

VARIABILITY OF NEEDLE MORPHO-ANATOMY OF NATURAL *Pinus heldreichii* POPULATIONS FROM SCARDO-PINDIC MOUNTAINS

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Eight morpho-anatomical properties of two-year-old needles of *Pinus heldreichii*
(Bosnian pine) from the Scardo-Pindic mountain massif in Serbia (Kosovo, Mt. Ošljak)
and North Macedonia (Mt. Galičica) were investigated. All measured characteristics,
except for needle length, were inspected on mid-needle cross-section. Cross-sections were
obtained with razorblade, while measurements were performed with *Leica-Gallen III* light
microscope. The mean values of the analyzed characters were as follows: 5.91 cm (needle
length), 1.35 mm (needle width), 0.85 mm (needle thickness), 25.05 μm (cuticle +

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epidermis thickness), 69.90 μm (height of hypodermal cells), 21.76 μm (resin duct diameter), 3.4 (number of hypodermis layers), and 3.6 (number of resin ducts). *P. heldreichii* needles also had 2-5 hypodermis layers and 0-12 resin ducts. The highest variation was in the number of resin ducts (CV=27%). In comparison with previously investigated needles from the Dinaric mountains, *P. heldreichii* needles from the Scardo-Pindic massif were shorter and had a thicker layer of hypodermis. PCA and CA visualize partial segregation of *P. heldreichii* populations between the two mountain massifs.

Keywords: Bosnian pine, needle morphology, needle anatomy, PCA, Cluster Analysis

INTRODUCTION

The Bosnian pine (*Pinus heldreichii* Christ.) or "white bark pine" is a tertiary relict and Balkan subendemit which naturally occupies fragmented areas of high mountains in southern Italy, Bosnia and Herzegovina, Serbia, Montenegro, Bulgaria, North Macedonia, Albania, and Greece (VIDAKOVIĆ, 1982). This two-needle pine has several varieties: 1) var. *typica* Markgraf, wide spread in Albania and Greece, 2) var. *leucodermis* (ant.) Markgraf in Montenegro, 3) var. *panicci* Fukarek described in south-western Serbia and northern Montenegro and 4) var. *longiseminis* Papaioannou in North Macedonia (VIDAKOVIĆ, 1982). Two forms, *vivipara* in Serbia and *leucodermoides* as well two spontaneous hybrids: *P. x mugodermis* and *P. x nigradermis* in Bosnia and Herzegovina and transitional forms between *typica* and *leucodermis* in North Macedonia were also described (TUČOVIĆ and STILINOVIĆ, 1971; FUKAREK, 1960; FUKAREK and NIKOLIĆ, 1974; NIKOLOVSKI and MATVEJEVA, 1975, respectively).

Investigation of morpho-anatomical properties of conifers was sometimes significant in resolving problems in distinguishing hybrids, species, sub-species, populations and provenances (CALAMASSI *et al.*, 1988; KRIEBEL and FOWLER, 1965; MERGEN, 1958; SNYDER and HAMAKER, 1978).

There were a lot of papers dealing with the morpho-anatomical structure of conifer needles depending on environmental factors (JOKANOVIĆ *et al.*, 2018; VESELINOVIĆ *et al.*, 2017; etc). Needles of *P. heldreichii* populations from the Dinaric massif were also examined (NIKOLIĆ *et al.*, 2014). To the best of our knowledge, this is the first population study of morphometry and anatomy of needles of *P. heldreichii* from the Scardo-Pindic mountains (Mt. Ošljak and Mt. Galičica). Differences in needle properties between Scardo-Pindic and Dinaric populations were examined, too.

MATERIALS AND METHODS

Material collection

Two-year-old needles were collected from two natural Scardo-Pindic populations of *P. heldreichii*: 1) in South Serbia (Kosovo, Mt. Ošljak, Šara massif) and 2) in North Macedonia (Mt. Galičica). Measurements were conducted on 16 trees from Mt. Ošljak and 8 trees from Mt. Galičica with ten repetitions (needles) per tree (ca 240 needles). Locations of the analyzed Scardo-Pindic populations, as well as four populations from the Dinaric Mountains previously studied by NIKOLIĆ *et al.* (2014), were also presented in Fig. 1. Details on the environmental conditions of the selected *P. heldreichii* populations have already been reported (NIKOLIĆ *et al.*, 2014).

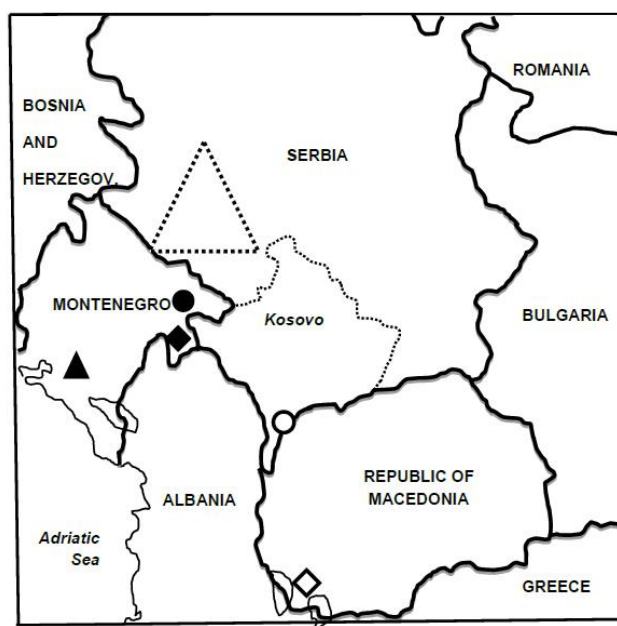


Fig. 1. Location of analyzed populations of *P. heldreichii*: ▲ - Mt. Lovćen, ◆ - Mt. Zeletin, ● - Mt. Bjelasica, △ - Mt. Zlatibor-Mt. Pešter; ○ - Mt. Ošljak, and ◇ - Mt. Galičica

Morpho-anatomical properties of the needles

All the measured characteristics, except for needle length, were inspected on mid-needle cross-section (Fig. 2), since this is the region with the most resin ducts (LIN *et al.*, 2001). Cross-sections were obtained with razorblade, while measurements were performed with *Leica-Gallen III* light microscope. Details on the microscopic analyses are given in Figure 2.

Table 1. Morpho-anatomical properties of *P. heldreichii* needles from Scardo-Pindic mountains

Needle characteristics	Range (min - max)	$X \pm S_x$	CV
1 Needle length (cm)	3.4 - 8.1	5.91 \pm 0.76	20%
2 Needle width (μm)	856.51 - 1886.51	1494.27 \pm 3.93	4%
3 Needle thickness (μm)	80.51 - 1815.96	855.57 \pm 4.26	5%
4 Cuticle + epidermis thickness (μm)	9.25 - 69.33	25.05 \pm 0.20	7%
5 Height of hypodermal cells (μm)	29.15 - 140.84	69.90 \pm 0.44	13%
6 Resin duct diameter (μm)	0 - 103.41	46.91 \pm 0.36	25%
7 Number of hypodermis layers	2 - 4	3.35 \pm 0.03	6%
8 Number of resin ducts	0 - 12	3.58 \pm 0.03	27%

Notes: X, arithmetic mean; S_x , standard error of the mean; CV, coefficient of variation.

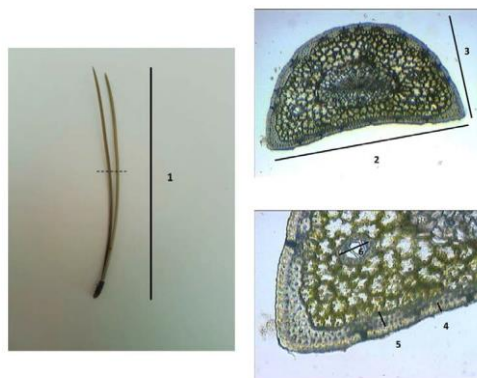


Fig. 2. Needles of *Pinus heldreichii* (1) and its cross section. Numbers 2-6 denote the anatomical characters (as listed in Table 1).

Statistical analyses

Statistically analysed morpho-anatomic characteristics of the needles are given in Tables 1 and 2. One-way analyses of variance (ANOVA), homogenous groups obtained by LSD test ($P \leq 0.05$), Linear correlation, Principal component analyses (PCA) and Cluster analyses (CA) ('Nearest Neighbor method' - squared Euclidean distance) were carried out using *Statgraphics Plus* version 5.0 (Statistical Graphics Corporation, USA).

RESULTS

On cross-section, Bosnian pine needles are lunate, rarely triangular (rarely, three needles appear in the same sheet) (Figs. 2,3). Resin ducts are immersed in mesophyll (internal type). The average values of the needle characteristics of *P. heldreichii* from the Scardo-Pindic mountains are presented in Table 1. Inserted coefficient of variation values (CV, Table 1) showed that the number of resin ducts (CV=27%) was the most variable property.



Fig. 3. Different shapes of *Pinus heldreichii* needles from Scardo-Pindic mountains (cross section).

Variance analysis ($P \leq 0.05$, LSD test) established the existence of significant differences among populations in all the needle characteristics analysed, especially in needle length and width (Table 2).

Table 2. Morpho-anatomical properties of needles of Bosnian pine of natural populations from Mt. Ošljak and Mt. Galičica

	Needle traits	Mt. Ošljak		Mt. Galičica	
		Range (min – max)	X ± Sx	Range (min – max)	X ± Sx
1	Needle length (cm)	4.70 – 8.10	6.44 ± 0.65 b*	3.40 – 7.20	4.83 ± 1.16 a
2	Needle width (µm)	856.51 – 1705.36	1434.49 ± 4.30 a	1397.01 – 1886.00	1613.81 ± 4.75 b
3	Needle thickness (µm)	80.51 – 1391.16	834.64 ± 4.29 a	752.08 – 1815.96	897.43 ± 7.07 b
4	Cuticle + epidermis thickness (µm)	12.50 – 69.33	24.72 ± 0.21 a	9.25 – 68.78	25.72 ± 0.43 a
5	Height of hypodermal cells (µm)	29.15 – 140.84	65.60 ± 0.51 a	38.08 – 110.03	78.51 ± 0.71 b
6	Resin duct diameter (µm)	0 – 93.37	43.00 ± 0.67 a	0 – 103.41	54.47 ± 1.07 b
7	Number of hypodermis layers	2 - 4	3.26 ± 0.04	2 - 4	3.55 ± 0.06
8	Number of resin ducts	0 - 12	3.20 ± 0.18	0 - 9	4.34 ± 0.27

*a, b: homogenous groups.

Table 2 points that the population from Mt. Ošljak has significantly longer and narrower needles, as well as lower needle thickness, height of hypodermal cells and resin duct diameter. The population from Mt. Galičica has a little higher cuticle+epidermis thickness. Needle width and thickness, height of hypodermis cells, height of hypodermis cells and resin duct diameter tend to rise in the populations in a north-east direction (Table 2, Fig. 1). Coefficients of variations were also added in Table 1.

Correlation analysis of the morpho-anatomical characteristics pointed almost weak correlations between the analysed traits (Table 3). Only two correlations: between needle width (NW) and needle thickness (NT), and between resin duct diameter (RDD) and number of resin ducts (NRD), are nearly moderately strong ($r = 0.45$ and 0.43 , respectively). Needle length (NL) is in a weak negative correlation with all the traits, especially with the height of hypodermal cells (HHC) ($r = -0.34$).

PCA results were obtained from all eight morpho-anatomical characteristics (Fig. 4). PCA showed that the first two axes represent 57.14% of the information and visualizes a particular separation between the populations from the Scardo-Pindic and Dinaric massifs. The Serbian population emerged as most distant from Scardo-Pindic mountains.

Table 3. Corellation coefficients of needle traits of *P. heldreichii* from Scardo-Pindic mountains. *NL – needle length; NW – needle width; NT – needle thickness; CET – cuticle + epidermis thickness; HHC - height of hypodermal cells; RDD – resin duct diameter; NHL - number of hypodermis layers ; NRD – number of resin ducts

NW	-0.17						
NT	-0.02	0.45					
CET	-0.02	0.07	0.07				
HHC	-0.34	0.29	0.11	0.08			
RDD	-0.07	0.26	0.08	0.13	0.14		
NHL	-0.15	0.22	0.04	-0.06	0.24	0.00	
NRD	-0.01	0.30	0.27	-0.03	0.08	0.43	0.02
	*NL	NW	NT	CET	HHC	RDD	NHL

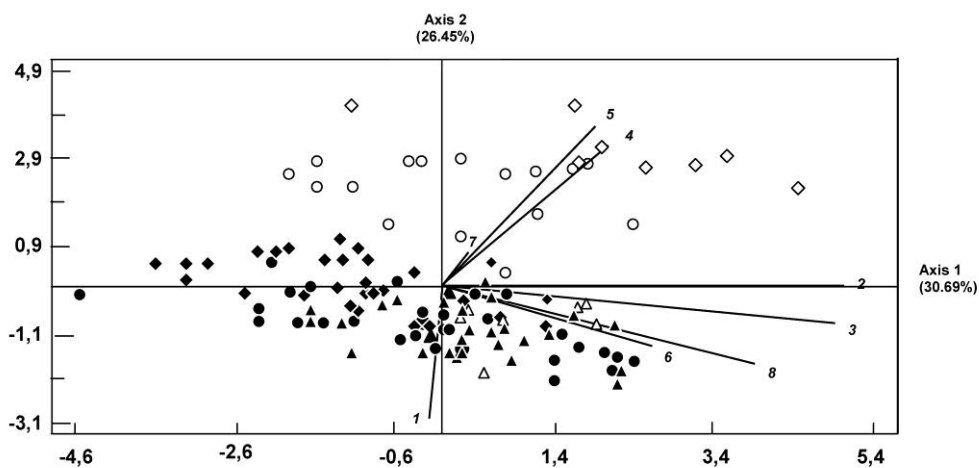


Fig. 4. Principle-component analysis (PCA) of eight morpho-anatomical traits of *Pinus heldreichii*: needle length (1), needle width (2), needle thickness (3), cuticle + epidermis thickness (4), height of hypodermal layer (5), resin duct diameter (6), number of hypodermic layers (7) and number of resin ducts (8). Symbols refer to populations are given in Fig. 1.

Cluster analysis was conducted using the same morpho-anatomical properties (Fig. 5). The cluster analysis clearly separated the populations of Dinaric and Scardo-Pindic massifs, with the Serbian population being the most distant.

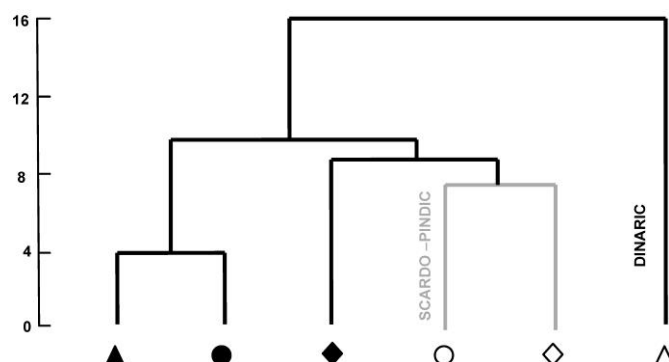


Fig. 5. Dendrogram of eight morpho-anatomical traits of *Pinus heldreichii* based on a 'Nearest Neighbor method' (squared Euclidean distance). Symbols refer to populations are given in Fig. 1.

DISCUSSION

The needles of *P. heldreichii* from the Scardo-Pindic mountains are significantly smaller and have smaller resin ducts compared with needles from Dinaric mountains, but somewhat higher values of needle width (NIKOLIĆ *et al.*, 2014). There are no great differences in needle thickness between the Bosnian pines from these two massifs. Our needles from Mt. Ošljak are shorter than those from the same mountain measured by GUDESKI *et al.* (1975), and needles from Prokletije (STEVANOVIĆ-JANEŽIĆ and VILOTIĆ, 1998), Greece (PAPAIOANNOU, 1975) and Bulgaria (YURUKOV *et al.*, 2005; PANAYOTOV *et al.*, 2010, 2011), too. Needle width is a little smaller than the findings of GUDESKI *et al.* (1975), similar to Serbian needles and smaller than the findings of POPNIKOLA (1978) and NIKOLIĆ *et al.* (2014). Our population from Mt. Ošljak also has a higher thickness of the hypodermal layer. On the other hand, our needles from Mt. Galičica are shorter than all the other findings. Its mean value of resin duct diameter is similar to the other results. The great variability in the number of hypodermis layers and number of resin ducts, as well as presence and absence of resin ducts on the basal (inner) side of the needle may confirm our previous assumptions (based on the hypothesis of FUKAREK and VIDAKOVIĆ, 1965, after FUKAREK, 1979) that we found *P. leucodermis* Antoine trees and those created by its crossbreeding with different subspecies of *P. nigra* (*P. nigra* x *P. leucodermis*). The proof is a great variation in number of hypodermis layers (1-5) and number of resin ducts (0-12). CV is also the highest in number of resin ducts (27%).

CONCLUSIONS

Limestones are the basic geological substrata, both in the Scardo-Pindic and Dinaric populations of Bosnian pine (NIKOLIĆ *et al.*, 2007, 2015). According to the cluster analysis presented in this paper, the closest populations are those whose geological substrata contain shists in addition to limestone: the Scardo-Pindic population Galičica (NIKOLIĆ *et al.*, 2015) and

Dinaric population Zeletin (NIKOLIĆ *et al.*, 2007). The most distant is the Serbian population Zlatibor-Pešter (Dinaric massif), where several geological substrata were found (NIKOLIĆ *et al.*, 2007). The influence of geological substrata on the morpho-anatomical characteristics of plants has long been recognized (JAKOVLJEVIĆ *et al.*, 2013, and refs. cited therein). Besides, the morpho-anatomy of plants is also influenced by climate conditions (KUZMANOVIĆ *et al.*, 2011, and refs. cited therein), which we will study in greater detail in another paper.

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**VARIJABILNOST MORFO-ANATOMIJE IGLICA PRIRODNIH POPULACIJA
Pinus heldreichii SA ŠARSKO-PINDSKIH PLANINA**

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Izvod

Ispitivano je 8 morfo-anatomskih osobina dvogodišnjih iglica *Pinus heldreichii* (munike) Šarsko-Pinskog planinskog masiva u Srbiji (Kosovo, planina Ošljak) i Severnoj Makedoniji (planina Galičica). Sve merene osobine, osim dužine četina, su ispitivane na poprečnom preseku na sredini četine. Poprečni preseki su sečeni žiletom, dok su njihova merenja sprovedena na *Leica-Gallen III* svetlosnom mikroskopu. Srednje vrednosti analiziranih osobina bile su sledeće: 5.91 cm (dužina iglice), 1.35 mm (širina iglice), 0.85 mm (debljina iglice), 25.05 μm (debljina kutikule i epidermisa), 69.90 μm (visina hipodermalnih ćelija), 21.76 μm (prečnik smonog kanala), 3.4 (number of hypodermis layers), and 3.6 (number of resin ducts). Takođe, iglice *Pinus heldreichii* su imale 2-5 slojeva hipodermisa i 0-12 smonih kanala. Najveće variranje je u broju smonih kanala (CV=27%). U poređenju sa prethodno ispitivanim iglicama sa Dinarskih planina, iglice *P. heldreichii* Šarsko-Pinskog masiva su kraće i sa debljim hipodermalnim slojem. PCA and CA vizualizuju delimično razdvajanje populacija *P. heldreichii* između ova dva planinska masiva.

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