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Chemophenetic investigation of epicuticular *n*-alkanes in *Juniperus* L. section *Juniperus* L. from the Balkans

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ABSTRACT:

Junipers are one of the most diverse groups of gymnosperms today. They are adapted to arid areas of the world. In this study, we analysed the chemical composition of the leaf *n*-alkanes of 170 individuals belonging to *Juniperus communis* subsp. *communis*, *J. communis* var. *saxatilis*, *J. deltooides* and *J. macrocarpa* from the Balkans. Localities with at least two sympatric populations were chosen to study the chemical composition in order to limit the influence of environmental factors on the chemical composition. The leaf *n*-alkanes were extracted from the leaves with *n*-hexane and analysed using GC-MS. In all of the samples, *n*-C33 was the dominant alkane, with *n*-C29, *n*-C31 and *n*-C35 comprising over 80% of the *n*-alkane profile. The percentages of these dominant *n*-alkanes were taxon-specific, thus indicating their chemotaxonomic significance. In all the multivariate statistical analyses, samples belonging to different taxa were separated from each other. While the *n*-alkane profile can be used as a chemotaxonomic tool in the differentiation of these four taxa, it did not carry a phylogenetic signal. Instead, it reflected ecological adaptations, showing how different taxa have adapted to different arid environments within the study area.

Keywords:

cuticle, *n*-alkanes, junipers, chemotaxonomy, *Juniperus communis*, *Juniperus deltooides*, *Juniperus macrocarpa*

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INTRODUCTION

Juniperus L. is one of the most diverse genera of gymnosperms, second only to the genus *Pinus* (TUTIN *et al.* 1976; FARJON 2010; FARJON & FILER 2013). With around 60 different taxa, it inhabits mostly poor soils, predominantly in arid areas. It is also one of the rare gymnosperm genera which can be found in both the northern and southern hemispheres (FARJON 2001; ADAMS 2011). It owes its success to two features – its ability to thrive in arid environments, and the production of fleshy seed cones which are easily dispersed over long distances. The pollen and seed dispersion mechanisms in this genus are such that they ensure the almost constant flow of genes even between isolated populations, so all genetic diversity studies of junipers have shown high genetic diver-

sity and low differentiation of populations, even when the distribution is fragmented over vast areas (ADAMS *et al.* 2003; MICHALCZYK *et al.* 2010; MAZUR *et al.* 2021). Based on morphological and molecular data, the genus is split into three sections, the monotypic *Caryocedrus* Endlicher section, the subulate-leaf section *Juniperus* L. and the scalelike-leaf section *Sabina* (Miller) Spach. The phylogenetic relationship within each section remains unclear, with two major clades existing in the *Juniperus* section – red seed cone junipers related to *Juniperus oxycedrus* L. and blue seed cone junipers related to *Juniperus communis* L. (ADAMS 2011).

The cuticle is the outermost layer of every land plant. This important feature was one of the critical adaptations of land plants to life outside aquatic ecosystems. The cuticle is composed of an insoluble protein matrix made of

cutin, and a nonpolar amorphous wax mixture (YEATS & ROSE 2013). Based on their chemical composition and morphological features, these waxes are arranged into layers – the intracuticular and epicuticular wax. While there might be certain differences in the chemical composition of these two layers, this is due to the physicochemical properties of the cuticular wax components (SASANI 2021). The intracuticular layer is instrumental in water-proofing the epidermis, thus effectively reducing transcuticular transpiration. In contrast, the epicuticular layer can form different 3D structures, thus providing additional functions in plant-environment interactions (BARTHLOTT & NEINHUIS 1997). Studies in the past decade have shown that the chemical composition of the epicuticular layer is genetically determined and local chemotypes adapted to different chemotypes exist, thus showing the adaptive significance of this layer (RAJČEVIĆ *et al.* 2020 and ref. cited therein; CERDA-PÉÑA & CONTRERAS 2022; NIKOLIĆ *et al.* 2023). However, these studies have focused mainly on the differentiation of populations of individual species in relation to environmental and genetic factors, rather than their chemotaxonomic significance at the species level within this section.

Based on previous research (*cf.* Supplemental Figure S3 in RAJČEVIĆ *et al.* 2023) we hypothesise that the epicuticular wax composition can be used to separate samples from different taxa from the section *Juniperus*. Hence, the aim of this study was to analyse the epicuticular leaf *n*-alkanes in the section *Juniperus* from the Balkan peninsula using only sympatric populations so as to further limit the influence of environmental variability. The locations were chosen across the Balkans, taking into consideration different climate types according to the Koppen-Geiger classification (BECK *et al.* 2018) (*cf.* Table 1).

MATERIALS AND METHODS

Plant material. The plant material was collected in the field from 2009 to 2019 from wild-growing populations of *Juniperus deltoides* R.P. Adams (JD), *J. macrocarpa* Sm. (JM), *J. communis* L. var. *communis* (JCC), and *J. communis* L. var. *saxatilis* Pall. (JCS). Ten samples were taken from each population of adult individuals (main stem diameter above 2 cm) from undamaged branches at approximately 1/3 of the height of the individual in late summer (August–September). Branchlets with fully developed leaves (ca. 10 cm) were packed in paper bags and air-dried in a dark and very well-ventilated room prior to epicuticular wax extraction. Voucher specimens for all populations were deposited in the University of Belgrade Herbarium (BEOU). A list of localities and their geographic data is presented in Table 1.

To extract epicuticular waxes, approximately 1 g of leaves from each individual was washed briefly (1 min)

with 7 mL of *n*-hexane. The extract was evaporated in a fume hood, and purified using a microscale Florisil column with 5 mL of *n*-hexane. The obtained extracts were then concentrated under vacuum to 0.5 mL and analysed using GC-MS-FID (RAJČEVIĆ *et al.* 2023).

The GC/MS and GC/FID analyses. The analysis was performed using a 7890A apparatus equipped with an auto-injection system (Agilent 7683B Series), an inert 5975C XL EI/CI mass-selective detector (MSD) and a flame ionisation detector (FID) connected by a cap flow technology 2-way splitter with make-up, and an HP-5 MS capped column (30 m, 0.25 mm i.d., film thickness 0.25 mm). The GC oven temperature was programmed from 60 to 300°C at a rate of 3°C/min and held for 10 min. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). An auto-injection system (Agilent 7683B Series Injector) was employed to inject 1 µl of the sample. The sample was analysed in the splitless mode. The injector temperature was 250°C and the detector temperature 300°C. MS data was acquired in the EI mode with a scan range of 30–550 *m/z*, source temperature 230°C, and quadrupole temperature 150°C; the solvent delay was 3 min. A library search along with mass spectral deconvolution and extraction were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software version 2.64.113.71, using retention index (RI) calibration data analysis parameters with a ‘strong’ level and a 10% penalty for compounds without an RI. The search was performed against our own library, containing 4972 spectra. The relative abundance of the *n*-alkanes was calculated from the signal intensities of the homologues in the GC/FID traces.

Statistical analysis. Statistical analysis was performed using Past 4.11b software (HAMMER *et al.* 2001). To study the differentiation of the populations and taxa, Principal Component Analysis (PCA), Multivariate ANOVA (MANOVA), Discriminant Analysis (DA) and Hierarchical Cluster Analysis (HCA) were carried out on the raw (untransformed) data.

RESULTS

The chemical composition of the epicuticular leaf *n*-alkanes. Fourteen to sixteen *n*-alkanes ranging from 20 to 35 C atoms were detected and identified in 170 analysed samples. In all the samples, longer chain *n*-alkanes (C29–C35) were the most abundant, comprising over 85.0–99.1% of the total composition. In all the samples, C33 was the dominant *n*-alkane, however, its percentage varied depending on the locality and species. The highest abundance of C33 was detected in *J. communis* var. *communis*, while the lowest was found in *J. macrocarpa* and *J. deltoides* from Trogir. Three other *n*-alkanes

Table 1. Geographic information of the analysed populations of junipers in the Balkans

Locality	Country	Taxa ¹	Latitude [°N]	Longitude [°E]	K-G	BEOU
Mt. Durmitor	Montenegro	JCC, JCS	43.165	19.150	Dfb	17898, 16822
Mt. Kopaonik	Serbia	JCC, JCS, JD	43.312	20.914	Dfb	17408, 17409, 17410
Mt. Bistra	North Macedonia	JCC, JCS	41.625	20.682	Dfb	17216, 16818
Mt. Pindus	Greece	JCC, JD	39.907	20.782	Dsb	17174, 17179
Prilep	North Macedonia	JCC, JD	41.386	21.684	Bsk	18030, 18031
Rhodopes Mts	Bulgaria	JCC, JD	41.899	24.930	Dfb	17401, 17402
Mt. Suva	Serbia	JCC, JCS	43.197	22.144	Dfb	17396, 17398
Trogir	Croatia	JD, JM	43.569	16.246	Csa	17208, 18032

¹ JCC – *Juniperus communis* var. *communis*, JCS – *J. communis* var. *saxatilis*, JD – *J. deltooides*, JM – *J. macrocarpa*. Bsk – Arid cold steppe, Csa – Temperate with dry and hot summers, Dfb – cold without a dry season with warm summer, Dsb – Cold with dry and warm summers. K-G – Koppen-Geiger climate type (Beck *et al.* 2018); BEOU – University of Belgrade Herbarium voucher numbers.

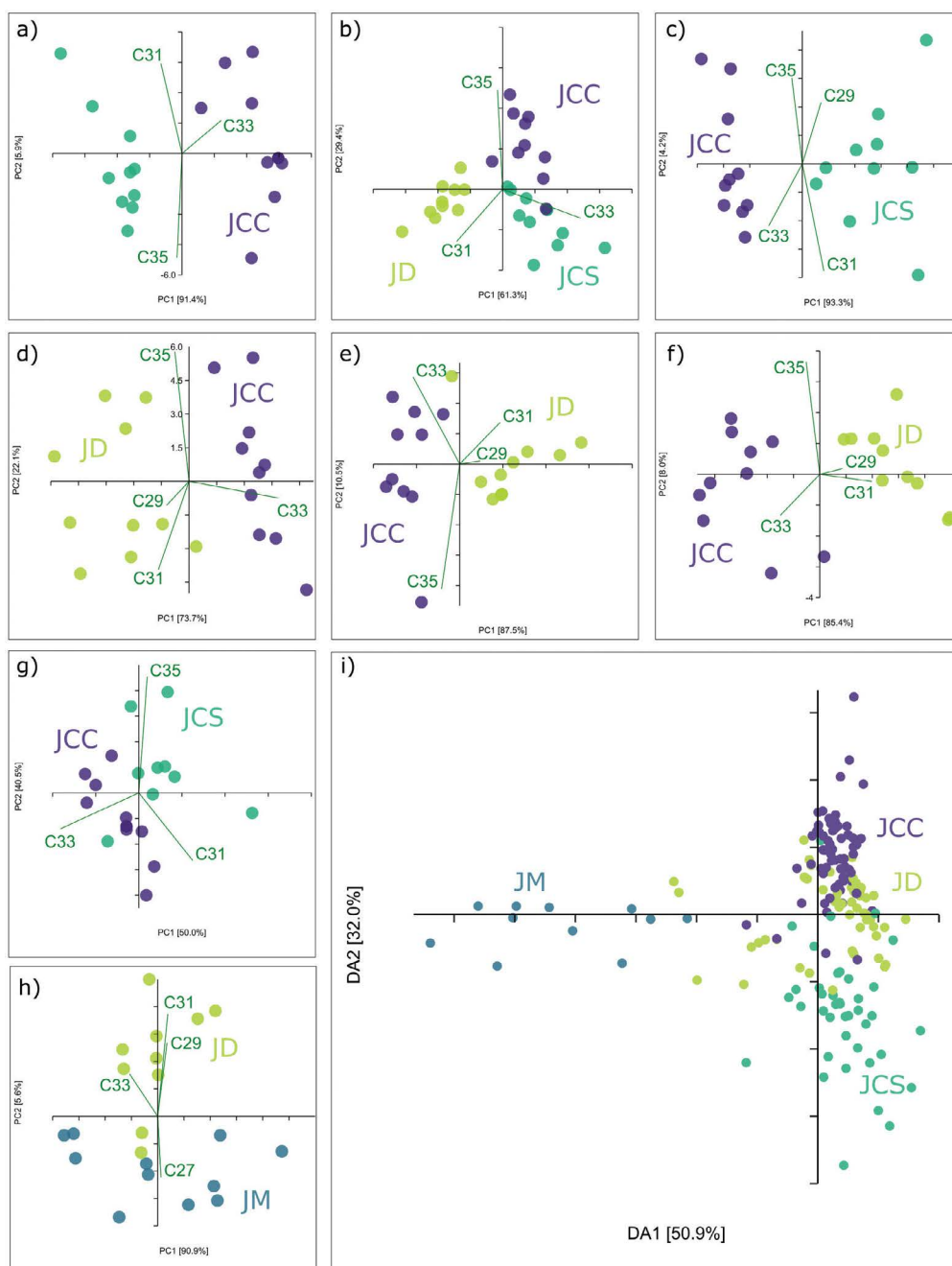


Fig. 1. a-h) PCA scatter plots of the leaf *n*-alkane profiles of individual localities of the Balkan junipers from the *Juniperus* section; a) Mt. Durmitor, b) Mt. Kopaonik, c) Mt. Bistra, d) Mt. Pindus, e) Prilep, f) Rhodopes Mts., g) Mt. Suva, h) Trogir; i) DA scatter plot of the leaf *n*-alkanes with taxa as groups; JCC – *Juniperus communis* var. *communis*, JCS – *J. communis* subsp. *saxatilis*, JD – *J. deltooides*, JM – *J. macrocarpa*.

Table 2. Leaf epicuticular *n*-alkane profiles of sympatric populations of junipers in the Balkans.

Locality	Mt. Durmitor		Mt. Kopaonik			Mt. Bistra		Mt. Pindus	
Taxon ¹	JCC	JCS	JCC	JCS	JD	JCC	JCS	JCC	JD
C20	tr	-	-	-	-	0.4±0.2	-	-	-
C21	0.2±0.1	-	-	-	-	0.5±0.2	-	0.1±0.0	0.1±0.0
C22	0.6±0.3	0.5±0.1	0.5±0.5	0.8±0.5	0.6±0.4	0.6±0.7	0.4±0.1	0.1±0.0	0.2±0.1
C23	0.8±0.4	0.5±0.2	tr	2.1±1.1	tr	0.4±0.1	0.6±0.2	0.3±0.1	0.4±0.1
C24	0.8±0.4	0.4±0.1	tr	tr	tr	0.4±0.1	0.5±0.2	0.6±0.2	1.5±1.6
C25	0.6±0.3	0.6±0.2	0.1±0.3	tr	tr	0.5±0.1	0.9±0.3	0.6±0.2	0.6±0.2
C26	0.3±0.2	0.4±0.1	0.1±0.3	tr	0.2±0.6	0.4±0.1	0.6±0.2	0.5±0.1	0.7±0.3
C27	0.5±0.3	1.4±0.4	tr	tr	0.2±0.7	1.2±0.2	2.5±0.6	0.5±0.1	0.6±0.2
C28	0.2±0.1	0.8±0.2	0.2±0.5	0.1±0.2	0.8±0.2	0.5±0.1	1.4±0.3	0.4±0.1	1.2±0.5
C29	3.2±1.1	8.4±1.0	2.0±0.6	1.9±1.1	6.2±2.1	5.9±1.3	17.0±4.0	1.9±0.3	4.4±0.8
C30	0.4±0.1	1.2±0.2	0.1±0.3	0.5±0.9	1.5±2.1	0.6±0.1	1.5±0.2	0.6±0.2	0.6±0.1
C31	9.9±3.4	18.5±3.6	6.5±1.5	9.0±2.4	15.2±2.9	10.6±2.4	23.4±4.2	7.3±1.3	11.1±1.7
C32	3.0±0.5	3.2±0.2	2.0±0.4	3.1±0.5	2.8±0.6	2.6±0.4	3.2±0.3	2.6±0.2	2.3±0.2
C33	74.4±3.1	56.1±2.6	69.4±3.2	73.2±4.5	60.6±2.0	64.1±1.4	44.1±5.7	72.6±1.8	63.8±3.0
C34	1.6±0.9	1.5±0.2	5.5±1.9	4.0±1.2	3.5±1.2	2.0±0.4	0.8±0.2	2.6±0.5	2.3±0.5
C35	3.5±2.9	6.4±1.2	13.6±3.4	5.3±2.8	8.3±1.9	9.2±3.5	3.1±1.3	9.4±2.9	10.2±2.4
F	9.863		12.42			17.68		19.36	
<i>p</i>	0.020		9.04×10 ⁻⁹			0.007		0.002	
Locality	Prilep		Rhodopes Mts.		Mt. Suva		Trogir		
Taxon ¹	JCC	JD	JCC	JD	JCC	JCN	JM	JD	
C20	-	-	0.1±0.1	-	-	-	-	-	
C21	-	-	0.2±0.1	-	-	-	-	-	
C22	-	0.1±0.0	0.6±0.1	0.2±0.1	0.3±0.3	1.2±0.9	0.3±0.1	0.2±0.1	
C23	0.1±0.0	0.3±0.2	1.2±0.3	0.5±0.2	0.2±0.3	0.2±0.3	0.6±0.1	0.3±0.1	
C24	0.1±0.0	0.2±0.2	1.2±0.3	0.5±0.2	tr	tr	1.0±0.3	0.5±0.2	
C25	0.2±0.1	0.2±0.2	0.8±0.3	0.4±0.1	tr	tr	2.3±0.9	1.3±0.5	
C26	0.2±0.1	0.2±0.1	0.4±0.2	0.2±0.1	0.2±0.3	0.1±0.2	2.2±0.9	1.3±0.5	
C27	0.4±0.2	0.6±0.2	0.7±0.6	0.6±0.2	0.1±0.2	tr	4.8±1.5	2.9±0.6	
C28	0.2±0.1	0.4±0.1	0.3±0.2	0.3±0.1	0.7±0.3	0.5±0.3	3.6±1.3	2.4±0.6	
C29	2.1±0.4	5.9±1.5	2.3±0.7	5.1±0.9	3.1±1.0	2.8±0.6	11.8±4.0	13.7±3.0	
C30	0.3±0.1	0.7±0.2	0.5±0.2	0.6±0.1	0.6±0.6	0.6±0.4	3.1±0.8	2.7±0.4	
C31	6.9±1.4	14.0±2.8	6.7±1.1	13.2±1.8	8.9±2.7	9.3±3.6	19.9±4.6	21.9±2.6	
C32	2.4±0.4	2.4±0.3	2.5±0.3	2.1±0.2	3.1±0.6	3.2±0.4	3.0±0.4	2.9±0.3	
C33	74.0±2.2	65.8±3.2	70.7±1.9	66.0±1.1	70.8±2.0	66.2±3.6	44.1±12.1	45.5±4.8	
C34	2.6±0.3	1.9±0.3	2.6±0.3	1.9±0.2	2.8±0.8	3.2±1.5	0.9±0.4	1.1±0.2	
C35	10.5±2.5	7.3±1.7	9.2±1.8	8.2±1.4	9.1±2.8	12.6±2.8	2.6±1.2	3.1±0.5	
F	11.31		17.48		3.453		11.73		
<i>p</i>	0.015		0.019		0.120		0.007		

¹ JCC – *Juniperus communis* var. *communis*, JCS – *J. communis* var. *saxatilis*, JD – *J. deltooides*, JM – *J. macrocarpa*; F, *p* – Results of MANOVA test. For each component the percentage is given as a mean ± SD values (n=10), tr - trace amounts (< 0.1%)

were also significantly present in the samples – C29, C31 and C35. In almost all of the *J. communis* var. *communis* and var. *saxatilis* populations, C35 followed by C31 were present in higher concentrations in the *n*-alkane profile, although the climatic influence was evident. For example, continental populations (Serbia, Bulgaria, and North Macedonia) exhibited higher concentrations of C35, while those growing closer to coastal climates had

more abundant C31, and lower C35 (Croatia and Montenegro). The same was observed for *J. deltooides*, however, in this species the overall domination of C33 in the *n*-alkane profile was lower, so the concentrations of C31 and C29 were much higher in comparison with the former two taxa. *J. macrocarpa* was studied from only one coastal locality, where the *n*-alkane profile was weakly dominated by C33, and high percentages of C29 and

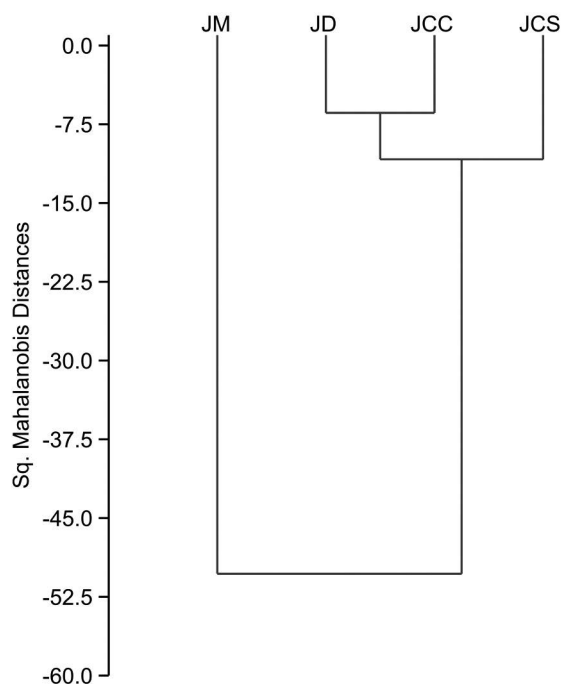


Fig. 2. HCA dendrogram (Sq. Mahalanobis distances, UPGMA) of the leaf *n*-alkane profile of four studied junipers JCC – *Juniperus communis* var. *communis*, JCS – *J. communis* subsps. *saxatilis*, JD – *J. deltooides*, JM – *J. macrocarpa*.

C31. Interestingly, the mid-length *n*-alkanes (C25–C28) were present in much higher percentage than the other species.

Statistical analysis. To study the differentiation of the taxa while minimising the influence of environmental factors, each of the sympatric populations was analysed separately (Fig. 1a–h). Since the studied species show only partial overlap in distribution, all but one of the localities had only two taxa present. There were no localities where all four of the studied taxa grew sympatrically. The PCA analysis showed moderate to strong separation based on the taxa pairs. For example, the lowest separation was found in the *J. deltooides*–*J. macrocarpa* pair growing in Trogir. In this locality individuals were separated strongly between taxa, but the separation was on the second axis only (5.6%). In all the other localities, the taxa separated strongly on the first axis (73.7–93.0%). In the locality where *J. communis* var. *communis*, *J. c.* var. *saxatilis* and *J. deltooides* grow sympatrically (Mt. Kopaonik), there is clear separation between the taxa (Fig. 1b). *Juniperus deltooides* separated from both varieties of *J. communis* on the first axis (61%), while var. *communis* and var. *saxatilis* separated on the second axis only. MANOVA for each of the populations showed a statistically significant difference between pairs of species at each locality, with the exception of *J. deltooides*–*J. macrocarpa* in Trogir (Table 2). In the population from

Mt. Kopaonik, Hotelling’s post-hoc test showed a more pronounced separation of *J. deltooides* from *J. communis* var. *communis* and var. *saxatilis* ($p = 0.01$ and 0.02 , respectively) than between the two varieties of *J. communis* ($p = 0.08$).

Discriminant analysis with all of the samples and taxa as groups showed the strongest separation of the *J. macrocarpa* *n*-alkane profile, while all the other profiles separated on the second axis. The confusion matrix showed the correct placement of 91.2% of all the individuals to the corresponding taxa. The highest placement was observed for *J. macrocarpa* (100%), and the lowest for *J. deltooides* (88%). Interestingly, the *J. deltooides* *n*-alkane profile exhibited an intermediate character, with the samples of this species positioned between var. *communis* and var. *saxatilis*. MANOVA also confirmed the statistically significant separation of all the taxa, as also shown in Hotelling’s post-hoc test between each pair of taxa. The squared Mahalanobis distances obtained in this test were plotted using HCA. This confirmed the strong separation of *J. macrocarpa* from all the other taxa. However, the *n*-alkane profile of *J. communis* var. *communis* was much more similar to that of *J. deltooides* than *J. c.* var. *saxatilis* (Fig. 2).

DISCUSSION

The presented results of the *n*-alkane profiles of four junipers from the Balkans show a similar profile to all other junipers, characterised by the dominance of C33, and a higher percentage of long-chain alkanes (RAJČEVIĆ *et al.* 2023 and ref. cited therein). The chemical profiles of *J. communis* var. *saxatilis* and *J. deltooides* correspond very closely to those previously published from the same localities, but from different individuals, showing both the chemical stability of *n*-alkanes over time, and their relative similarity among individuals. It is worth noting that the variability of the *n*-alkane profiles was taxon-specific (RAJČEVIĆ *et al.* 2020 and ref. cited therein). Even those samples collected from two varieties of *J. communis* showed significant separation in almost all the statistical tests.

As shown in previous investigations, the leaf *n*-alkane profile is dependent, at least in part, on the geographic distribution of the population, with the most striking differences observed between continental and coastal populations of *J. deltooides*. However, discriminant analysis also showed that the *n*-alkane composition was much more dependent on the taxon than on the geographical distribution of the population. Bearing that in mind, leaf *n*-alkane profiles not only show taxon-specific distribution and variability, but also reflect the evolutionary history of the taxa. *Juniperus communis*, a species with panarctic distribution, has the biggest distribution range in junipers, and possibly also among conifers. However, the species is more continental in its distribution, and

prefers colder climates (ADAMS 2011; FARJON & FILER 2013). Two varieties differ not only in terms of morphological characteristic, but also their distribution. *J. communis* var. *saxatilis* can only be found in colder climates, i.e. at high altitudes above the tree line in areas below 50° N, and at lower altitudes at higher latitudes, extending down to the sea level in the far north. On the other hand, both *J. deltooides* and *J. macrocarpa* have circum-Mediterranean distribution, and can be found predominantly in warmer (Mediterranean) climates. Bearing that in mind and taking previous findings into consideration, one would expect the Mediterranean taxa to have higher percentages of longer-chain *n*-alkanes since they grow in areas with hot dry summers. However, *J. communis* varieties, which are predominantly continental species, have a much higher percentage of longer-chain *n*-alkanes than the former taxa, which stands in contrast to angiosperms such as savory and *clinopodium* (DODOŠ *et al.* 2019 and refs. cited therein; JANKOVIĆ *et al.* 2024). This confirms the hypothesis that aridity exerts a much greater influence on the *n*-alkane composition than average or maximum temperatures (DODD & POVEDA 2003; RAJČEVIĆ *et al.* 2023 and refs. cited therein). Additionally, the morphology of the leaves and habitus of *J. communis* var. *saxatilis* is such that the species is better adapted to life above the tree line, so it appears that the cuticular layer is less derived under less stringent conditions than in var. *communis*. Nevertheless, this research has demonstrated that the leaf *n*-alkane profile is a valuable chemotaxonomic character for separating taxa within the section *Juniperus*, as also noted in the previous limited investigation of the section *Sabina* (RAJČEVIĆ *et al.* 2023). Discriminant analysis with individuals from different populations across the Balkans indicated their significant separation from each other. Most of the individuals (over 91%) were correctly placed to their corresponding taxa in the confusion matrix. However, the position of each taxon in the dendrogram did not correspond to the phylogenetics of the group (*J. deltooides* is phylogenetically closer to *J. macrocarpa* than *J. communis*, cf. ADAMS 2011), but rather to the ability of each taxon to adapt to a different type of arid area. It is interesting to note that *J. macrocarpa* has a very fragmented distribution in the Mediterranean region, with small isolated populations scattered from Spain to Israel exclusively in the coastal areas, while *J. deltooides* can be found much deeper in the Mediterranean peninsulas (ADAMS 2011). Also, unlike *J. deltooides*, *J. macrocarpa* is a tree, while even the oldest *J. deltooides* individuals grow as bushes. The unusual distribution of *J. macrocarpa* might even be linked to its very specific adaptations to the Mediterranean environment that once existed (ADAMS 2011).

CONCLUSIONS

The chemical composition of the leaf *n*-alkanes of four junipers from the section *Juniperus* in the Balkans shows taxon-specific variability. Studies of sympatric populations of different junipers have shown that leaf *n*-alkane composition is strongly determined by genetic factors, however, environmental factors also play a significant role over time, selecting chemotypes which are better adapted to the local climate. While all the taxa could be separated based on leaf *n*-alkane composition, the observed similarities do not reflect the phylogeny of the group.

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REZIME



Botanica
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Hemofenetička studija epikutikularnih alkana *Juniperus* L. sekcije *Juniperus* L. sa Balkana

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Juniperus je danas jedan od najraznovrsnijih rodova golosemenica. Prilagođen je sušnim područjima sveta. U ovoj studiji analizirali smo hemijski sastav *n*-alkana izolovanih sa površine listova 170 jedinki *Juniperus communis* subsp. *communis*, *J. communis* var. *saxatilis*, *J. deltoides* i *J. macrocarpa* sa Balkana. Za proučavanje hemijskog sastava izabrani su lokaliteti na kojima se nalaze barem dva taksona u simpatiji kako bi se ograničio uticaj faktora sredine na hemijski sastav. Epikutikularni alkani su isprani sa listova *n*-heksanom i analizirani korišćenjem gasne hromatografije sa masenom spektroskopijom. U svim uzorcima, *n*-C33 je bio dominantan alkan, pri čemu *n*-C29, *n*-C31 i *n*-C35 čine preko 80% profila *n*-alkana. Udeli dominantnih *n*-alkana bili su specifični za takson, odnosno pokazali su hemotaksonomski značaj. U svim multivarijantnim statističkim analizama, uzorci koji pripadaju različitim taksonima su odvojeni jedan od drugog. Dok se profil *n*-alkana može koristiti kao hemotaksonomski karakter u diferencijaciji ova četiri taksona, on nije nosio filogenetski signal, već ekološki – pokazujući kako su se različiti taksoni prilagodili različitim sušnim sredinama u oblasti istraživanja.

Ključne reči: kutikula, *n*-alkani, kleka, hemotaksonomija, *Juniperus communis*, *Juniperus deltoides*, *Juniperus macrocarpa*

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