

POPULATION DIVERSITY OF *n*-ALKANES IN THE NEEDLE CUTICULAR WAX OF RELICTS *PINUS HELDREICHII* AND *P. PEUCE* FROM THE SCARDO-PINDIC MOUNTAINS

Biljana Nikolić^{1*}, Vele Tešević², Iris Đorđević³, Marina Todosijević², Zorica Mitić⁴, Srdjan Bojović⁵, Petar D. Marin⁶

^{1*} Institute of Forestry, Kneza Višeslava 3, 11000 Belgrade, Serbia

² Faculty of Chemistry, University of Belgrade, Studentski trg 16, P.O. Box 158, 11000 Belgrade, Serbia

³ Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000 Belgrade, Serbia

⁴ University of Niš, Faculty of Sciences and Mathematics, Department of Biology and Ecology, Višegradska 33, 18000 Niš, Serbia

⁵ Institute for Biological Research “Siniša Stanković”, University of Belgrade, Boulevard Despota Stefana 142, 11060 Belgrade, Serbia

⁶ Faculty of Biology, Institute of Botany and Botanical Garden “Jevremovac”, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

smikitis@gmail.com

The variability of *n*-alkanes in the needle cuticular wax of *Pinus heldreichii* and *P. peuce* in two natural populations from the Scardo-Pindic mountains was investigated for the first time. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS) analyses of two-year-old needles were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass spectrometer detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology 2-way splitter with make-up. An HP-5MS capillary column was used. *n*-Alkanes ranged from C₁₉ to C₃₃. In *P. heldreichii* the most abundant were C₂₃, C₂₅ and C₂₇ (16.3, 15.6 and 12.8 % on average, respectively), while in *P. peuce* they were C₂₉, C₂₅, and C₂₇ (16.5, 15.3 and 13.5 % on average, resp.). Mid-chain and long-chain *n*-alkanes prevailed in both species. Principle component analysis (PCA) and Cluster analyses of five and six *n*-alkanes, respectively, showed divergence of the Scardo-Pindic populations from the Dinaric ones.

Keywords. *Pinus heldreichii*; *P. peuce*; *n*-alkanes, diversity, principle component analysis (PCA), cluster analysis

ПОПУЛАЦИСКИ ДИВЕРЗИТЕТ НА *n*-АЛКАНИ ВО ВОСОК ОД КУТИКУЛИ НА ИГЛИЧКИ ОД РЕЛИКТНИ *PINUS HELDREICHII* И *P. PEUCE* ОД СКАРДО-ПИНДИСКИТЕ ПЛАНИНИ

За прв пат беше истражена варијабилноста на *n*-алкани во восок од кутикули на иглички од *Pinus heldreichii* и *P. peuce* во две природни популации од скардо-пиндискиот планински масив. Беа извршени гасна хроматографска (GC) и гасна хроматографска/масено-спектрометриска (GC–MS) анализа, со употреба на Agilent 7890A GC опремен со инертен масен спектрометриски детектор (MSD) 5975C XL EI/CI и пламенен јонизациски детектор (FID), поврзани со капиларна проточна технологија со двонасочен сплитер со надоместување на гасови. Беше употребена капиларна колона HP-5MS. *n*-Алканите беа во опсегот од C₁₉ до C₃₃. Кај *P. heldreichii* најзастапени беа C₂₃, C₂₅ и C₂₇ (просечно 16,3 %, 15,6 % и 12,8 %, соодветно), додека кај *P. peuce* тоа беа C₂₉, C₂₅, and C₂₇ (просечно 16,5 %, 15,3 % и 13,5 %, соодветно). И кај двата вида преовладуваа *n*-алкани со средни и долги низи.

Анализата на главните компоненти (PCA) и кластер-анализата од соодветно пет и шест *n*-алкани покажа различност на скадро-пиндиските од динарските популации.

Клучни зборови: *Pinus heldreichii*; *P. peuce*; *n*-лакани; диверзитет; PCA; кластер-анализа

1. INTRODUCTION

Pinus heldreichii Christ. (Bosnian pine) is a Tertiary relict and a subendemic two-needle species. It grows naturally on the Balkan Peninsula and in southern Italy [1]. Several varieties, forms, and interspecific natural hybrids of *P. heldreichii* have been described [1].

Pinus peuce Griseb. (Macedonian pine) is a Tertiary relict and Balkan endemic five-needle species [1]. It grows naturally only on the Balkan Peninsula (on the high mountains of Bulgaria, North Macedonia, Montenegro, Serbia, Kosovo, Albania and the south of Greece).

These two pines grow alone or together on high mountains with different geologic substrata: limestones, silicates or schists [2, 3]. In addition, owing to their decorative characteristics, they are often present in horticulture parks in Serbia.

n-Alkanes were used in fundamental [4, 5] or applied investigations [6] as well as in chemotaxonomic studies [7, 8].

Leaf *n*-alkanes studies have already shown population divergence in both pine species from the Dinaric mountains [9, 10]. The aim of this study was to examine the population diversity of leaf *n*-alkanes of *P. heldreichii* and *P. peuce* originating from the Scardo-Pindic mountains and to compare them with previously examined populations originating from the Dinaric Alps [9, 10]. To the best of our knowledge, this is the first report of population variability of *n*-alkanes of *P. heldreichii* and *P. peuce* originating from the Scardo-Pindic mountains.

2. EXPERIMENTAL SECTION

Plant material. Two-year-old needles from two localities in the Scardo-Pindic mountains for both *P. heldreichii* (Mt. Baba – 8 trees; Mt. Šara – 16 trees) and *P. peuce* (Mt. Galičica – 10 trees; Mt. Šara – 14 trees) were collected from around the lower third of the unshaded tree crown at the end of the photosynthetic active season. Details about these populations have been already published [11, 12]. Twigs with needles were stored in polyethylene bags (with labels of the sample plot, date of collection, and age of the needles), and were transported to the freezer (–20 °C) in a hand fridge.

Extraction and isolation of needle wax. The concentrated extracts were chromatographed on a

small-scale column using a Pasteur pipette filled with silica gel 60 previously activated at 120 °C [13]. Wax was obtained by elution with 5 ml of *n*-hexane. Wax samples were stored at –20 °C until further analysis.

Chemicals and reagents. Hexane (HPLC grade) and silica gel 60 (0.2 – 0.5 mm) were purchased from Merck (Darmstadt, Germany).

GC and GC-MS analysis. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) analyses were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass spectrometer detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology 2-way splitter with make-up. An HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 60 °C to 300 °C at a rate of 3 °Cmin⁻¹ and held for 10 min. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). We used an auto-injection system (Agilent 7683B Series Injector) to inject 1 μl of the sample. The sample was analyzed in the splitless mode. The injector temperature was 250 °C and the detector temperature 300 °C. MS data were acquired in EI mode with scan range of 30–550 *m/z*, source temperature 230 °C, and quadrupole temperature 150 °C; solvent delay was 3 minutes.

Identification of *n*-alkanes. The components were identified based on their retention index and comparison with reference spectra (Wiley and NIST databases) as well as by the retention time locking (RTL) method and the RTL Adams data base. The retention indices were experimentally determined using the standard method of Van Den Dool and Kratz [14] involving retention times of *n*-alkanes injected after the sample under the same chromatographic conditions. The relative abundance of the *n*-alkanes (Tables 1, 2) was calculated from the signal intensities of the homologues in the GC-FID traces.

Calculations of CPI and ACL values. The carbon preference index of total odd-numbered and even-numbered LNAs (CPI_{total}) was calculated by the formula of Mazurek and Simoneit [15]. The average chain length of total odd-numbered and even-numbered LNAs (ACL_{total}) was calculated by using the formula of Poynter and Eglinton [16]. In order to compare the obtained results with those from literature sources, CPI_{25–33}, CPI_{20–36}, CPI_{15–21}, and CPI_{25–31} were also calculated by using the formula

of Bray and Evans [17] as well as ACL_{23-35} by using the formula of Poynter and Eglinton [16]. Relative proportions of short-, mid-, and long-chain *n*-alkanes ($n-C_{18-20}$, $n-C_{21-24}$ and $n-C_{25-33}$, respectively) were also calculated [18].

Statistical analysis. The calculation of mean values (\bar{X}), standard deviations (SD), test for normality (χ^2 test), one-way analyses of variance (ANOVA), Levene's test, principal-component analyses (PCA) and cluster analysis were all carried out with the software Statgraphics Plus (version 5.0; *Statistical Graphics Corporation*, U.S.A.).

3. RESULTS AND DISCUSSION

Variability of *n*-alkanes. *n*-Alkanes in *P. heldreichii* and *P. peuce* from the Scardo-Pindic mountains ranged from C_{19} to C_{33} (Table 1). The range of *P. heldreichii* var. *leucodermis* grown in nurseries is narrower (C_{18} – C_{31}) [8]. Some pines from the same subsection *Pinus*: *P. mugo* var. *pumilio*, *P. sylvestris*

and *P. sylvestris* var. *iberica* had a range of *n*-alkanes different from *P. heldreichii* [8]. A wider range was found in *P. mugo* (C_{18} – C_{33}) and a narrower range in *P. nigra* (C_{21} – C_{33}) and *P. thunbergii* (C_{27} – C_{29}).

In *P. heldreichii* needles from the Scardo-Pindic mountains, the most abundant *n*-alkanes were four odd-numbered: C_{23} , C_{25} , C_{27} and C_{29} (16.3 %, 15.6 %, 12.8 %, and 11.1 % on average, respectively) (Table 1), very often regarded as C_{max} values (Table 2). Populations from Mt. Šara (II) had a higher content of *n*-alkanes C_{25} and C_{27} than the population from Galičica (I) (Fig.1). Populations of *P. heldreichii* from the Dinaric massif had similar amounts of C_{23} , C_{27} and C_{25} (12.2 %, 11.2 % and 10.8 % on average, resp.) [10]. The domination of several *n*-alkanes was also characteristic of *P. mugo* and *P. pinea* [8]. However, sometimes *P. heldreichii* var. *leucodermis* and some other pines were rich in only one *n*-alkane: C_{31} [8], C_{33} [19] or C_{29} [20].

Table 1

Chemical composition of *n*-alkanes of *Pinus heldreichii* and *Pinus peuce* needles (%)

<i>n</i> -Alkanes	Populations											
	<i>P. heldreichii</i>				<i>P. peuce</i>				<i>P. heldreichii</i>		<i>P. peuce</i>	
	Mt. Galičica		Mt. Šara		Mt. Baba		Mt. Šara		Average		Average	
	I		II		I		II		I-II		I-II	
	\bar{X}^a	SD^b	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
C_{19}	0.36	0.07	0.35	0.18	0.39	0.13	0.51	0.11	0.36	0.15	0.45	0.13
C_{20}	1.25	0.28	1.35	0.45	1.01	0.23	1.15	0.24	1.32	0.40	1.08	0.24
C_{21}	4.34	0.63	4.50	0.87	3.05	0.73	3.51	0.60	4.44	0.78	3.28	0.70
C_{22}	7.69	0.78	7.62	0.48	5.49	1.17	5.91	1.21	7.64	1.26	5.70	1.19
C_{23}	16.61	2.36	16.11	1.28	13.47	2.18	13.45	1.40	16.29	1.69	13.46	1.80
C_{24}	10.59	1.15	10.53	1.17	7.80	1.23	7.34	1.00	10.55	1.14	7.57	1.13
C_{25}	14.04	1.55	16.44	1.61	14.66	1.88	16.00	2.02	15.60	1.95	15.33	2.04
C_{26}	5.28	0.41	4.94	0.42	4.54	0.43	4.09	0.37	5.06	0.44	4.31	0.45
C_{27}	11.80	1.39	13.34	2.18	12.50	1.46	14.47	1.12	12.80	2.05	13.48	1.62
C_{28}	4.23	0.52	3.80	0.36	4.99	0.75	3.96	0.44	3.95	0.46	4.47	0.80
C_{29}	11.51	2.33	10.91	1.16	17.59	4.13	15.45	1.73	11.12	1.63	16.52	3.30
C_{30}	1.70	0.51	1.32	0.61	2.94	1.67	2.68	0.81	1.45	0.60	2.81	1.30
C_{31}	5.54	1.80	5.16	1.21	5.61	2.26	6.31	1.81	5.29	1.41	5.96	2.04
C_{32}	1.78	0.47	1.46	0.40	2.34	0.80	2.07	0.50	1.57	0.44	2.20	0.67
C_{33}	3.30	1.78	2.16	0.42	3.62	0.92	3.09	0.78	2.56	1.20	3.36	0.88

^aMean value; ^bStandard deviation

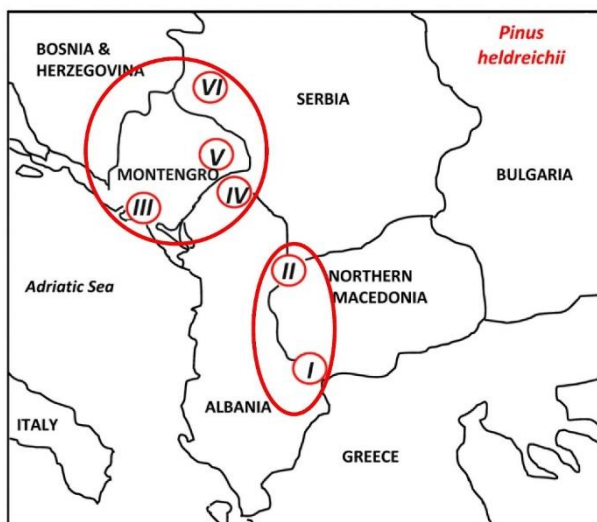


Fig. 1. Graphical illustration of *Pinus heldreichii* populations. Scardo-Pindic massif: Population I: Mt. Baba; Population II: Mt. Šara; Dinaric massif: Population III: Mt. Lovćen; Population IV: Mt. Zeletin; Population V: Mt. Bjelasica; Population VI: Mts. Zlatibor–Pešter;

In *P. peuce* needles, the most abundant were four odd-numbered *n*-alkanes: C_{29} , C_{25} , C_{23} , and C_{27} (16.5 %, 15.3 %, 13.5 %, and 13.5 % on average, respectively) (Table 2). Populations from Mt. Šara (II) had a higher content of *n*-alkanes C_{25} and C_{27} than the population from Mt. Baba (I) (Fig. 2). Populations of *P. peuce* from the Dinaric massif had similar amounts of C_{29} , C_{25} , C_{27} , and C_{23} (15.5 %, 11.1 %, 10.6 %, and 10.5 % on average, resp.) [21]. The domination of several *n*-alkanes was also characteristic of *P. cembra* var. *glauca* [21].

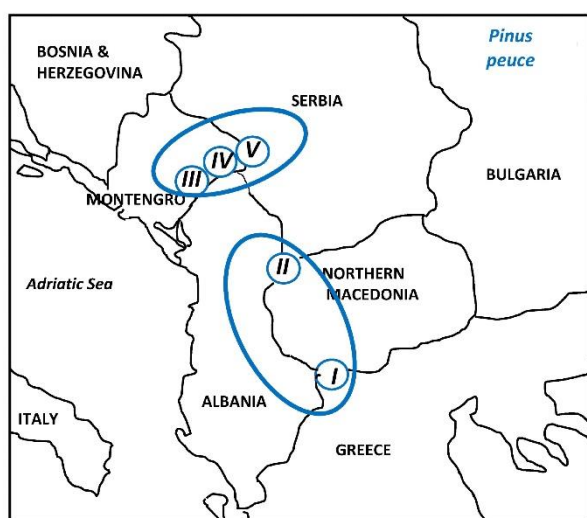


Fig. 2. Graphical illustration of *Pinus peuce* populations. Scardo-Pindic massif: Population I: Mt. Galičica; Population II: Mt. Šara; Dinaric massif: Population III: Mt. Zeletin; Population IV: Mt. Sjekirica; Population V: Mt. Mokra Gora.

Variability of CPIs and ACLs of *n*-alkanes.

For the calculation of the amounts of long-chain *n*-alkanes (LNAs), i.e., CPIs and ACLs of *n*-alkanes of *P. heldreichii* and *P. peuce* from the Scardo-Pindic mountains (Table 2), the relative values from Table 1 were used. Maximum CPI values of long-chain *n*-alkanes of *P. heldreichii* from Scardo-Pindic mountains are 2.9 (CPI_{20–36}), 4.2 (CPI_{25–31}), and 4.2 (CPI_{25–33}) (Table 2). Short-chain *n*-alkanes (CPI_{15–21}) ranged from 1.7 to 2.8 (2.2 on average) and exhibited odd/even predominance (OEP) (because CPI < 1 indicates EOP, CPI > 1 denotes OEP) [18]. However, in our previous investigations, populations from Montenegro also showed OEP, while the ones from Serbia exhibited EOP. The CPI_{total} of *P. heldreichii* from the Scardo-Pindic mountains is narrower but on average higher (1.6–2.8; 2.1 on average) than in the ones from the Dinaric massif (0.8–3.1; 1.6 on average) [18]. The ACL_{total} of *P. heldreichii* from the Scardo-Pindic mountains ranged from 24.9 to 26.5 (25.7 on average) and ACL_{23–35} ranged from 26.0 to 27.5 (26.4 on average) (Table 3). The ACL_{total} of *P. heldreichii* from the Dinaric mountains ranged from 20.9 to 26.5 (24.4 on average) and ACL_{23–35} ranged from 23.1 to 28.2 (26.1 on average) [9] (Table 2). The relative proportion of short-chain *n*-alkanes of *P. heldreichii* from the Scardo-Pindic mountains is small (n -C_{18–20} = 1.7 %), while long-chain *n*-alkanes dominate over mid-chain ones (59.4 % and 39.0 %, resp.) (Table 2). Short-chain *n*-alkanes of *P. heldreichii* from the Dinaric mountains were significant (n -C_{18–20} = 12.6 %) and varied individually to a great extent (from 0.0 to 26.8) [9]. Slight domination of long-chain (n -C_{25–33} = 49.6 %) over mid-chain *n*-alkanes (n -C_{21–24} = 37.9 %) was found, too.

Maximum CPI values of long-chain *n*-alkanes of *P. peuce* from the Scardo-Pindic mountains are 2.2 (CPI_{20–36}), 3.0 (CPI_{25–31}), and 3.0 (CPI_{25–33}) (Table 2) which are equal or smaller than in *P. peuce* from the Dinaric massif (2.3, 1.2, and 1.9, resp.) [10]. Short-chain *n*-alkanes (CPI_{15–21}) ranged from 1.0 to 4.3 (2.0 on average) and exhibited odd/even predominance (OEP) (because CPI < 1 indicates EOP, CPI > 1 denotes OEP). Short-chain *n*-alkanes (CPI_{15–21}) from the Dinaric massif ranged from 0.1 to 1.1 (0.6 on average) and exhibited even/odd predominance (EOP) [10]. The ACL_{total} of *P. peuce* from Scardo-Pindic mountains ranged from 24.9 to 26.6 (25.9 on average) and ACL_{23–35} ranged from 26.0 to 27.5 (26.6 on average) (Table 2). The ACL_{total} of *P. peuce* from the Dinaric mountains ranged from 0.9 to 5.5 (2.3 on average) and ACL_{23–35} ranged from 18.4 to 27.7 (25.7 on average) [10].

The relative proportion of short-chain *n*-alkanes of *P. peuce* from the Scardo-Pindic mountains is small ($n\text{-C}_{18-20} = 1.7\%$), while long-chain *n*-alkanes dominate over mid-chain ones (61.8% and 35.0%, resp.) (Table 3). Short-chain *n*-alkanes of *P. peuce* from the Dinaric mountains were also low ($n\text{-C}_{18-20} = 5.9\%$) and varied individually (from 0.0 to 20.1) [10]. Strong domination of long-chain ($n\text{-C}_{25-33} = 65.1\%$) over mid-chain *n*-alkanes ($n\text{-C}_{21-24} = 28.8\%$) was found.

Population diversity in regard to n-alkane variability. For multi-variation principle-component analysis (PCA), five *n*-alkanes of 88 trees of *P. heldreichii*

were selected (according to normal distribution, χ^2 , $P \geq 0.05$ and *Levene's* test, $P \geq 0.05$). In this study, previously examined populations from Montenegro and Serbia were added [9]. PCA revealed that the first two principal axes represent 68.7% of the total information (Fig. 3). Populations of *P. heldreichii* from the Scardo-Pindic mountains (I and II) overlapped and were clearly separated from populations Zeletin (IV) and Bjelasica (V). *n*-Alkanes which influenced the population diversity of *P. heldreichii* are presented in Table 3.

Table 2

Analytical data for *n*-alkanes in needles of *Pinus heldreichii* and *Pinus peuce* (%)

Population	C _{range}	C _{max}	CPI _{total} ^c	CPI ₂₅₋₃₃ ^d	CPI ₂₀₋₃₆ ^e	CPI ₁₅₋₂₁ ^f	CPI ₂₅₋₃₁ ^g	ACL _{total} ^h	ACL ₂₃₋₃₅ ⁱ	<i>n</i> -C ₁₈₋₂₀	<i>n</i> -C ₂₁₋₂₄	<i>n</i> -C ₂₅₋₃₃	
										Short-chain	Mid-chain	Long-chain	
In % of total <i>n</i> -alkanes (C ₁₉₋₃₃)													
<i>P. heldreichii</i>													
I	Range	19–33	23, 25, 27, 29	1.6–2.3	2.1–3.4	1.7–2.4	1.9–2.7	2.0–3.2	25.2–26.5	26.0–27.5	1.3–2.1	34.0–44.4	53.5–64.6
	Average		23	2.1	2.8	2.1	2.2	2.7	25.7	26.5	1.6	39.2	59.2
II	Range	19–33	25, 23, 27, 29	1.6–2.8	2.4–4.2	1.7–2.9	1.7–2.8	2.2–4.2	24.9–25.9	26.0–26.6	1.0–2.9	31.5–45.6	51.6–67.3
	Average		25	2.2	3.2	2.3	2.2	3.2	25.6	26.3	1.7	38.8	59.5
I–II	Range	19–33	23, 25, 27, 29	1.6–2.8	2.1–4.2	1.7–2.9	1.7–2.8	2.0–4.2	24.9–26.5	26.0–27.5	1.0–2.9	31.5–45.6	51.6–67.3
	Average		23	2.1	3.0	2.2	2.2	3.0	25.7	26.4	1.7	39.0	59.4
<i>P. peuce</i>													
I	Range	19–33	29, 25, 23, 27	1.5–2.7	2.5–4.1	2.2–3.4	0.1–0.9	2.4–4.1	25.8–27.1	26.4–27.6	0.8–2.0	20.0–37.8	60.3–79.2
	Average		29	2.0	3.1	2.5	0.5	3.3	26.4	26.9	1.4	29.8	68.8
II	Range	19–33	25, 29, 27, 23	1.8–2.6	2.8–4.9	2.1–4.1	0.2–1.1	2.7–4.9	25.9–26.6	26.4–27.3	1.0–2.2	26.5–34.2	63.6–71.8
	Average		25	2.1	3.6	2.8	0.6	3.4	26.2	26.8	1.7	30.2	68.1
I–II	Range	19–33	29, 25, 27, 23	1.6–2.8	2.1–4.9	1.7–4.1	0.2–2.8	2.0–4.9	24.9–26.6	26.0–27.5	1.0–2.9	26.5–45.6	51.6–71.8
	Average		29	2.1	3.3	2.5	1.4	3.2	25.9	26.6	1.7	35.0	61.8

^cCPI_{total} = $\sum \text{odd } C_n / \sum \text{even } C_n$ [15], C_n is the concentration of *n*-alkane containing *n* carbon atoms;

^dCPI₂₅₋₃₃ = $[\sum (C_{25} - C_{33})\text{odd} / \sum (C_{24} - C_{32})\text{even} + \sum (C_{25} - C_{33})\text{odd} / \sum (C_{26} - C_{34})\text{even}] / 2$ [17],

^eCPI₂₀₋₃₆ = $[\sum (C_{20} - C_{36})\text{odd} / \sum (C_{19} - C_{35})\text{even} + \sum (C_{20} - C_{36})\text{odd} / \sum (C_{21} - C_{37})\text{even}] / 2$ [17],

^fCPI₁₅₋₂₁ = $[\sum (C_{15} - C_{21})\text{odd} / \sum (C_{14} - C_{20})\text{even} + \sum (C_{15} - C_{21})\text{odd} / \sum (C_{16} - C_{22})\text{even}] / 2$ [17],

^gCPI₂₅₋₃₁ = $[\sum (C_{25} - C_{31})\text{odd} / \sum (C_{24} - C_{30})\text{even} + \sum (C_{25} - C_{31})\text{odd} / \sum (C_{26} - C_{32})\text{even}] / 2$ [17],

^hACL_{total} = $(\sum C_n \times n) / \sum C_n$ [16],

ⁱACL₂₃₋₃₅ = $(23 \times C_{23} + 25 \times C_{25} + 27 \times C_{27} + 29 \times C_{29} + 31 \times C_{31} + 33 \times C_{33} + 35 \times C_{35}) / (C_{23} + C_{25} + C_{27} + C_{29} + C_{31} + C_{33} + C_{35})$ [16].

Table 3

n-Alkanes (also quoted in Fig. 4) which influenced the diversity of *Pinus heldreichii* populations

<i>Pinus heldreichii</i>					
Scardo-Pindic mountains *			Dinaric mountains **		
Mt. Galičica	Mt. Šara	Mt. Lovćen	Mt. Zeletin	Mt. Bjelasica	Mt. Zlatibor–Pešter
I	II	III	IV	V	VI
C ₂₃ , 16.61%	C ₂₅ , 16.44%	C ₂₃ , 13.48%	C ₂₃ , 12.35%	C ₂₅ , 11.38%	C ₂₇ , 12.23%
C ₂₅ , 14.04%	C ₂₃ , 16.11%	C ₂₈ , 3.96%		C ₂₇ , 11.23%	C ₂₈ , 4.13%
C ₂₇ , 11.80%	C ₂₇ , 13.34%				

* - original data, ** - literature data [9]

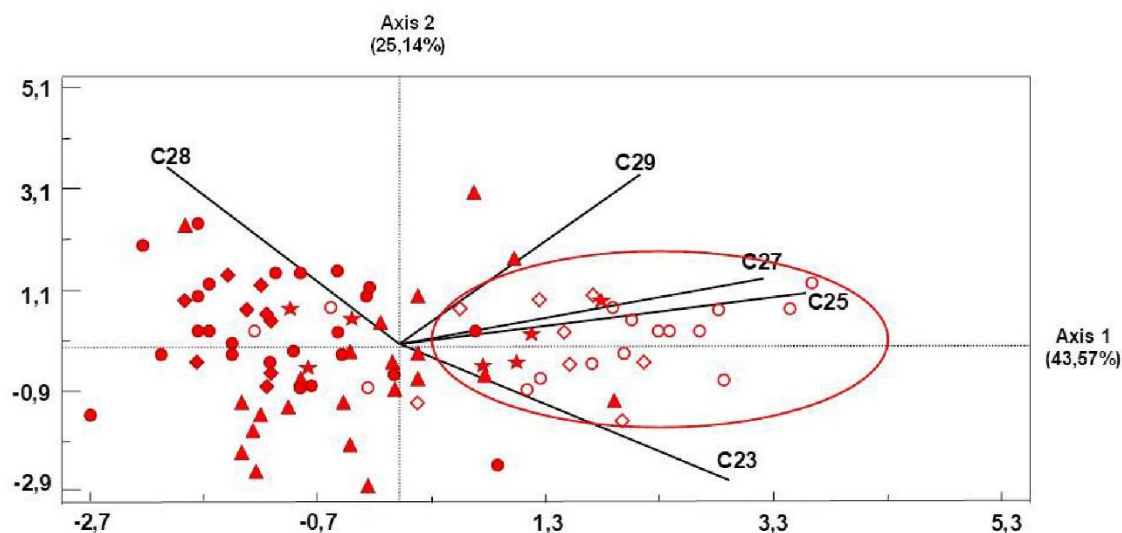


Fig. 3. Principle-component analysis of five selected *n*-alkanes isolated from 88 Bosnian pine-tree samples from six populations. *n*-Alkanes: C₂₃, C₂₅, C₂₇, C₂₈, and C₂₉. Population I: Mt. Baba ◊; Population II: Mt. Šara ○; Population III: Mt. Lovćen ▲; Population IV: Mt. Zeletin ◆; Population V: Mt. Bjelasica ●; Population VI: Mts. Zlatibor-Pešter ★

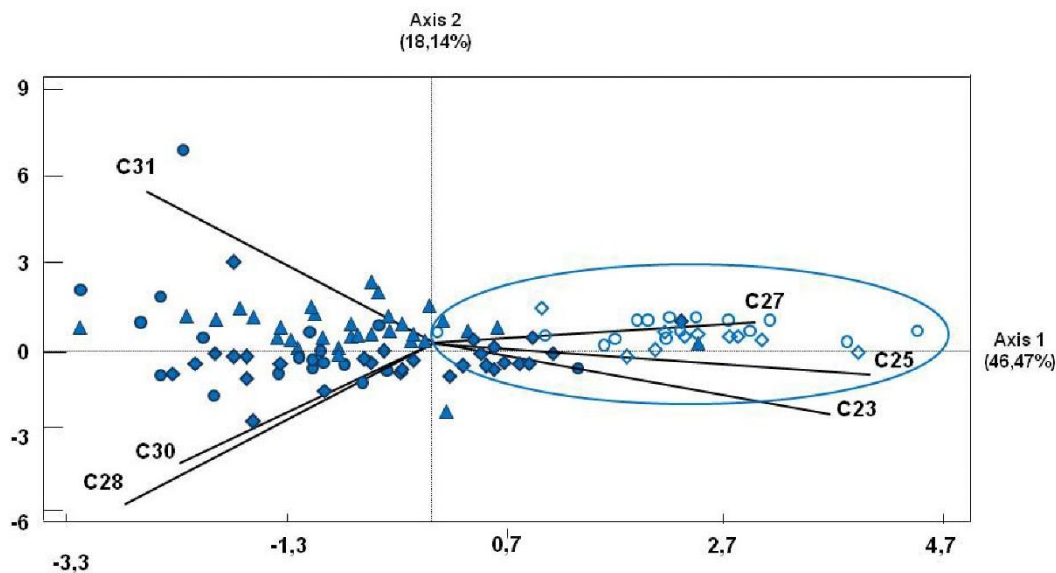


Fig. 4. Principle-component analysis of five selected *n*-alkanes isolated from 109 Macedonian pine-tree samples from five populations. *n*-Alkanes: C₂₃, C₂₅, C₂₇, C₂₈, C₃₀ and C₃₁. Population I: Mt. Galičica ◊; Population II: Mt. Šara ○; Population III: Mt. Zeletin ▲; Population IV: Mt. Sjekirica ◆; Population V: Mt. Mokra Gora ●

Table 4

n-Alkanes (also quoted in Fig. 5) which influenced the diversity of *Pinus peuce* populations

<i>Pinus peuce</i>				
Scardo-Pindic mountains *		Dinaric mountains **		
Mt. Baba	Mt. Šara	Mt. Zeletin	Mt. Sjekirica	Mt. Mokra Gora
I	II	III	IV	V
C ₂₉ , 17.59 %	C ₂₅ , 16.00 %	C ₂₉ , 14.44 %	C ₂₉ , 16.44 %	C ₂₉ , 15.71 %
C ₂₅ , 14.66 %	C ₂₉ , 15.45 %	C ₂₃ , 11.91 %	C ₃₁ , 9.95 %	C ₃₁ , 9.87 %
C ₂₃ , 13.47 %	C ₂₇ , 14.47 %	C ₂₅ , 11.57 %	C ₃₀ , 4.11 %	C ₃₀ , 4.83 %

* - original data, ** - literature data [10]

For PCA of *P. peuce* populations, six *n*-alkanes of 109 trees of *P. peuce* were selected (according to normal distribution, χ^2 , $P \geq 0.05$ and Levene's test, $P \geq 0.05$). In this analysis previously examined populations from Montenegro and Serbia were added [9]. PCA revealed that the first two principal axes represent 64.9 % of the total information (Fig. 4). Populations of *P. peuce* from the Scardo-Pindic mountains (I and II) overlapped and were clearly separated from other populations: Zetletin (III) and Bjelasica (IV) and Mokra Gora (V). *n*-Alkanes which influenced population diversity of *P. peuce* are presented in Table 4.

Cluster analyses. Cluster analyses of populations of both species confirm the separation obtained by PCA (Figs. 5 and 6).

It should be noted that needles of *P. heldreichii* and *P. peuce* were sampled in the late summer or early autumn. However, in some literature, the needles of some pines were collected in spring [8, 19, 20]. The range of seasonal variation of *Pinus* needles is still unknown. Furthermore, the carbon isotopic composition of *n*-alkanes is surely under the influence of environmental factors [22–24].

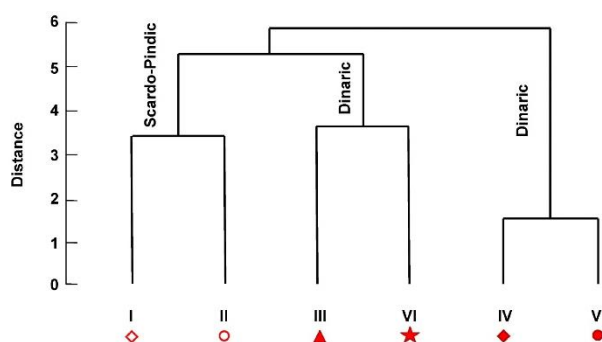


Fig. 5. Dendrogram based on a 'nearest-neighbor method' (square Euclidean distance) of the studied populations I–VI (mean values) of *P. heldreichii*. The numbers on the vertical axis refer to the distance level calculated on the basis of differences between population contents of selected components.

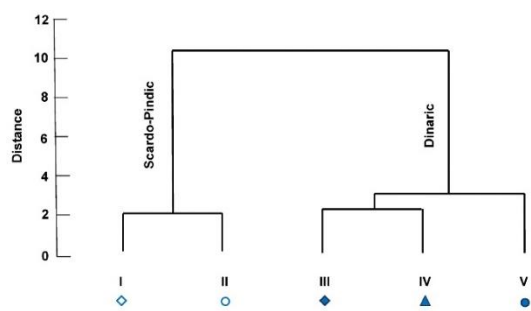


Fig. 6. Dendrogram based on a 'nearest-neighbor method' (square Euclidean distance) of the studied populations I–V (mean values) of *P. peuce*. The numbers on the vertical axis refer to the distance level calculated on the basis of differences between population contents of selected components.

4. CONCLUSIONS

The populations of *P. heldreichii* and *P. peuce* from the Dinaric (previous results) and Scardo-Pindic mountains (present data) diverge in terms of *n*-alkane composition. Strong domination of long-chain over mid-chain *n*-alkanes was found in both species. Differences in *n*-alkane range and abundance were confirmed in some other pine species and their varieties [8]. These differences could be conditioned by genetic differentiation and/or the influence of various ecological factors.

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