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Hot-spots of the genus *Aconitum* in the Ukrainian Carpathian Region

Introduction

The genus *Aconitum* in the World flora comprises about 300–400 species distributed mainly in the temperate regions of the Northern Hemisphere (Li, Kadota, 2001; Mitka et al., 2021). In the Carpathians is located one of the main centres of the genus diversity in Europe (Mitka, 2003). The Ukrainian Carpathian Region (UCR), being part of the Eastern Carpathians and including surrounding foothills and lowlands, is subdivided into 57 geomorphological mesoregions (Novikov, 2021a). In general, in the UCR, there are present 21 unequally distributed *Aconitum* taxa of the specific and infraspecific level, including ten species of the native flora (Novikoff et al., 2016; Onyshchenko et al., 2021).

The preliminary analysis of the genus *Aconitum* distribution in the Ukrainian Carpathians was published in 2011 (Novikoff, Mitka, 2011). However, only some of the localities were included in our previous research, and no modern GIS-based approaches were applied. The aim of this research was to reveal main centres of the genus *Aconitum* diversity and distribution (so-called hot-spots) in the UCR. Here we represent the results of the genus' hot-spot analysis basing on the most comprehensive data gathered from herbaria, field surveys, and published sources.

Material and methods

In general, 2054 *Aconitum* occurrences from the UCR were included in the analysis and shared as a GBIF dataset (Novikov, 2021b). The data mining was conducted from all available data sources, including published materials, databases, herbarium

collections, and personal field surveys during 2007–2019. In particular, data gathered personally or distantly from the 27 herbaria (see Tab. 1 – Appendix 1) were involved in the analysis.

The spatial distribution analysis was entirely performed in the QGIS 3.10.2 environment (*QGIS Development Team, 2020*) with an installed hot-spot analysis plug-in (Oxoli et al., 2017) and using Getis-Ord G_i^* statistics implementation (Getis, Ord, 1992; Ord, Getis, 1995). For the purposes of the hot-spot analysis, a 10×10 km hexagonal grid was preliminarily constructed in QGIS. Hexagons were chosen due to the most reduced edge effect biasing the spatial data (Birch et al., 2007).

The species richness (SR) was calculated per each of 57 delimited geomorphological mesoregions of the UCR that served as operational geographical units (OGUs) throughout all the investigation. However, SR poorly performs for analysis of OGUs (Albuquerque, Beier, 2015). Therefore, an analysis of rarity-weighted richness (RWR) following Williams et al. (1996) has been performed too.

Similarly RWR, which has found its wider application for the calculation of the weighted endemism (Crisp et al., 2001), we applied another technique that is also widely used in the analysis of endemism areas. The principles of Parsimony Analysis of Endemism (PAE) allow delimiting the areas of endemism (in our case – areas of *Aconitum* species concentration) and analysing the OGUs similarity basing on the matrices with taxa presence/absence serving as character states (Morrone, 1994; Oliveira et al., 2015; Fattorini, 2017; Li et al., 2017) were extrapolated to our research. To perform PAE, the OGUs having zero presence (eight OGUs) of the *Aconitum* were excluded, and, at the same time, a hypothetical OGU having zero presence of investigated taxa was introduced as a zero-outgroup. The maximum parsimony analysis was performed in the TNT 1.5 environment (Goloboff, Catalano, 2016) with tree bisection reconnection (TBR) set as collapsing rule and 1000 replications. The consensus tree was obtained using the majority rule set to 50% cut-off. After that, the consensus tree was exported to Mesquite 3.70 (Maddison, Maddison, 2021), where it was rooted to zero-outgroup, set up to display the bootstrap support, visualised, and clades were coloured.

Results and discussion

Species richness and general distribution pattern

Most of the registered occurrences were distributed within the Outer (76.77%) and Inner Eastern Carpathians (20.16%), considering together the Carpathian Mountain Range in the strict sense, while the adjacent sub-mountainous territories comprised only 3.07% from a total number of occurrences. Namely, 54 occurrences (2.63%) were scattered

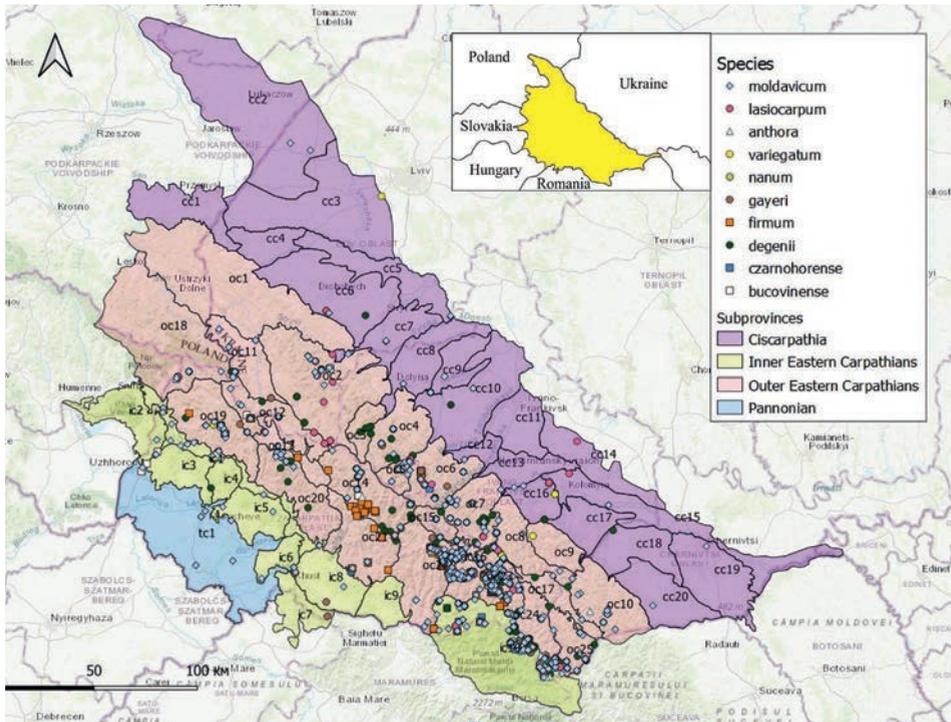


Fig. 1. Distribution of all registered *Aconitum* occurrences in the UCR (For abbreviations see Tab. 2 – Appendix 1)

in the Ciscarpathia and only nine (0.44%) – in the Pannonian province corresponding to the Transcarpathia (Fig. 1).

The OGUs oc23 (Chornohora – 720 occurrences, all ten species registered), oc22 (Svydovets – 234 occurrences, all ten species registered), ic10 (Maramures – 363 occurrences, nine species registered), oc6 (Syvuliansko-Stanymyrski Gorgany – 60 occurrences, eight species registered), oc14 (Torunsko-Bertianski Gorgany – 77 occurrences, eight species registered), and oc19 (Polonyna Rivna – 51 occurrences, eight species registered) demonstrated the highest SR (Fig. 2A–B – Appendix 2). As we can see, the number of occurrences per OGU (in other words – sampling frequency) does not strictly correlate with SR. For example, Chornohora demonstrated both the highest sampling frequency and species richness. However, another OGU with a relatively high sampling frequency (oc12 – Waterdivided Mountain Range) having 69 registered occurrences comprises only five different *Aconitum* species. It is important to note that Waterdivided Mountain Range is often considered the part of Eastern Beskyds (= Bieszczady Wschodnie) following the classification of Kondracki (1989, 2002). However, here Waterdivided Mountain Range is considered as independent mesoregional

OGU, following Ukrainian approach (Slyvka, 2001; Hiletskiy, 2012; Novikov, 2021a). Similarly, oc7 (Dovbushanski Gorgany) having 60 registered occurrences comprises only five different *Aconitum* species too.

Sampling frequency and SR can be affected by several distorting factors, i.e., higher popularity of certain OGUs among collectors; repeated collection from the same populations; absence of the information from the hardly accessible places (Baltanás, 1992). To correctly analyse the distribution data, it is important to treat all test areas equally, i.e. assuming that all species are detected and that the detectability of the different species is the same (Boulinier et al., 1998). We could not be sure that all *Aconitum* species were detected for each certain OGU. However, the high number of registered occurrences relating to a low number of tested species analysed at the high spatial resolution allowed to minimise the distortion effect (Graham, Hijmans, 2006; Gotelli, Colwell, 2011; Chao, Chiu, 2016).

Considering the limitations of the SR analysis and unequal rarity of the analysed *Aconitum* species (from ten studied species, six are threatened and with limited distribution in the UCR – see Novikov (2021b) for details), other advanced data processing techniques were applied. We calculated the RWR index for all OGUs and found that oc23, oc22, and ic10 had the highest RWR values, while oc6, oc14, and oc19 also demonstrated a relatively high RWR level (over 0.5). Hence, the RWR results were found to be concordant with SR outcomes. Additionally, oc21 (Polonyna Krasna) showed the RWR value very close to 0.5 points (0.48), while other analysed OGUs differed much distinctly and did not reach 0.3 RWR value (Fig. 2C – Appendix 2).

Hot-spot analysis

Getis-Ord G_i^* statistics allowed to primarily reveal four hot-spot centres in the UCR (Fig. 3A – Appendix 2): 1 – located mainly in the OGUs oc23, oc22, and ic10 (i.e., Chornohora, Svydovets, and Maramures); 2 – located mainly in oc12 and oc19 (i.e., Waterdivided Mountain Range and Polonyna Rivna); 3 – located mainly in the oc6 (i.e., Syvuliansko-Stanymyrski Gorgany); 4 – located mainly in the oc14 (Torunsko-Bertianski Gorgany). Normalisation of the hot-spot analysis eliminated the last two hot-spots (Fig. 3B – Appendix 2). Hence, also taking into account the data on the species richness and sampling frequency, we can conclude that there are two centres of the genus *Aconitum* diversity and distribution within the UCR. The main centre is located in the south-eastern part of the UCR and covers Svydovets, Chornohora, and Maramures. At the same time, the second small isolated centre exists in the Polonyna Rivna and Waterdivided Mountain Range. A similar result was obtained while studying the centres of phenetic coherence of the *Aconitum* sect. *Aconitum* (= *Napellus*) in the Eastern Carpathians, based on Mahanalobis distances among regions' centroids in Discriminant Function Analysis (Mitka, 2002). Here, the Svydovets-Negrovets region and Chornohora formed

closely related regions in terms of the taxon morphological variability. Another centre (partially outside the Ukrainian Carpathians) is in Maramures and Bistrica Mts-Čeahlău region (Mitka, 2002).

The high *Aconitum* diversity and distribution in the Svydovets, Chornohora, and Maramures are not surprising due to the highest altitudes in the region. These mountain ranges reach the alpine belt and generally have the highest floristic diversity (Malynovskiy, 1991; Tasenkevich, 2003; Cherepanyn, 2017). Moreover, in the Maramures is located one of the main areas of plant endemism in the Carpathians, partly spread to the north-west and separated by a strong barrier from surrounding mountain ranges (Pawłowski, 1970; Tasenkevich, 2014; Hurdu et al., 2016). Much engaging is the presence of a small hot-spot of the genus *Aconitum* in the Waterdivided Mountain Range and Polonyna Rivna. In the Ukrainian Carpathians, Waterdivided Mountain Range is not as high as other surrounding ranges and is considered a relatively older geomorphological construction hosting the relict floristic complexes and unique plant taxa (Popov, 1949; Stoyko et al., 1997). Moreover, Mt. Pikui, located in this mountain range, has the beech dwarf forest delimiting a short subalpine belt with rocky outcrops, making unique isolated habitats (Stoyko et al., 1997). In particular, here exists isolated and the most abundant in the UCR population of the *Aconitum bucovinense* Zapal. (Novikoff, Mitka, 2011). There are no reports about such unique floristic conditions on the Polonyna Rivna, and most analysed *Aconitum* occurrences related to Runa-Plai Mt. and its slopes.

Cladistics analysis

Maximum parsimony analysis following the principles of PAE has been performed to reveal the similarity of the analysed OGUs basing on their species composition. Li et al. (2017) reported that PAE analysis at the multiple taxa level is not well-performing. Therefore we cut off all taxa at the same (i.e., species) level.

Most of the analysed OGUs did not demonstrate a statistically significant difference in PAE (perhaps, due to a low number of studied species). It showed eight well-distinguishing clades of *Aconitum* diversity in the UCR (Fig. 4A – Appendix 2, marked on by color). The OGUs oc23 (Chornohora) and oc22 (Svydovets) were found to be the most outstanding (bootstrap support = 1) and sharing the common node with clades ic10 (Maramures) and oc14 (Torunsko-Bertianski Gorgany). Interestingly, that floristically poor regions oc19 (Polonyna Rivna) and oc21 (Polonyna Krasna) appeared to be distinctly separated from oc20 (Polonyna Borzhava) that belongs to the same mountainous group. Moreover, PAE did not allow for the distinguishing oc12 (Waterdivided Mountain Range) that was found to be distinct in the previous analyses. Despite this, PAE amplified the clades of OGUs oc5, oc6, and oc14 belonging to different mesoregions of the Gorgany Mts. Extrapolation of the PAE results on

the map (Fig. 4B – Appendix 2) confirmed that there exists the main centre of the genus *Aconitum* diversity in the UCR with its core in the Chornohora and Svydovets that is spread to adjacent mountain ranges. The second isolated centre of *Aconitum* diversity was revealed in the Polonyna Rivna, which is also partly concordant with mentioned above findings. Perhaps, a continuous distribution pattern of the genus *Aconitum* from the SE toward NW of the UCR existed before. The gap in the oc20 (Polonyna Borzhava) could arise as a result of anthropogenic pressure. In the Polonyna Borzhava, just like in Polonyna Rivna and Polonyna Krasna, the forest level was artificially lowered, and open spaces were overgrown by *Vaccinium myrtillus* L. (Felbaba-Klushina, Bizilya, 2015).

Conclusions

Different methods of spatial distribution analysis were tested for the genus *Aconitum* in the UCR. It was found that Chornohora and Svydovets Mts., with a significant contribution of Maramures, form the core of the genus diversity and distribution in this region. Basing on maximum parsimony analysis (adopted PAE), it was found that Chornohora and Svydovets Mts. form the most distinct clades with high bootstrap support. The Polonyna Rivna was found to have surprisingly high RWR value and clustering together with Polonyna Krasna, while Polonyna Borzhava disrupts these two OGUs from the main hot-spot of the genus in the SE part of the UCR. Perhaps, a continuous gradient of *Aconitum* diversity and/or distribution from SE toward NW of the UCR existed before, and the gap in Borzhava secondarily resulted from anthropogenic transformation.

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Conflict of interest

The authors declare no conflict of interest related to this article.

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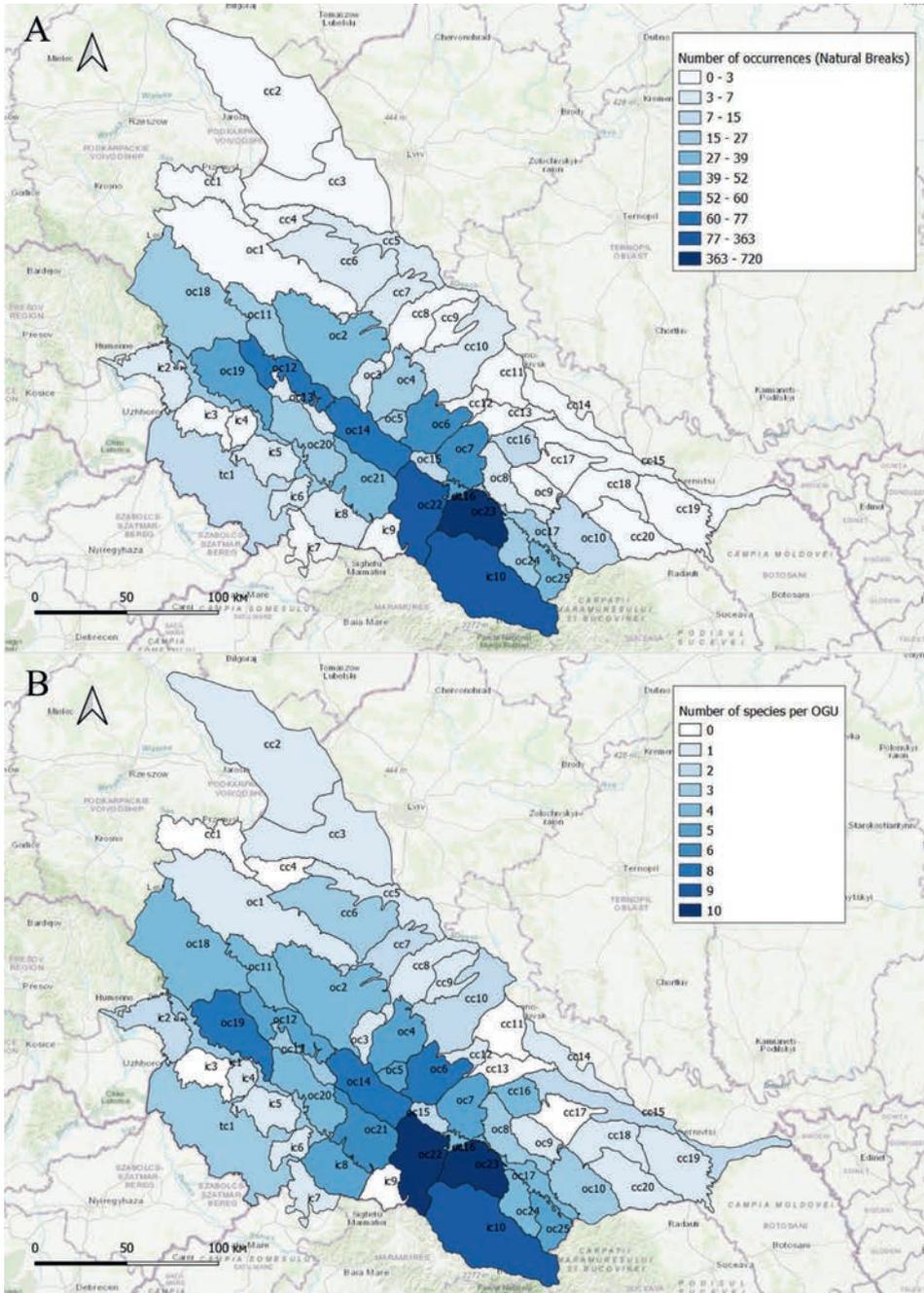
Tab. 2. Distribution of the *Aconitum* species in of the UCR mesoregions designated according to Novikov (2021a)

OGUs ID	OGUs (mesoregions)	OGU synonyms	Number of discovered occurrences																	
			Number of noticed species					Number of discovered occurrences												
			subgen. <i>Lycototomum</i> DC.	subgen. <i>Anthora</i> DC.	subgen. <i>Cammarum</i> DC.	subgen. <i>Aconitum</i>														
cc1	Temgorod Plateau	Plaskowyż Tamogrodzki	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc2	Peremyshel-Dobromyr Highland	Pogórze Przemyskie	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc3	Sian-Dniester Lowland	Oversian Basin	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
cc4	Stryvigor-Bolozivka Highland	Stryvigor Highland	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc5	Upper-Dniester Depression	Dniester-Svicha Lowland	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc6	Drohobych Highland	Bolekhiv-Zhuravne region	3	2	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
cc7	Morshyn Highland	Dolyno-Bolokhivskiy region	2	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc8	Zalissia Highland	Limnytsia region	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc9	Rozhniativ-Kalush Depression	Lukva Highland	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc10	Prylukva Highland	Mizhbystriske Horboghiria, Gvizdtske Horboghiria	2	3	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
cc11	Bystrytsia Depression		0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cc12	Interbystrytsia Highland		1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

oc5	Bratkovski Gorgany	2	15	1	1	3	-	-	-	-	-	1	1
oc6	Yasinia Depression	4	14	2	2	21	2	-	3	8	4	6	6
oc7	Vorokhta-Putyla Valley	4	33	4	4	17	3	3	-	-	-	-	-
oc8	High Bieszczady	4	4	-	-	1	1	-	-	-	-	-	-
oc9	Bukovske Vrchy	8	-	-	-	-	-	1	-	-	-	-	-
oc10	Polonyna Runa	4	11	-	-	1	-	1	-	-	-	-	-
oc11	Polonyna Borzhava	6	8	1	-	7	2	-	-	-	-	-	-
oc12	Polonyna Krasna	10	26	5	-	1	-	-	33	-	3	-	-
oc13	Svydovets	10	3	2	-	-	-	-	-	-	-	1	-
oc14	Chornohora	4	12	-	-	1	15	1	-	7	4	17	19
oc15	Gryniava	5	3	-	-	-	7	-	-	-	-	-	-
oc16	Tsyrokh-Borzava Valley	3	34	2	-	-	14	-	-	1	-	-	-
oc17	Vygorlat	2	10	2	-	-	10	-	1	-	-	-	-
oc18	Makovytsia	0	7	12	5	1	-	-	-	-	-	-	-
oc19	Syniak	1	17	8	-	4	6	1	6	-	2	5	-
oc20	Velykiy Dil	1	17	1	-	-	5	-	-	1	-	-	-
oc21	Tupiy	1	7	-	-	4	-	-	2	2	15	1	-
oc22	Oas	1	70	2	8	37	7	3	2	16	55	34	-
oc23	Tereblia Massif	5	178	7	24	137	32	1	6	178	63	83	-
oc24	Apsyhtsia Massif	0	13	-	1	10	3	-	-	-	-	-	-
oc25	Maramures Mts.	9	25	3	1	8	1	-	-	-	-	-	-
tc1	Transcarpathian Lowland	3	6	-	-	-	-	-	2	-	-	-	1
	Tysa Lowland, Prytysenska Dolina, Tysenska Dolina												
	Marmarosh Mts.												
	Gutyn												
	Berezne-Lipshansk Valley												

Tab. 1. List of applied herbaria's acronym following Thiers (2021)

No.	Acronym	Herbarium	Location
1.	B	Botanischer Garten und Botanisches Museum Berlin, Zentraleinrichtung der Freien Universität Berlin	Germany, Berlin
2.	BM	The Natural History Museum	U.K. England, London
3.	BP	Hungarian Natural History Museum	Hungary, Budapest
4.	BRNU	Masaryk University	Czech Republic, Brno-Bohunice
5.	CHER	Yu. Fedcovich Chernivtsi National University	Ukraine, Chernivtsi
6.	CL	Babes-Bolyai University	Romania, Cluj-Napoca
7.	GJO	Universalmuseum Joanneum	Austria, Graz
8.	HBG	University of Hamburg	Germany, Hamburg
9.	JE	Friedrich Schiller University Jena	Germany, Jena
10.	KRA	Jagiellonian University	Poland, Kraków
11.	KRAM	W. Szafer Institute of Botany, Polish Academy of Sciences	Poland, Kraków
12.	KW	M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine	Ukraine, Kyiv
13.	KWHA	National Academy of Sciences of Ukraine	Ukraine, Kyiv
14.	KWHU	O. V. Fomin Botanical Garden of the Taras Shevchenko National University of Kyiv	Ukraine, Kyiv
15.	LI	Upper Austrian State Museum	Austria, Linz
16.	LW	Ivan Franko National University of Lviv	Ukraine, Lviv
17.	LWKS	Institute of Ecology of the Carpathians	Ukraine, Lviv
18.	LWS	State Museum of Natural History	Ukraine, Lviv
19.	O	University of Oslo	Norway, Oslo
20.	OHN	Biological Museum, Oskarshamn	Sweden, Oskarshamn
21.	PR	National Museum in Prague	Czech Republic, Praha
22.	PRC	Charles University, Prague	Czech Republic, Praha
23.	SAV	Slovak Academy of Sciences	Slovakia, Bratislava
24.	SIB	Natural History Museum	Romania, Sibiu
25.	UU	Uzhhorod National University	Ukraine, Uzhhorod
26.	WU	Universität Wien	Austria, Wien
27.	ZT	Eidgenössische Technische Hochschule Zürich	Switzerland, Zürich



description Fig. 2AB on the next page

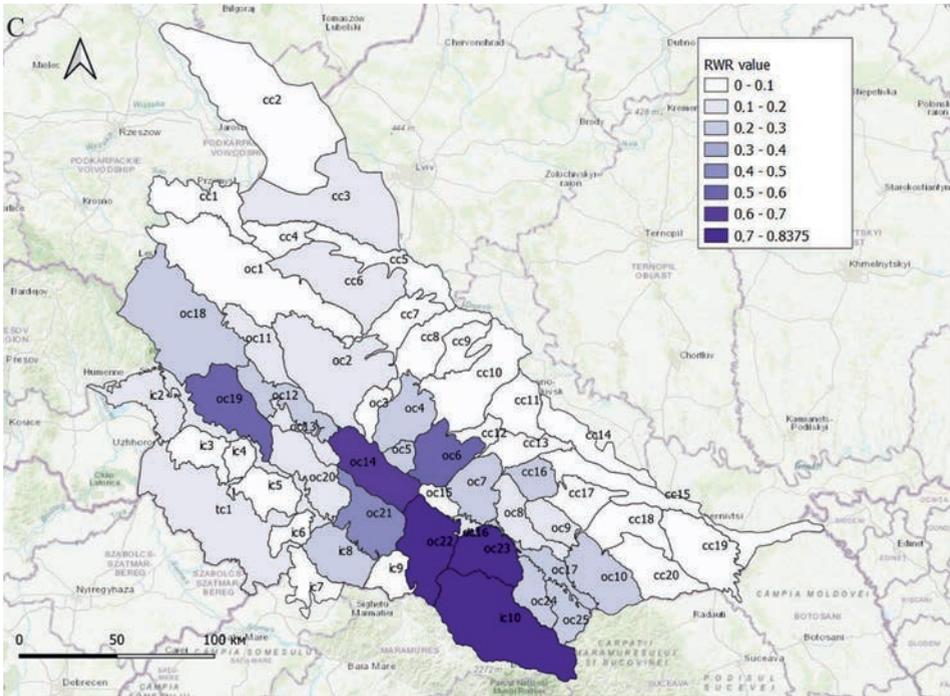


Fig. 2. The number of registered occurrences (A), species richness (B), and rarity weighted richness (C) of the genus *Aconitum* in the Ukrainian Carpathian Region

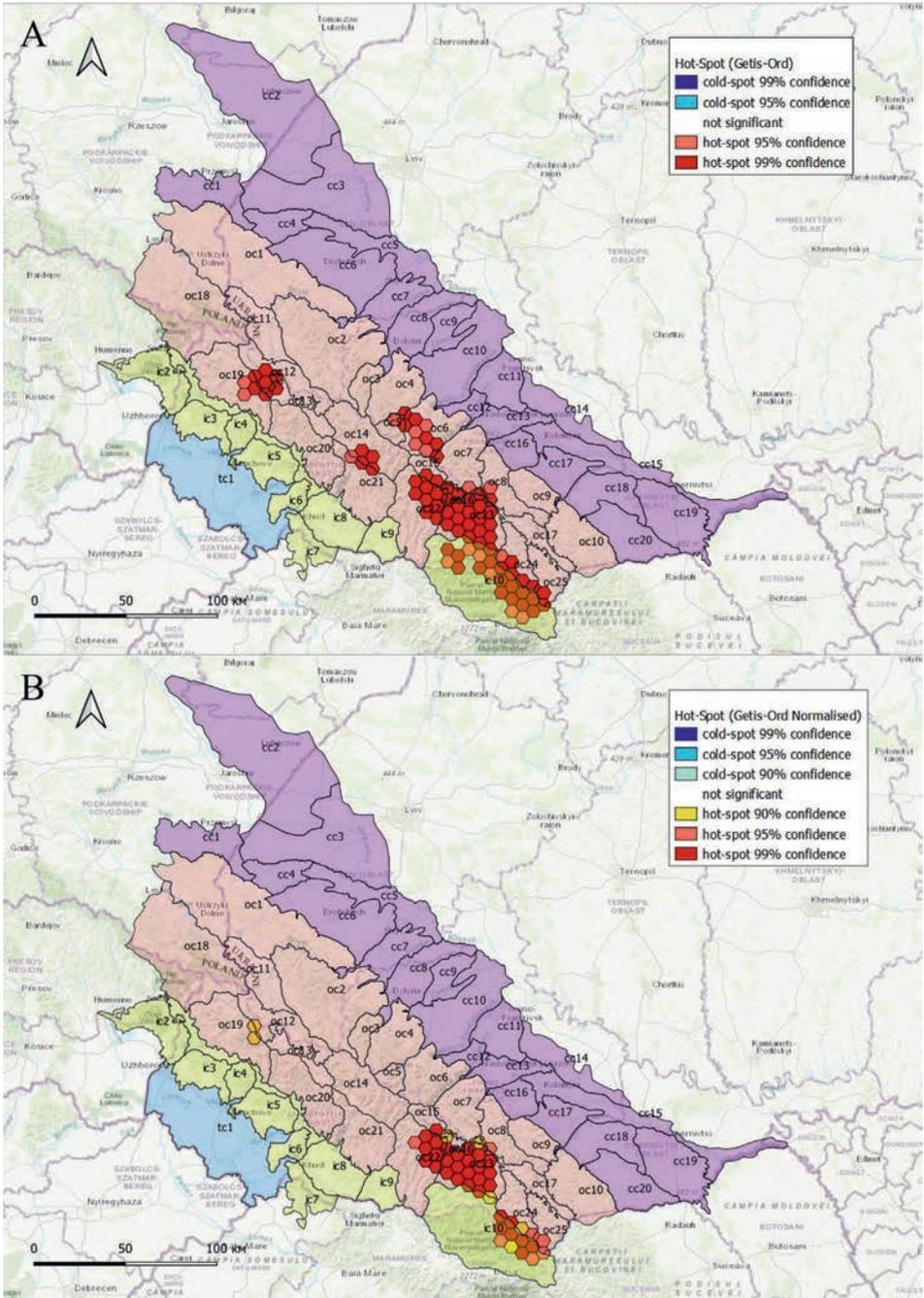


Fig. 3. Standard (A) and normalised (B) Getis-Ord hot-spot analysis of the *Aconitum* distribution in the UCR.

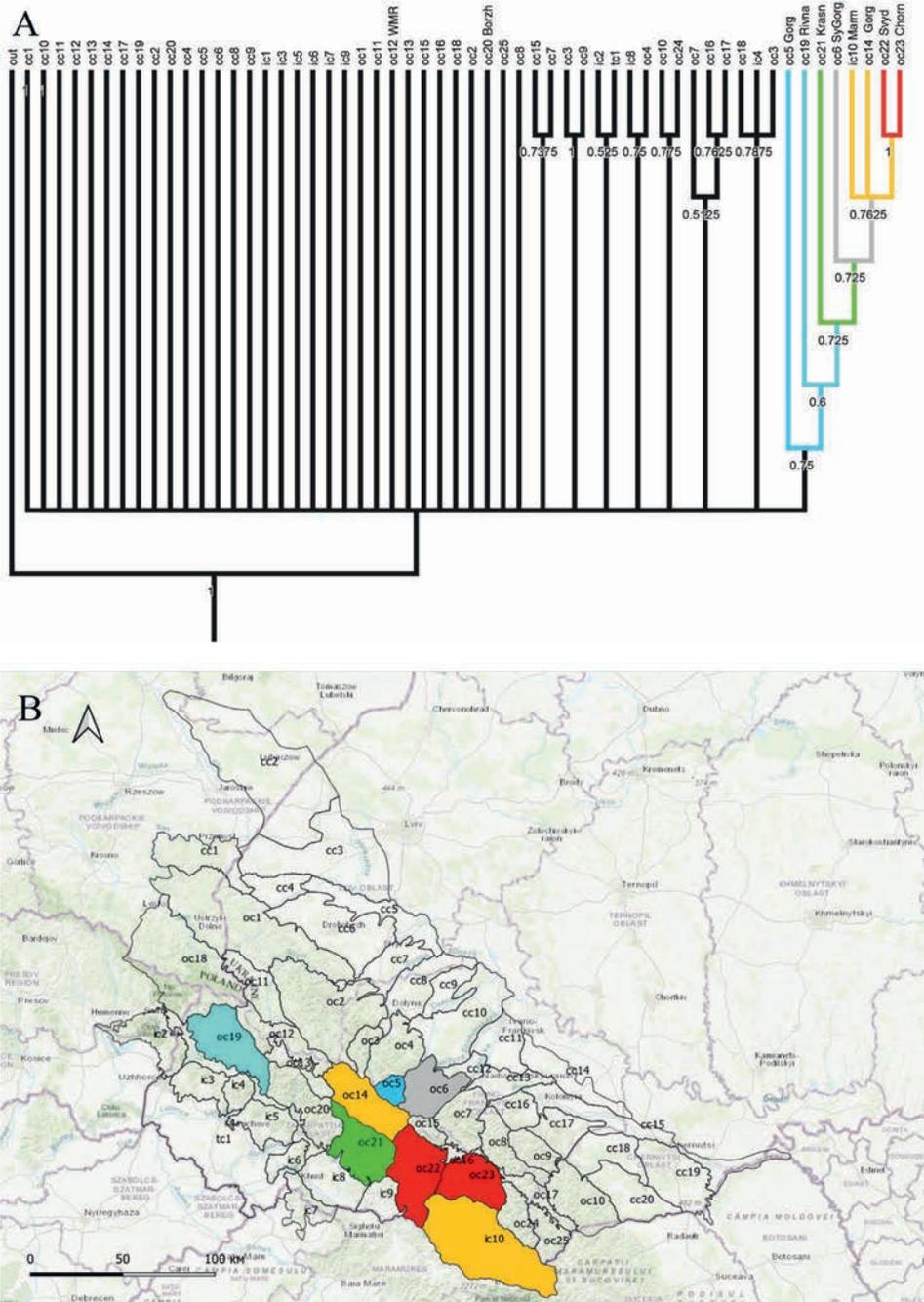


Fig. 4. Cladogram of the adopted PAE analysis of the *Aconitum* diversity in the UCR (A) extrapolated to the map (B)

Abstract

Basing on 2054 georeferenced occurrences of ten *Aconitum* species, the initial map of the species richness per each of 57 operational geographic units (OGU) of the Ukrainian Carpathian Region (UCR) has been developed. Next, to avoid unequal data distribution, we counted absolute species presence per OGU, based on which we calculated 'rarity-weighted richness' (RWR) and performed parsimony analysis. As a result, we found that Chornohora and Svydovets made a strongly supported cluster with the highest *Aconitum* species diversity and demonstrated the highest RWR values, hence being the main hot-spot of the genus distribution in the UCR. This main hot-spot also spreads to adjacent mountain ranges, including Maramures and Gorgany. Probably, a continuous gradient of *Aconitum* taxonomic richness from southeast toward northwest of the UCR existed before. The gap in Polonyna Borzhava, located between Polonyna Rivna and Polonyna Rivna, can be secondarily resulted by the high level of anthropogenic transformation of this region. Contrary, the high RWR value and distinct clustering of the Polonyna Rivna supported the presence of a local isolated *Aconitum* hot-spot related to two prominent mountain peaks located here – Runa-Plai Mt. (in Polonyna Rivna) and Pikui Mt. (in adjacent Waterdivided Mountain Range).

Key words: *Aconitum*, biogeography, hot-spot analysis, Ranunculaceae, Ukrainian Carpathians

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Hot-spoty rodzaju *Aconitum* w Ukraińskim Karpaccim Regionie

Streszczenie

Na podstawie analizy 2054 georeferencyjnych wystąpień dziesięciu gatunków rodzaju *Aconitum* opracowano wstępną mapę bogactwa gatunkowego w każdym z 57 operacyjnych jednostek geograficznych (OGU) Ukraińskiego Karpacciego Regionu (UKR). Następnie, aby uniknąć nierównego rozkładu danych, policzyliśmy bezwzględną obecność gatunków w każdym OGU, obliczyliśmy bogactwo ważone rzadkością (RWR) i wykonaliśmy analizę parsymonii. W rezultacie stwierdziliśmy, że Czarnohora i Svydovets utworzyły silnie poparte ugrupowanie o najwyższym bogactwie gatunków *Aconitum* i wykazywały najwyższe wartości RWR, będąc tym samym głównym hot-spotem rozmieszczenia rodzaju w UCR. Ten główny hot-spot rozciąga się również na sąsiednie pasma górskie, w tym Maramures i Gorgany. Prawdopodobnie w UKR istnieje gradient bogactwa taksonomicznego *Aconitum* z południowego wschodu na północny zachód. Luka w Borżawie, położonej między Równą a Krasą, może być wtórnie spowodowana wysokim stopniem antropogenicznej transformacji tego regionu. Natomiast wysoka wartość RWR i wyraźna odrębność Połoniny Rivna wskazuje na obecność lokalnego, izolowanego hot-spotu rodzaju *Aconitum*, związanego z dwoma jej głównymi szczytami górskimi – Runa-Plai (na Połoninie Rivna) i Pikui (na Werchowynskim Wododilnim Chrebetie).

Słowa kluczowe: *Aconitum*, biogeografia, analiza hot-spotów, Ranunculaceae, Karpaty Ukraińskie

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