. waldsteinii* is limited to the southeastern Carpathians. The new findings by these authors unreservedly refute the existence of the species *C. waldsteinii* in the region of the Dinaric and Eastern Alps.

This issue presents a challenge that is effectively solved through advanced modelling techniques. Species Distribution Models (SDMs) are used to estimate a species' current and future geographic distribution and environmental niche. Today, MaxEnt is the most commonly used model and has been used in studies assessing the effects of climate change on species distribution, species richness, invasive species, endemism hotspots, as well as in estimating the range and protection status of rare species (Cunningham et al., 2009; Wan et al., 2021; Qazi et al., 2022). Additionally, QGIS is widely used in scientific research across various disciplines due to its capabilities for handling spatial data and conducting geospatial analysis. This method is helpful for visualising urban development (Zaki et al., 2022), tree species mapping (Choudhury et al., 2020), forest inventory (Pica et al., 2022), tracking outbreaks, and analysing the spread of infectious diseases (da Silva et al., 2021), but also for asses distribution patterns and predictive models for plant species (Cursach et al., 2020).

The objectives of this study were to contribute to the revision of the current species count within the genus *Cirsium* in Bosnia and Herzegovina (B&H) and to detect potential localities of allopatric and sympatric populations of newly described *C. greimleri* using predictive modelling methodology.

MATERIALS AND METHODS – *Materijal i metode*

Input data

Herbarium collections data from the National Museum of Bosnia and Herzegovina (SARA), relevant literature sources (Kušan, 1956; Bjelčić et al., 1983; Mišić, 1984; Barudanović, 2003; Đug, 2004) and field investigations were used for the estimation of the potential distribution of *C. greimleri* (ex *C. waldsteinii*) in Bosnia and Herzegovina (Supplement Table 1). All references for the species *C. waldsteinii* in Bosnia and Herzegovina were treated as *C. greimleri* in this study. Additionally, available distribution data of *C. rivulare* and *C. erisithales*, as *C. greimleri* frequent hybridization partners (Heimerl, 1884; Fritsch, 1906; Khek, 1908; Benz, 1922; Leute and Zeitler, 1967; Bureš et al., 2018), were used to detect sympatric populations.

Unfortunately, herbarium and literature data regarding the geographic locations of detected populations in B&H for the investigated species were often incomplete. Consequently, reliability statuses were assigned as follows: (1) unreliable – indicating the absence of a narrower locality and/or altitude, and (2) reliable – indicating the presence of a narrower locality and/or altitude. All obtained initial parameters for the target species were utilized in designing the distribution map and predictive modelling of ecological niches.

The areal map and predictive ecological niches

The Desktop version of QGIS Hannover 3.16.16 was used to create the area map. Climate data were extracted from the WorldClim 2.1 database, which includes 19 bioclimatic variables in the form of raster layers at a resolution of 30 arc-seconds (Fick & Hijmans, 2017; https://www.worldclim.org). This global database contains data obtained through the interpolation of temperature and precipitation values from over 3.500 national meteorological stations included in the global network. The data were derived from a 30-year average (1970-2000) and are widely used (Hijmans et al., 2005; Fick & Hijmans, 2017).

MaxEnt software functions by predicting the potential spatial distribution based on existing occurrence data (Phillips et al., 2006; Dai et al., 2022). An ecological niche model was created using the maximum entropy met-

Table 1. Investigated *Cirsium greimleri* populations

Tabela 1. Istraživane populacije Cirsium greimleri

Locality	GPS-N	GPS-E	Altitude (m)	Exposition
Mt. Igman, RavnaVala (rainforest reserve)	43°44'22"	18°16'23"	1257	N
Mt. Jahorina, Bistrica-Paljevina	43°44'51"	18°33'48"	1454	N-NW
Mt. Jahorina, Bistrica-Mušak	43°44'49"	18°33'55"	1460	
Mt. Jahorina, Grahov Dol	43°44'35"	18°34'00"	1478	

hod in MaxEnt 3.4.4. software (Phillips et al., 2006), and the results were projected into geographic space, producing a predictive map. On this map, pixel values range from 0 to 1, with cooler (blue) to warmer (red) colors indicating habitat favorability. Values closer to 1 signify more favorable habitats, while values closer to 0 indicate less favorable habitats for *C. greimleri*.

In the graphic representation of model accuracy, the x-axis represents specificity, ranging from 0 to 1, indicating the proportion of locations correctly identified as species-absent. The y-axis shows sensitivity (1 – omission rate), also on a scale from 0 to 1, reflecting the proportion of locations where the species' presence was accurately predicted.

Fieldwork

According to the created preliminary distribution map, four possible localities of the newly described diploid species *C. greimleri* were selected (Table 1): the Ravna Vala microhabitat with an allopatric population, and three sympatric populations on Jahorina Mt. (with *C. erisithales* and *C. rivulare*). The field investigations were carried out between May and September of 2022. and 2023.

Genome size

Three to six individuals per population were sampled for genome size estimation (Table 1). The nuclear genome size was determined according to Bourge et al. (2018). Fresh leaves of the internal standard species, *Petunia hybrida* (Hort.) PxPc6 (2C=2.85 pg) (Marie & Brown, 1993), were shredded with *C. greimleri* dried leaves in a sterile plastic Petri dish with 600 µL of cold GiF buffer for nuclear isolation. The GiF buffer included 45 mM MgCl2, 30 mM sodium citrate, 60 mM MOPS acid pH 7, 0.1% PVP 10 000, 10 mM sodium metabisulfite (Na2S2O5), RNA-se (2.5 U/mL), and 0.1% Triton X-100. To remove cell and tissue debris, the nuclei suspension was filtered through a nylon filter (r=30 or r=50 µm). Nuclei were stained with 100 µg/ml propidium iodide (PI) for at least 5 minutes at 4°C.

To obtain mean genome size values, approximately 5.000 stained nuclei were analysed for each sample using a CytoFLEX S cytometer with 561 nm excitation, 26 mW, and emission through a 610/20 nm band-pass filter (Beckman Coulter-Life Science, United States). Samples for each population had three to six separately measured individuals, each with replication. CytExpert 2.3 software was used for histogram analysis. The mean value (± st. dev.) of 2C DNA was calculated by comparing the linear relationship between the fluorescent signals of the stained nuclei of the examined species and the internal standard. Genome size values are expressed in absolute units of 2C (pg), monoploid values of 1Cx (pg and in Mbp), recalculated using the conversion factor 1 pg DNA = 978 Mbp (Doležel et al., 2003).

RESULTS AND DISCUSSION – *Rezultati i diskusija*

Bureš et al. (2018) state that *Cirsium waldsteinii* is widespread in the Eastern and Southern Carpathians, with no distribution in the Eastern Alps and Dinaric region. Also, they suggest that *C. greimleri* is distributed across southeastern Austria, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, and Serbia. Therefore, all data published for Bosnia and Herzegovina (Bjelčić et al., 1983; Kušan, 1956; Mišić, 1984; Barudanović, 2003; Đug, 2004) and SARA's specimens were treated as *C. greimleri*. According to the available literature data, SARA specimens and field work a total of 40 localities of the species *C. greimleri* were detected in B&H (Supplement Table 1).

The distribution map and habitat suitability of *C. greimleri* are presented in Figure 1. Based on the conducted software analysis, logistic output data were obtained.

The formation of the given distribution was primarily influenced by the Mean Temperature of the Wettest Quarter (Bio8 variable; 51.8%). The following variables include the Mean Temperature of the Driest Quarter (Bio9; 30.2%) and Precipitation of the Wettest Quarter (Bio16; 9.4%). According to our findings, habitats for the potential spread of *C. greimleri* in B&H engage areas from the high mountains to the subalpine belt. This is in agreement with Bureš et al. (2018) conclusions about *C. greimleri* habitat ecology. These authors state that *C. greimleri* occupies ecological niches within high-mountain and subalpine ecosystems with a preference for open high-mountain and subalpine park-like forest/woodlands, as well as moist slopes of forested valleys and shaded forest roads, at altitudes ranging from 800 to 2.000 m above sea level.

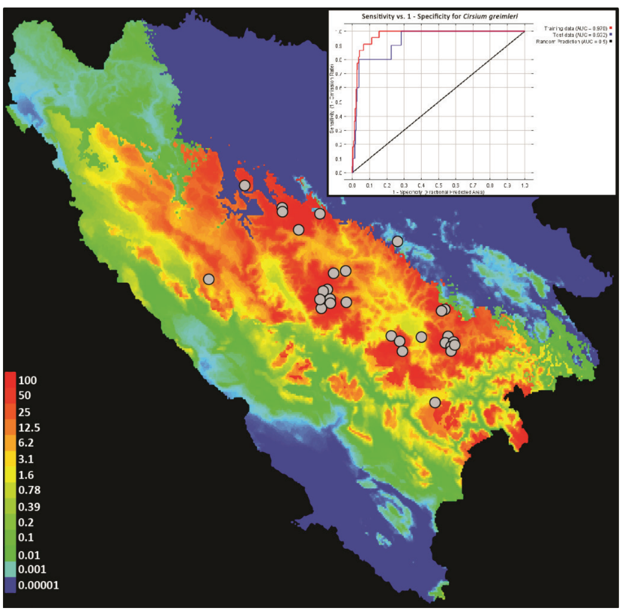

Figure 1. Recorded localities for *C. greimleri* in B&H (see Supplement Table 1) overlaid by SDM (Species Distribution Model)

Karta 1. Evidentirane populacije C. greimleri *u B&H (vidjeti Dodatnu tabelu 1), predstavljene kroz distributivni model vrste*

Detected *Cirsium greimleri* populations (Supplement Table 1) with obtained habitat suitability using the MaxEnt software, including a graphic representation of the model accuracy, are presented in Figure 1. Some of the localities are not visible on the map of this scale due to their proximity. In the graphic representation of the model accuracy (Figure 1), the ROC curve shows the relationship between the true positive rate (sensitivity) and the false positive rate (specificity) for different model cut-offs, using them as evaluation criteria. Small differences in omission rates and their standard deviations, which indicate a good match of the model with literature data were visible. An AUC value of 0.970 means the model is highly accurate and capable of distinguishing between regions with the presence of species with those without them. Additionally, the graph shows the omission gap between the modelled and observed values.

The obtained potential distribution model of *C. greimleri* and its predictive ecological niches (Figure 1) confirmed that the Alpine biogeographical region of the B&H is an optimal habitat and potential zone for further research and monitoring to better understand the spread of the species.

The case of selected allopatric and sympatric Cirsium greimleri populations

Figure 2 presents localities for the three target species according to the Supplement Table 1.

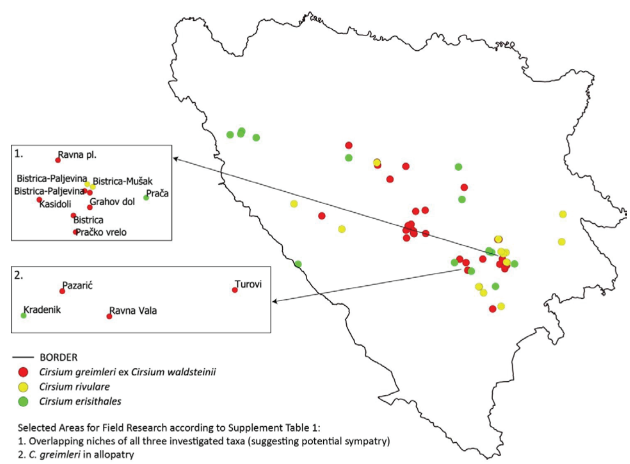

Figure 2. Distribution of the investigated *Cirsium* species in Bosnia and Herzegovina

Karta 2. Distribucija istraživanih Cirsium *vrsta na području Bosne i Hercegovine*

C. greimleri in Bosnia and Herzegovina has been recorded at 40, *C. rivulare* at 13, and *C. erisithales* at 15 sites (Figure 2). *C. greimleri* preferences for moist slopes of forested valleys and shaded forest roads (Bureš et al. 2018) occasionally overlap with *C. rivulare* and *C. erisithales* habitats ranging from moderate elevation foothills to mid-level mountainous areas with preferences for moist environments (Gajić, 1975; Bjelčić et al., 1983). Niche-overlapping that includes preferences for high moisture content in the air and soil in habitats such as forest hydrophilous fringe communities might explain why these species are frequently found in sympatric populations.

According to the Figure 2, two areas were selected: one with an allopatric population of *C. greimleri*, and the other with sympatric populations of all three analysed species. Field research confirmed the presence of the

C. greimleri allopatric population at the Ravna Vala site (Table 1, Figure 2). The individuals from the population Ravna Vala exhibited uniform leaf and flower morphology. In three Jahorina Mt. localities *C. greimleri* individuals showed significantly different morphological features compared to the Bureš et al. (2018) diagnosis. Namely, individuals with very variable leaf blade shapes: from narrower and less deeply lobed to deeply lobed median cauline leaves, were discovered in these populations (Figure 3).

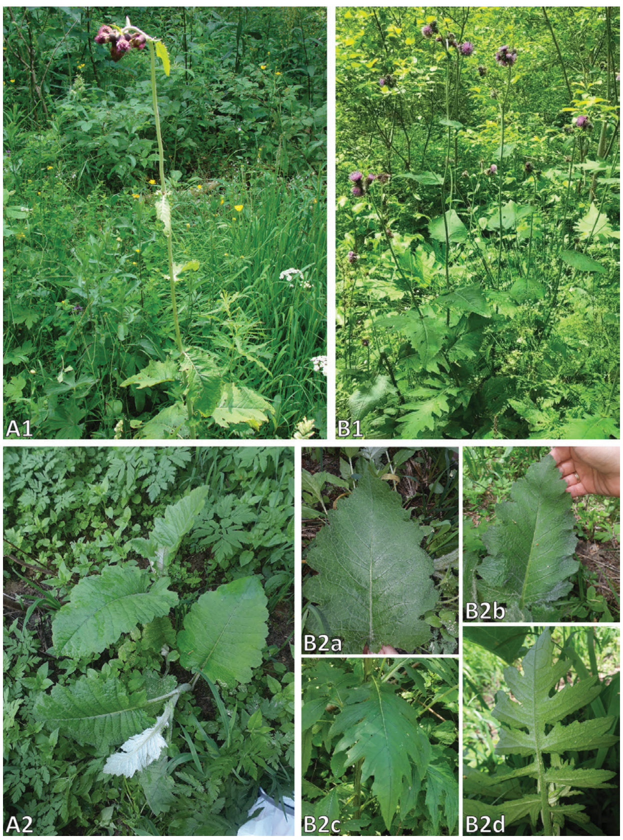

Figure 3. *Cirsium greimleri* on A) Igman Mt. A1: Individual in allopatric population. A2: Leaf morphology; B) Jahorina Mt. B1: Individuals in sympatric population. B2a-B2d: Leaf morphology variations.

Slika 3. Cirsium greimleri *na planini A) Igman. A1: Individua u alopatričnoj populaciji. A2: Izgled lista; B) Jahorini. B1: Indvidue u simpatričnoj populaciji. B2a-B2d: Varijacije u morfologiji lista.*

A more detailed analysis of the herbarium *C. greimleri* specimens from SARA, labelled as *C. waldsteinii*, revealed that some specimens have uncommon leaf morphological features, particularly in upper and medium cauline leaves as we found in studied sympatric populations. Unfortunately, flower colour, the stable taxonomic feature in genus *Cirsium* (Wagenitz, 1987; Yildiz et al., 2016; Bureš et al., 2018), could not be determined due to the long storage of these exsiccates. The observed morphological variability is most likely a consequence of the presence of a hybrid species of *C. greimleri* as one of the parents. Namely, *Cirsium* species, particularly those from the type's section, frequently hybridise (Wagenitz 1987; Bureš et al., 2004, 2010; Stöhr 2006; Segarra-Moragues et al., 2007) and *C. greimleri* is the most common among them with a significant frequency of hybrids in natural populations compared to the "pure" individuals (Bureš et al., 2018). Obtained predictive modelling results for *C. greimleri* and distribution map (Figures 1 and 2) of its most common possible hybridisation partners (*C. erisithales* and *C. rivulare*; Heimerl, 1884; Fritsch, 1906; Khek, 1908; Benz, 1922; Leute & Zeitler, 1967; Bureš et al., 2018) are used for highlighting of potential sympatric populations in B&H.

The appearance of sympatry further complicates the determination of the species *C. greimleri* and the assessment of its distribution. This is especially evident in localities such as Jahorina, Treskavica and Zvijezda Mts. The results of the predictive analysis have confirmed the actual distribution of the studied species. This methodology is justified for investigating complex ecological interactions in vivo.

Genome size

The nuclear DNA amount values for all analysed individuals varied in the range from 1.72 to 2.25 pg (Table 2), which corresponds to the diploid ploidy level. The genome size in all populations, except in Bistrica-Paljevina, was uniform. In the mentioned population, two significantly different genome size values were observed (1.76-1.82 pg and 2.22-2.25 pg). Individuals from Bistrica-Paljevina with higher genome size values had similar leaf morphology and ruby red capitulum as individuals from the allopatric *C. greimleri* population (marked with grey) which is in accordance with Bureš et al. (2018) diagnosis. In all other cases, individuals with lower 2C values showed great variability in leaf blade shapes (Figure 3).

According to Bureš et al. (2018), genome size values for *C. greimleri* vary from 1.87 to 2.17 pg whereas 2C values of Bosnian samples varied from 1.88 to 1.98 pg. The obtained finding of the range of genome size variation in this study is wider than the stated results in Bureš et al. (2018). Additionally, our results suggest that the mean 2C value in the allopatric population of *C. greimleri* (2.18 pg; Ravna Vala) is higher than the highest value reported by Bureš et al. (2018) joined the species. Based on the results presented in Table 2, Figures 2 and 3, it is possible to infer that individuals with variable leaf morphology and lower nuclear DNA values may be the offspring of sympatric hybridisation.

Table 2. The nuclear DNA amount values for *Cirsium greimleri* populations (individuals with uniform morphological features in gray)

Tabela 2. Vrijednosti nuklearne DNK za populacije Cirsium greimleri (individue sa ujednačenim morfološkim karakteristikama su označene sivom bojom)

Locality	Sample	2C (pg)	2C (pg)±SD	CV%	1C (pg)	1C (Mpb)
Jahorina Mt., Bistrica-Paljevina	1	2.23	2.24±0.01	0.65	1.12	1,095
	2	2.22				
	3	2.25				
	4	1.82	1.80±0.04	2.00	0.90	1,760
	5	1.81				
	6	1.76				
Igman Mt., RavnaVala	1	2.19	2.18±0.04	1.82	1.09	2,132
	2	2.14				
	3	2.15				
	4	2.17				
	5	2.24				
Jahorina Mt., Bistrica-Mušak	1	1.83	1.80±0.02	1.35	0.90	1,760
	2	1.77				
	3	1.82				
	4	1.80				
	5	1.80				
Jahorina Mt., Grahov Dol	1	1.78	1.79±0.05	2.65	0.90	1,751
	2	1.84				
	3	1.72				
	4	1.82				
	5	1.83				
	6	1.76				

During the hybridisation process, as rapid genomic changes, the gain or loss of DNA is commonly evident. Among the genera with a high hybridisation rate is the genus *Cirsium*, within which *C. greimleri* is characterized by a particular "interspecific promiscuity" (Bureš et al., 2018). The hybridisation often leads to the genetic erosion of the species and the loss of one of the hybrid parents in sympatric populations, which is a possible case in this study.

CONCLUSION – *Zaključak*

This study confirms the findings of Bureš et al. (2018), who determined the presence of *C. greimleri* in Bosnia and Herzegovina at the investigated localities. This species in B&H predominantly occurs in sympatric populations with related species, as was also observed in this study. The consequence of the above is challenging taxonomic differentiation of the genus. The study also demonstrated that the MaxEnt model yields reliable results even with limited samples. Therefore, predictive modelling can be utilized to study sympatric populations, which can improve the efficiency of fieldwork.

REFERENCES – *Literatura*

Ackerfield, J., Keil, D.J., Hodgson, W.C., Simmons, M.P., Fehlberg, S.D., Funk, V.A. (2020). Thistle be a mess: Untangling the taxonomy of Cirsium (Cardueae: Compositae) in North America. *Journal of Systematics and Evolution*, 58 (6), 881-912. DOI: 10.1111/jse.12692

Adanson, M. (1763). *Familles des plantes*. Paris: chez Vincent.

Barudanović, S. (2003). Ekološko-vegetacijska diferencijacija lišćarsko-listopadnih šuma planine Vranice. Doktorska disertacija.

Benz, R. (1922). *Die Vegetationsverhältnisse der Lavanttaler Alpen*. Wien: Verlag der Zoologisch-botanischen Gesellschaft.

Bjelčić, Ž., Beck-Mannagetta, G., Maly, K. (1983). Cirsium (pp. 99-102). In: *Flora Bosnae et Herzegovina, vol 4: Sympetalae*. (ed. Slišković T.). GZM BH, SOUR Svjetlost, Trebinje (in Bosnian).

Bourge, M., Brown, S.C., Šiljak-Yakovlev, S. (2018). Flow cytometry as a tool in plant sciences, with emphasis on genome size and ploidy level assessment. *Genetics & Applications, 2*(2), 1-12. DOI: 10.31383/ga.vol2iss2pp1-12

Bureš, P., Wang, Y.F., Horova, L., Suda, J. (2004). Genome size variation in Central European species of Cirsium (Compositae) and their natural hybrids. *Annals of Botany, 94,* 353363. DOI: 10.1093/aob/mch151

Bureš, P., Šmarda, P., Rotreklová, O., Oberreiter, M., Burešova, M., Konečný, J., Knoll, A., Fajmon, K., Šmerda, J. (2010). Pollen viability and natural hybridisation of Central European species of Cirsium. *Preslia, 82*, 391-422.

Bureš, P., Šmerda, J., Michálková, E., Šmarda, P., Knoll, A., Vavrinec, M. (2018). Cirsium greimleri: a new species of thistle endemic to the Eastern Alps and Dinarides. *Preslia, 90,* 105134. DOI: 10.23855/preslia.2018.105

Candolle, A.P. (1837). *Prodromus systematis naturalis regni vegetabilis* (vol. 6). Paris: sumptibus sociorum Treuttel et Würtz.

Candolle, A.P., Duby, J.E. (1828). *Botanicon gallicum*, (2nd ed., Vol. 2). Paris: Ve Desray.

Cassini, H. (1826). Eriolepis. In: Cuvier, F. (Ed.): "*Dictionnaire des sciences naturelles",* (pp. 480-482, vol. 41). Paris & Strasbourg: F.G. Levrault; Paris: Le Normant.

Charadze, A.L. (1963). Cirsium Mill. In Bobrov, E.G., Cherepanov, S.K. (Eds). *Flora of the USSR*, Vol. XXVIII (pp. 63.270). Moscow, USSR: Izdatel'stvo Akademii Nauk SSSR.

Choudhury, M.A.M., Marcheggiani, E., Despini, F., Costanzini, S., Rossi, P., Galli, A., Teggi, S. (2020). Urban tree species identification and carbon stock mapping for urban green planning and management. *Forests, 11*(11), 1226. DOI: 10.3390/f11111226

Cunningham, H.R., Rissler, L.J., Apodaca, J.J. (2009). Competition at the range boundary in the slimy salamander: using reciprocal transplants for studies on the role of biotic interactions in spatial distribution. *The Journal of Animal Ecology*, 78, 52-62. DOI: 10.1111/j.1365-2656.2008.01468.x

Cursach, J., Far, A.J., Ruiz, M. (2020). Geospatial analysis to assess distribution patterns and predictive models for endangered plant species to support management decisions: a case study in the Balearic Islands. *Biodiversity and Conservation, 29*, 3393-3410. DOI: 10.1007/s10531-020-02029-y

da Silva, P.P., da Silva, F.A., Rodrigues, C.A.S., Souza, L.P., de Lima, E.M., Pereira, M.H.B., ... Gomes, M.Z.R. (2021). Geographical information system and spatial–temporal statistics for monitoring infectious agents in hospital: a model using Klebsiella pneumoniae complex. *Antimicrobial Resistance & Infection Control*, *10*(1), 92. DOI: 10.1186/s13756-021-00944-5

Dai, X., Wu, W., Ji, L., TIan, S., Yang, B., Guan, B., Wu, D. (2022). MaxEnt model based prediction of potential distributions of Parnassia wightiana (Celastraceae) in China. *Biodiversity Data Journal, 10*, e81073. https://doi.org/10.3897/BDJ.10.e81073

Davis, P.H., Parris, S.B. (1975). Cirisum Mill. In: Davis, P.H. (Ed). *Flora of Turkey and the East Aegean Islands* (pp. 370-412, Vol. 5). Edinburgh, UK: Edinburgh University Press.

Doležel, J., Bartoš J., Voglmayr, H., Greilhuber, J. (2003). Nuclear DNA content and genome size of trout and human. *Cytometry, 51*, 127-128.

Domac, R. (1967). *Ekskurzijska flora Hrvatske i susjednih područja*. Zagreb: Institut za Botaniku Sveučilišta u Zagrebu.

Đug, S. (2004). Diverzitet i konzervacija vegetacije subalpinskog pojasa planine Vranice. Doktorska disertacija.

Fick, S.E., Hijmans, R.J. (2017). WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302-4315. https://doi.org/10.1002/joc.5086

Fourreau, J.P. (1869). Catalogue des plantes qui croissant spontanément le long du cours du Rhône. *Annales de la Société Linnéenne de Lyon*, *2*(16), 301-404.

Fritsch, K. (1906). Über die in der Steiermark vorkommenden Arten und Hybriden der Gattung Cirsium. *Mitteilungen des Naturwissenschaftlichen Vereines für Steiermark, 43*, 404–410.

Gajić M. (1975). Genus *Cirsium*. In Josifović, M. (Ed.). *Flora SR Srbije VII* (pp. 196-220). Beograd: Srpska akademija nauka i umetnosti.

Gray, A. (1874). Contributions to the Botany of North America: A synopsis of the North American thistles. *Proceedings of the American Academy of Arts and Sciences*, *10*, 39-78.

Greene, E.L. (1892). Ecologaebotanicae, No. 1: New or noteworthy thistles. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *44*, 357-365.

Heimerl, A. (1884). Floristische Beiträge. *Zoologisch-Botanische Gesellschaft, 34*, 95–104.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25,* 1965-1978. DOI: 10.1002/joc.1276

Josifović, M (ed). (1970): *Flora SR Srbije*. Novi Sad: Srpska akademija nauka i umjetnosti.

Kadereit, J.W., Jeffrey, C. (2007). Flowering plants. Eudicots: Asterales. In Kubitzki, K. (Ed.): *"The families and genera of vascular plants"* (p. 13). Berlin: Springer.

Keil, D.J. (2006). Cirsium. In: *"Flora of North America Editorial Committee. Flora of North America North of Mexico"* (Vol. 19, pp. 95-164). New York, USA: Oxford University Press.

Khek, E. (1908). Seltene Cirsienbastarde aus Steiermark. *Allgemeine botanische Zeitschrift für Systematik, Floristik, Pflanzengeographie, 14,* 33–36.

Koch, K. (1851). Beiträgezueiner Flora des Orientes. *Linnea, 24*, 395-403.

Kušan, F. (1956). Sastav i raspored vegetacije na planini Kamešnici. *Godišnjak Biološkogi nstituta u Sarajevu*, IX, 1-2.

Lessing, C.F. (1832). *Synopsis generum Compositarum*. Berlin: sumtibus Dunckeri et Humblotii.

Leute, G., Zeitler, F. (1967). Nachträge zur Flora von Kärnten I. *Carinthia II 157/77*, 137–164.

Linnaeus, C. (1753). *Species Plantarum*, Vol. 2. Stockholm, Sweden: Laurentii Salvii.

Marie, D., Brown, S.C. (1993). A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell*, *78*(1-2), 41-51.

Michálková, E., Šmerda, J., Knoll, A., Bureš, P. (2018). Cirsium × sudae: a new interspecific hybrid between rare Alpine thistles. *Preslia, 90*, 347-365. DOI: 10.23855/preslia.2018.347

Miller, P. (1754). *The gardeners dictionary* (4th ed., 3 vols). London.

Mišić, Lj. (1984). Vegetacija livada i pašnjaka na planini Treskavici. Doktorska disertacija.

Necker, N.J. (1790). *Elementa botanica* (vol. 1). Paris: apud Bossange … typis societatis typographicae Neowedensis.

Petrak, F. (1979). Cirsium Mill. In: Rechinger, K.H. (Ed). *"Flora Iranica. Compositae III Cynareae"* (pp. 231-280, vol. 139a). Graz, Austria: Akademische Druck-u Verlagsanstalt.

Phillips, S.J., Anderson, R.P., Schapire, R.E. (2006). Maximum entropy modelling of species geographic distributions. *Ecological Modelling*, *190*, 231-259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Pica, A., Boja, F., Fora, C., Moatar, M., Boja, N. (2022). Advantages of using GNSS technology and QGIS software in inventory stands exploiters. *Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering, 11*, 434-443.

Qazi, A.W., Saqib, Z., Zaman-ul-Haq, Z. (2022). Trends in species distribution modelling in the context of rare and endemic plants: a systematic review. *Ecological Processes, 11*, 40. DOI: 10.1186/s13717-022-00384-y

Segarra-Moragues, J.G., Villar, L., López, J., Pérez-Collazos, E., Catalán, P. (2007). A new Pyrenean hybrid *Cirsium* (Asteraceae) as revealed by morphological and molecular analyses, *Botanical Journal of the Linnean Society, 154*(3), 421-434. DOI: https://doi.org/10.1111/j.1095-8339.2007.00668.x

Stöhr, O. (2006). Cirsium-Hybriden im Bundesland Salzburg. *Linzer biologische Beiträge, 38*, 189–216.

Sweet, R. 1825 ("1825–1827"). *The British flower garden* (vol. 2). London: published for the author by W. Simpkin and R. Marshall.

Tournefort, J.P. (1694). *Elements de Botanique* (Tome I). Paris, France: Masson et Cie.

Wagenitz, G. (1987). Cirsium Mill. em. Scop. In: Hegi, G. (Ed): *"Illustrierte Flora von Mitteleuropa"* (pp. 866–916, 2nd ed, Vol. 6/4). Berlin et Hamburg: Paul Parey Verlag.

Wan, J-N., Mbari, N.J., Wang, S-W., Liu, B., Mwangi, B.N., Rasoarahona, J.R. et al. (2021). Modelling impacts of climate change on the potential distribution of six endemic baobab species in Madagascar. *Plant Diversity, 43*(2), 117-124. DOI: 10.1016/j.pld.2020.07.001

Werner, K. (1996). Cirsium Mill. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Web, D.A. (Eds): *"Flora Europaea"* (pp. 232-242, Vol 4). Cambridge: University Press.

Yildiz, B., Arabaci, T., Dirmenci, T., Kostekci, S. (2016). A taxonomic revision of the genus Cirsium Mill. sect. Cirsium (Asteraceae: Cardueae) in Turkey. *Turkish Journal of Botany*, *40*, 514-530. doi:10.3906/bot-1503-35

Zaki, A., Buchori, I., Sejati, A.W., Liu, Y. (2022). An object-based image analysis in QGIS for image classification and assessment of coastal spatial planning. *The Egyptian Journal of Remote Sensing and Space Science, 25*(2), 349-359. http://dx.doi.org/10.1016/j.ejrs.2022.03.002

Supplement Table 1. Distribution of *Cirsium greimleri* (ex *C. waldsteinii*), *C. erisithales* and *C. rivulare* in Bosnia and Herzegovina

Dodatna Tabela 1. Distribucija vrsta Cirsium greimleri *(ex* C. waldsteinii*),* C. erisithales *i* C. rivulare *u Bosni i Hercegovini*

Locality	Species	Literature data, SARA's herbarium specimens and field investigation
Vranica Mt.	***C. greimleri***	Above Točila, 1.320 m, beech and conifer forest (Barudanović, 2003); Estuary of the Požarna, Mt.Vranica, 1.140 m, beech and conifer forest (Barudanović, 2003); Donja Bistrica, 610 m, mountain beech forest (Barudanović, 2003); Vrtače, 980 m, mountain beech forest (Barudanović, 2003); Estuary of the Požarna, 1.090 m, mountain beech forest (Barudanović, 2003); Estuary of the Požarna, 950 m, mountain beech forest (Barudanović, 2003); Above Kaćuni, 430 m, *Ulmo-Aceretum*, forest with *Symphyandra hofmannii* (Barudanović, 2003); Stream Zavol, 1.190 m, hygrophilous forest (Barudanović, 2003); Above Jezernica, 1.160 m, hygrophilous forest (Barudanović, 2003); Turkovići-Klisure, 1.160 m, hygrophilous forest (Barudanović, 2003); Borovnica stream, 870 m, hygrophilous forest (Barudanović, 2003); Zelena Gromila, 1.750 m, *Agrosti-Alnetum viridis* (Đug, 2004); Stražica, 1.700 m, *Athyrio-Alnetum viridis* (Đug, 2004); Zelena Gromila, 1.660 m, *Alnetum viridis* (Đug, 2004); Banjaluk, 1.600 m, *Alnetum viridis* (Đug, 2004); Bukov vrat, 1.545 m, *Salicetum waldsteinianae* (Đug, 2004); Ćoso, 1.830 m, *Pinetum mugi calciolum* (Đug, 2004); Podine, 1.870 m, *Pinetum mugi calciolum* (Đug, 2004); Nadkrstac, 2.105 m, *Pinetum mugi calciolum* (Đug, 2004).
Jahorina Mt.	***C. greimleri***	Kasidol, 1.360 m, ex Maly, 1903, SARA 43 791; Bistrica, Ravna planina in Pale, 1.250 m, ex Maly, 1907, SARA 43 792; In the forest near Pračko river spring, 1.460 m, *Abietum albae*, ex Maly, 1921, SARA 43 787; Bistrica-Paljevina, 1.454 m, N-NW, Muratović & Pustahija, 2022; Bistrica-Mušak, 1.460 m, Muratović & Pustahija, 2022; Grahov Dol, 1.478 m, Muratović & Pustahija, 2023.
	C. rivulare	In wet bushes, 840 m, ex Maly, 1919. SARA 43 801; ex Fiala, 1895. SARA 43 803; In the wet meadows near Pale, ex Maly, 1931. SARA 43 807.
	C. erisithales	Ravna planina towards Bistrica, 1.320 m, ex Maly, 1933. SARA 43 719.
Bjelašnica Mt.	***C. greimleri***	Babin Do, 1.260 m, ex Maly, 1906. SARA 43 788.
	C. erisithales	Hranisava, above Kradenik river spring, 1.000 m, ex Ritter, 1935. SARA 43 713; Along the stream, below Javornik spring, ex Bjelčić, 1950. SARA 43 714.
Pazarić	***C. greimleri***	Zovik near Pazarić, 1.300 m, ex Ritter, 1935. SARA 43 794.
Treskavica Mt.	***Cirsium greimleri***	Turovi, towards Kazani (Željeznica river), 1.218 m, Trakić, 2023 (personal communication).
	C. rivulare	Bara above Gvozno, 1.430 m, *Trollio-Juncetum*, Mišić, 1984; Veliko jezero, ex Popović, 1952. SARA 43 799; Veliko jezero, 1.550 m, ex Maly, 1911. SARA 43 808.
OzrenMt.	***C. greimleri***	Han Jezero, 1.200 m, ex Maly, 1908. SARA 43 782; In the valley Babin potok towards Han Toplica, 1.170 m, ex Maly, 1919. SARA 43 783.
	C. rivulare	On meadows between Han Jezero and Han Toplice, 1.270 m, ex Maly, 1922. SARA 43 796.
Vlašić Mt.	***C. greimleri***	Gujni Do, 1885. SARA 43 790; Above Paklarevo, Gujni do, Korićani, ex Brandis, 1884. SARA 43 780; Above Paklarevo, Gujni do, Korićani, ex Brandis, 1885. SARA 43 780.
	C. rivulare	Gujni do, 1885. SARA 43 802.
Zvijezda Mt.	***C. greimleri***	Bukovački spring, Vareš, Šarić, 2011; Pogar near Vareš, Šarić, 2014, 2016, 2020.
	C. erisithales	In Bobovac, ex Plavšić, 1940. SARA 43 718.
Zelengora Mt.	***C. greimleri***	In the valley Stabrovača on Mt. Husad and Ravna gora towards Jelašća, 1.380 m, ex Maly, 1931. SARA 43 786.
	C. rivulare	Konjska voda towards Jelašća, 1.400 m, ex Maly, 1931. SARA 43 797.
Hrbljina Mt.	***C. greimleri***	In Glamoč, ex Santorius, 1897. SARA 43 789.
Vučija Mt.	***C. greimleri***	Towards Nemila, Javorak, 1.400 m, ex Maly, 1913. SARA 43 784.
Igman Mt.	***C.greimleri***	Ravna Vala, 1.257 m, Muratović & Pustahija, 2022.
Mehorić village	***C. greimleri***	Mehorić near Kakanj, Šarić, 2017.
Borika	***C. rivulare***	Towards Oprašići (distr. Rogatica), 950 m, ex Maly, 1931. SARA 43 806.
Podžeplje	***C. rivulare***	On wet meadows near Podžeplje (near Rogatica), 950 m, ex Maly, 1923. SARA 43 805.

Locality	Species	Literature data, SARA's herbarium specimens and field investigation
Kupreško polje	***C. rivulare***	ex Lažetić, 1958. SARA 43 804.
Glamočko polje	***C. rivulare***	On marshy meadows, ex Ritter, 1954. SARA 43 798.
Trebević Mt.	***C. erisithales***	Alpinetum, ex Šilić, 1959. SARA 50 005; Dovlići, 1.560 m, ex Maly, 1924. SARA 43 722; 1.560 m, ex Maly, 1937. SARA 43 724.
Klekovača Mt.	***C. erisithales***	In Petrovac, ex Fiala, 1891. SARA 43 709; Drinić in Petrovac, ex Fiala, 1891. SARA 43 716; In Petrovac, ex Fiala, 1891. SARA 43 717.
Prača	***C. erisithales***	Banja stijena, 570 m, ex Maly, 1909. SARA 43 711.
Kamenica Mt.	***C. erisithales***	In the valley Gostović river, 350 m, ex Maly, 1920. SARA 43 712.
Osječenica Mt.	***C. erisithales***	In Petrovac, 1.800 m, ex Fiala, 1891. SARA 43 721.
Jajce	***C. erisithales***	Vrbas canyon near Jajce, ex Maly, 1908. SARA 43 726.
Kamešnica Mt.	***C. erisithales***	Pre-mountain beech forest, Kušan, 1956.

SAŽETAK

Prema najnovijim podacima, Dinaride i Istočne Alpe naseljava endemična, diploidna, novoopisana vrsta *Cirsium greimleri* umjesto, do sada navođene, tetraploidne *C. waldsteinii*. Ova studija, koristeći podatke iz literature, herbarijske zbirke SARA i terenskih istraživanja, potvrdila je prisustvo vrste *C. greimleri* u Bosni i Hercegovini na 40 lokaliteta. Upotrebom MaxEnt modela i GIS alata izrađena je karta rasprostranjenosti i ekoloških niša za novu bh. vrstu, pri čemu su ključni faktori za njenu distribuciju identificirani kao srednja temperatura najvlažnijeg i najsušnijeg kvartala te količina padavina. Model je pokazao visoku tačnost (AUC vrijednost 0.970), što ukazuje na sposobnost preciznog razlikovanja područja sa i bez prisustva vrste. Pored toga, model je ukazao na prisustvo vrsta sa kojima *C. greimleri* često hibridizira (*C. rivulare* i *C. erisithales*) na više lokaliteta. Terenskim istraživanjima je potvrđeno prisustvo diploidnih populacija istraživane vrste s rasponom variranja nuklearne veličine genoma od 1.72 do 2.25 pg. Također, na osnovu prezentiranog prediktivnog modela izvršena je provjera odabranih lokaliteta na kojima *C. greimleri* koegzistira sa *C. rivulare* i *C. erisithales*. Tako je na lokalitetu Bistrica-Paljevina uočena populacija u kojoj su individue imale značajno iskazanu morfološku varijabilnost, što je i odgovaralo dvjema vrijednostima veličine genoma (1.76-1.82 pg i 2.22-2.25 pg). Ova studija ukazuje na potrebe daljih istraživanja problema simpatrije *C. greimleri* i njenih srodnika te neophodnu taksonomsku reviziju ovog kompleksa.

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