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# Variation in needle anatomy of *Picea omorika* (Pinaceae) plants belonging to different gene pools in natural populations on Tara Mt. in Serbia

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**ABSTRACT:** *Picea omorika* (Serbian spruce) is a stenoendemic species whose current distribution range is restricted to refugial habitats in W Serbia and E Bosnia and Herzegovina. We analyzed variability in the anatomical structure of needles in populations from Tara Mt. which had previously been found to belong to two gene pools characterized by different history and levels of genetic diversity. Needle anatomy was investigated on transverse, sagittal and longitudinal sections and 28 characters were measured. Descriptive statistics, one-way and two-way ANOVA, principal component analysis (PCA), canonical discriminant analysis (CDA) and cluster analysis (UPGMA) were performed to describe the overall anatomical variability and relationships between individuals from three populations. We found that plants originating from different gene pools showed significant anatomical differences. The main differences between the populations were related to needle thickness and width, dimensions of the mesophyll, vascular bundle and endodermis, volume and number of the intercellular spaces and dimensions of resin ducts.

**Key words:** *Picea omorika,* stenoendemic, relict, needle anatomy, resin duct, variability

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## INTRODUCTION

*Picea omorika* (Panč.) Purkyne (Serbian spruce) is a tertiary relict of the Balkan Peninsula, described in 1876 by Josif Pančić as *Pinus omorika*. *Picea omorika* is a stenoendemic species whose current distribution range occupies approximately 10,000 km$^2$ (W Serbia and E Bosnia and Herzegovina, between 43°21¢ and 44°08¢ N and 18°37¢ and 19°45¢E). It is estimated that within its distribution range there are more than 30 natural populations, each comprising several hundred to several thousand trees (Ostojić 2005; Aleksić 2008). Within this restricted area, *P. omorika* inhabits almost exclusively north exposed slopes in the ravines, at altitudes ranging from 800 to 1700 m, that are therefore characterized by specific local microclimate conditions. Together with spruce, fir and beech, it forms coniferous or mixed coniferous-deciduous forests that grow on distinct geological substratum, such as limestone and serpentine (Gajić 1994).

Investigation of morphological traits of conifer needles is a habitual procedure in determining the taxonomical status of conifer plant samples, but some anatomical traits can also be helpful. Published data on the anatomy of needles of *P. omorika* plants grown in natural and cultivated populations report significant variability of needle characters (for a detailed review of literature see Nikolić *et al.* 2013)

Furthermore, Aleksić & Geburek (2010) carried out molecular analysis of mitochondrial DNA sequences on plant samples derived from ten natural populations

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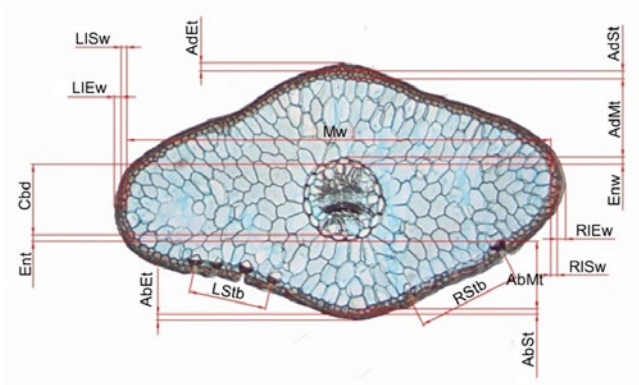


**Fig. 1.** Measured anatomical characters on the transverse section of *Picea omorika* needles (see Table 2 for acronyms of anatomical characters)

of *P. omorika*. They reported that despite the very small distribution range of *P. omorika* and its dispersal by wind, non-random distribution of haplotypes was observed, resulting in an unexpectedly high estimate of population differentiation, and high molecular variation assigned to variation among populations. Those findings suggest substantial isolation of populations and their partitioning into two gene pools characterized by different history and levels of genetic diversity (Aleksić & Geburek 2010).

Taking into account differences reported at the anatomical and molecular level and therefore divergence of the species' natural populations within a relatively small refugial area, the aims of this study were twofold: (1) to describe anatomical variability and provide an extensive anatomical description of needles sampled from plants grown in natural populations in Tara Mountain, and (2) to investigate whether needles of individuals from a natural population that contains different gene pools recognized by Aleksić and Geburek (2010) show some anatomical differences.

## MATERIALS AND METHODS

***Plant material.*** Plant material was collected during the late spring in 2013, from three populations on Tara Mt.: Zmajevački potok (ZP) and Vranjak (VR) belonging to gene pool A, and Studenac (ST) belonging to gene pool B (Table 1). Needles were sampled from the same populations and individuals that were subjects in detecting molecular distinctiveness in mtDNA (Aleksić, 2008; Aleksić & Geburek 2010). In this study it was examined weather the anatomical and molecular differences in studied *P. omorika* correspond. Three needles (oriented toward N, SE and SW) from each of 10 individuals per population were sampled and fixed in 50% ethanol. Therefore, 90 samples in total were used for preparation of permanent slides.

To describe the overall anatomical variability, sampling design included individuals from populations that grew in a variety of ecological conditions (substrate, habitat, altitude, canopy position). These populations of *P. omorika* inhabited ecologically different habitats, growing on different substrates (limestone and serpentine). They were constituents of coniferous and mixed coniferous-deciduous forests, growing at altitudes (700 / 950 / 1350 m a.s.l.) that cover almost the entire range of altitudes within which *P. omorika* normally occurs. Leaf samples were collected from different parts of the crown; from each plant, needles growing oriented towards N, SE and SW were sampled.

The anatomical structure of needles was analyzed on their transverse, sagittal and longitudinal sections obtained

**Table 1.** Sample provenances, herbarium voucher (acronyms follow Thiers 2014) and gene pools according to Aleksić & Geburek (2010)

| *Gene pool* | A | | B |
|---|---|---|---|
| *Site* | Zmajevački potok (ZP) | Vranjak (VR) | Studenac (ST) |
| *Latitude* | 43°51'35" | 43°51'54" | 43°53'24" |
| *Longitude* | 19°25'39" | 19°24'09" | 19°20'40" |
| *Mean Altitude (m)* | 900 | 750 | 1300 |
| *Habitat according EUNIS* | G3 Coniferous woodland // G3.1 [Abies] and [Picea] woodland | G4 Mixed deciduous and coniferous woodland // G4.6 Mixed [Abies] - [Picea] - [Fagus] woodland | G3 Coniferous woodland // G3.1 [Abies] and [Picea] woodland |
| *Phytocoenosis* | Coniferous *Picea omorika - Picea abies* forest | Mixed *Picea omorika -Picea abies - Abies alba - Fagus sylvatica* forest | Coniferous *Picea omorika-Picea abies* forest |
| *Substrate* | serpentinite | limestone | limestone |
| *Voucher* | BEOU-40013 | BEOU-40015 | BEOU-40014 |


**Fig. 2.** The dominant shape of needles: (A) rhomboidal, (B) triangular, (C) elliptical

using a cryostat Leica CM 1850, at -21°C. Cross-sections were made in the middle of the needle, and sagittal and longitudinal sections were done along entire needles. Sections were bleached in parazone, washed in water and double stained in safranin (1% w/v in 50% ethanol) and alcian blue (1% w/v, aqueous). Sections were observed and photographed with a Leica DFC295 digital camera and light microscope, Leica DMLS.

A total of 28 anatomical traits (Table 2, Fig. 1) were determined on cross sections, of which 23 were continuous, measured by software Leica Q Win (Leica Microsystems, Germany). The position of resin ducts was observed on needles previously softened in warm 10% KOH for 10 minutes and positioned between two slides.

The anatomical description is based on observation of permanent slides and results of morphometric study. The description of each anatomical trait within the text includes the minimum and maximum values, with the mean ± standard deviation in brackets.

***Basic characteristics of the sampled localities.*** The locality Zmajevački potok is situated in the village Zaovine, hamlet Lazici. Plants of *Picea omorika* grow on metamorphic serpentine rock (serpentinite peridotites) that occur in the form of bigger or smaller blocks within the surrounding limestone rocks. The shallow, skeletal brown soil contains fragments of the mother rock. Zmajevački potok is located at an altitude of 800-850 m, on a N-NE exposed slope (35-40°). The climate is represented by a 6.8°C mean annual temperature and 918 mm of precipitation. Characteristic vegetation includes the ass. *Piceetum omorikae-abietis serpentiniticum*, with *Picea omorika* and *Picea abies* as edificators of the tree floor (Gajić 1994).

The locality Vranjak is situated on the right bank of Beli Rzav river. The habitat is north-north west exposed. The substrate is represented by organogenic-skeletal calcareous black soil that develops on the Cretaceous limestone parent rock. The typical phytocenoses is the ass. *Omorikae Piceeto-Abieto-Fagetum* subass. *pinetosum* (Gajić 1994).

Locality Studenac is situated in the village Zaovine, north west from Zmajevački potok, in Gornje Karaklije hamlet. This habitat of *P. omorika* is on a NW exposed slope (35-60°), at 1255 to 1350 m a.s.l. Here, shallow soil develops on limestone stones and rocks and is rich in humus. At this locality two subassociations are present: *Piceetum omorikae-abietis calcicolum* subass. *pinetosum nigrae* and *Piceetosum omorikae-abietis calcicolum* subass. *typicum* (Gajić 1994).

***Statistical data processing.*** Statistical analyses were performed in Statistica 5.1 software and include descriptive statistics for each population, one-way and two-way ANOVA (populations and populations and orientation of needles as factors of variation, respectively), principal component analysis (PCA), canonical discriminant analysis (CDA) and cluster analysis (UPGMA) (StatSoft 1996). PCA based on a correlation matrix was performed on the complete data set to describe the overall anatomical variability and relationships between individuals from all populations, structure of the variability of samples and the contribution of individual characters in defining the structure of variability. One-way ANOVA followed by the Bonferroni test was used to check for differences in PC scores between the three populations. The hypothesis of anatomical separation of populations as a priori defined groups was tested by stepwise canonical discriminant analysis (CDA). Finally, the UPGMA (Unweighted Pair Group Method with Arithmetic mean) cluster analysis, based on Mahalanobis distances between all populations, was calculated.

## RESULTS

(1) ***Anatomical features of Serbian spruce needles.*** The dominant shape of needles was rhomboidal, with more or less accentuated angles, whereas triangular or nearly elliptical forms were rarely observed (Fig. 2).

Transverse sections through the mid part of needles showed thick-walled and lignified epidermal cells, sunken stomata, a single layer of hypodermal schlerenchyma cells, resin ducts, compact mesophyll tissue and one central vascular bundle bordered by one layer of large endodermal cells. In general, dimensions of the different anatomical characters observed on transverse sections of the mid part of the needle varied significantly (Table 2). Needle thickness ranged from 637.1 to 944.7 mm

**Table 2.** Anatomical characteristics of *Picea omorika* needles

| Character | Acronym | N | Mean | Min | Max | Std | SE |
|---|---|---|---|---|---|---|---|
| **Transverse section** (mm) | | | | | | | |
| [3]Needle thickness | Nt | 81 | 780.1 | 637.1 | 944.7 | 67.19 | 7.47 |
| [2]Needle width | Nw | 81 | 1422 | 1115 | 1832 | 135.8 | 15.09 |
| [4]Adaxial epidermis thickness | AdEt | 81 | 22.06 | 9.40 | 40.98 | 6.13 | 0.68 |
| Abaxial epidermis thickness | AbEt | 81 | 19.72 | 9.57 | 32.86 | 4.36 | 0.48 |
| Left lateral epidermis width | LlEw | 81 | 19.36 | 9.39 | 37.33 | 5.08 | 0.56 |
| Right lateral epidermis width | RlEw | 81 | 22.10 | 12.96 | 35.71 | 4.39 | 0.49 |
| [5]Adaxial schlerenchyma thickness | AdSt | 81 | 22.97 | 9.51 | 50.13 | 7.04 | 0.78 |
| Abaxial schlerenchyma thickness | AbSt | 81 | 17.79 | 6.20 | 37.31 | 5.51 | 0.61 |
| Left lateral schlerenchyma width | LlSw | 81 | 21.42 | 5.11 | 34.02 | 5.54 | 0.62 |
| Right lateral schlerenchyma width | RlSw | 81 | 21.59 | 9.80 | 42.75 | 6.20 | 0.69 |
| Adaxial mesophyll thickness | AdMt | 81 | 269.5 | 190.9 | 357.6 | 33.79 | 3.75 |
| Abaxial mesophyll thickness | AbMt | 81 | 176.4 | 125.5 | 263.13 | 26.63 | 2.96 |
| Mesophyll width | Mw | 81 | 1338 | 1038 | 1751 | 132.0 | 14.66 |
| Endodermis thickness | Ent | 81 | 20.65 | 8.65 | 34.04 | 5.17 | 0.57 |
| Endodermis width | Enw | 81 | 19.54 | 8.91 | 29.06 | 4.37 | 0.49 |
| Central bundle diameter | Cbd | 81 | 211.5 | 151.1 | 285.8 | 26.34 | 2.93 |
| Left stomata band | LStb | 81 | 274.2 | 47.67 | 447.8 | 73.20 | 8.13 |
| Right stomata band | RStb | 81 | 265.4 | 62.13 | 470.8 | 82.20 | 9.13 |
| [7]Resin ducts diameter | Rdd | 79 | 81.51 | 28.05 | 165.1 | 25.48 | 2.87 |
| Number of schlerenchyma layers | NoS | 81 | 1.33 | 1.00 | 2.00 | 0.47 | 0.05 |
| Number of endodermis cells | NoEn | 81 | 15.46 | 13.00 | 20.00 | 1.49 | 0.17 |
| Number of stomata | NoSt | 81 | 8.09 | 4.00 | 12.00 | 1.86 | 0.21 |
| [6]Number of resin ducts | NoRd | 81 | 1.79 | 0.00 | 2.00 | 0.47 | 0.05 |
| **Sagittal section** (mm) | | | | | | | |
| Resin duct length | Rdl | 64 | 824.7 | 148.1 | 2633 | 6635 | 82.86 |
| Resin duct maximum diameter | Rdmd | 64 | 70.45 | 29.00 | 139.1 | 21.83 | 2.73 |
| **Longitudinal section** ($mm^2$) | | | | | | | |
| Area of intercellular space | AIn | 81 | 0.14 | 0.04 | 0.28 | 0.05 | 0.01 |
| Area of the longitudinal section | ALS | 81 | 2.33 | 1.53 | 3.87 | 0.45 | 0.05 |
| Number of intercellular spaces | NoIn | 81 | 52.3 | 30.00 | 82.00 | 12.46 | 1.38 |

**Abbreviations:** N – number of plant samples, Mean – mean value, Min - minimum, Max - maximum, Std – standard deviation, SE - standard error of mean. Ordinal numbers in superscript in front of the character corresponding to the ordinal number of characters analyzed in Nikolić *et al.* 2013

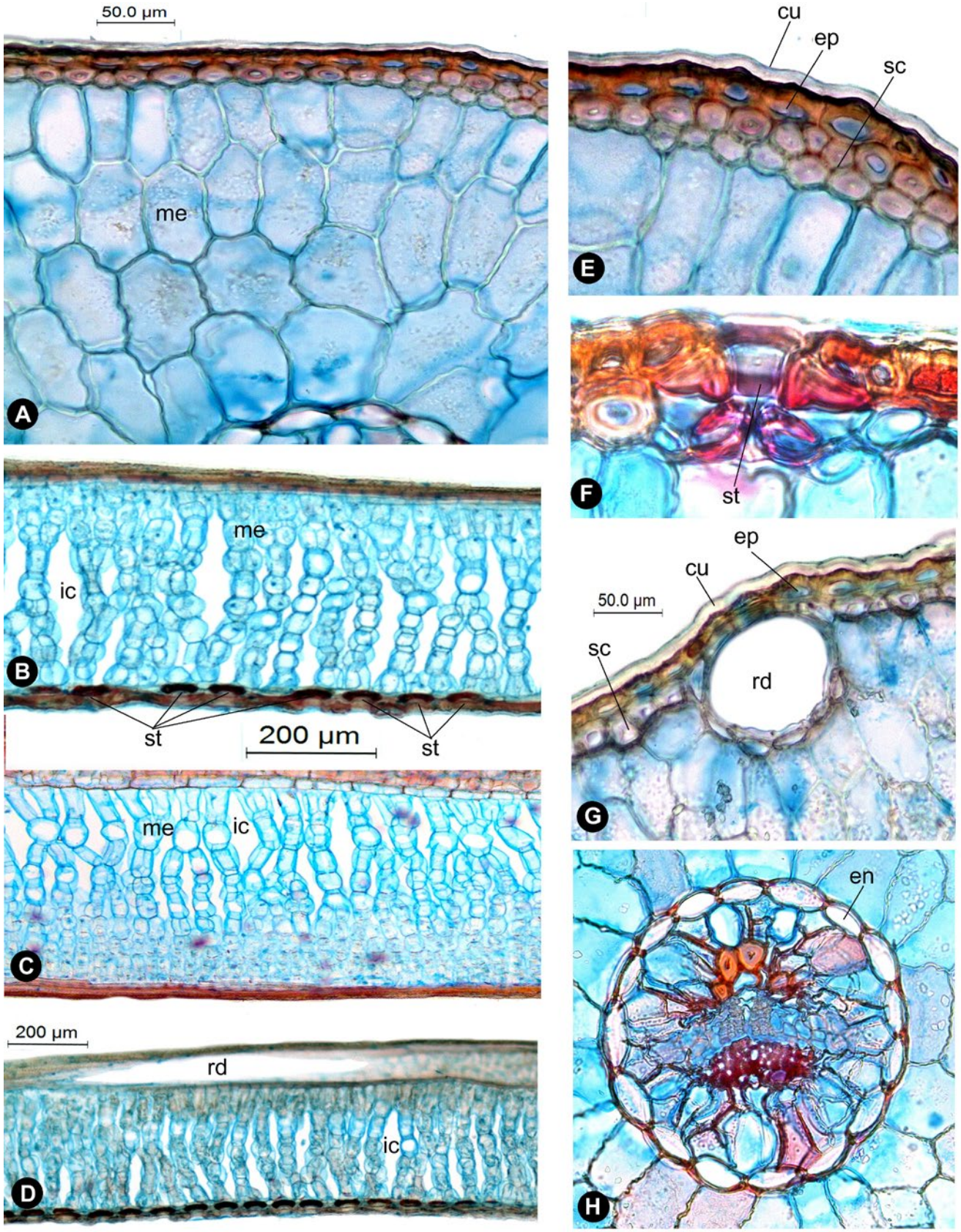


**Fig. 3.** Detailed anatomy of *Picea omorika* needles (me – mesophyll, cu – cuticle, ep – epidermis, sc – schlerenchyma, st – stomata, ic – intercellular spaces, rd – resin duct, en – endodermis)

(780.1±67.19 mm), and its width ranged from 1115 to 1832 mm (1422±135.8 mm). The *epidermis* was covered with a thick cuticle and consisted of one layer of thick-walled epidermal cells whose dimensions varied from 9.4 to 41.0 mm, being thicker on the adaxial (22.06±6.13 mm) than the abaxial (19.72±4.36 mm) and lateral leaf sides (Figs. 3A and 3E, Table 2). Stomata were sunken and aligned and formed 4-12 parallel lines along the abaxial side of the needle (Fig. 3F). The number of stomata strips varied along the leaf, mostly in dependence on the leaf width. *Schlerenchyma* tissue was composed of one, almost continuous cell layer that lay below the epidermis. It was absent only in the area of stomata bands and resin ducts (Fig. 3G). Sometimes, schlerenchyma cells were arranged to form a double-layer (Fig. 3E). The thickness of schlerenchyma cells ranged from 5.10 to 50.13 mm, with the mean between 17.79±5.51 mm on the abaxial needle side and 22.97±7.04 mm on the adaxial one (Table 2). Thus, dimensions of abaxial schlerenchyma cells were smaller than those on the adaxial and lateral sides of the needle. *Mesophyll* tissue was composed of plicate and closely packed cells that in the transverse plane formed very compactly organized photosynthetic tissue (Fig. 3A). The thickness of mesophyll tissue above the vascular bundle was 269.5±33.79 mm on the adaxial leaf side, and 176.4±26.63 mm below the vascular bundle on the abaxial side, and its width from the vascular bundle to the lateral edges of the needle was 1338±14.66 mm. In contrast to this very compact appearance of mesophyll tissue observed on leaf transverse sections, its organization on sagittal and longitudinal leaf sections was very different. The latter sections showed that mesophyll cells were very regularly organized to form distinct cell layers with very broad intercellular spaces in between, as shown in Figure 3 (B and C). On sagittal sections of the whole needle, 30 to 82 distinct intercellular spaces were present. Their areas varied from 0.04 to 0.28 mm$^2$, with a mean of 0.17±0.05 mm$^2$, thus equivalent on average to 17% of the leaf area of sagittal section of whole needles. *Resin ducts* were located directly below the adaxial leaf side, in contact with the epidermis (external type) and were positioned laterally with respect to the vascular bundle (Figs. 2A, 3D, 3G). In most needles, irrespective of population, they were present in the base as well as in the tip of the needle, whereas needles with resin ducts that extended continuously from the basis to the tip were rare. They were 148.1-2633 mm$^2$ long, with diameter ranging from 28.05 to 165.1 mm (Table 2). The *vascular bundle* was positioned centrally and was enclosed by transfusion tissue, surrounded by a well-defined endodermis (Fig. 3H). The number of thick-walled endodermal cells on the transverse section varied from 13 to 20, with mean thickness and width of 20.65±5.17 mm and 19.54±4.37 mm, respectively (Table 2). The diameter of vascular bundle varied from 151.1 to 285.8 mm, following the general leaf dimensions.

(2) ***Anatomical differentiation of the population of Serbian spruce in Tara Mt.*** One-way analysis of variance (ANOVA) followed by the Bonferroni test showed significant differences ($P < 0.05$) in nine anatomical traits of *P. omorika* needles from the three populations belonging to the two different gene pools (Table 3). Two-way ANOVA also showed a significant effect of population for these traits, but no effect of the orientation of needles or interaction between population and orientation of needles for any of the analyzed anatomical traits. Major differences in anatomical characters of needles between the three populations were identified by PCA, which showed that the first nine eigenvalues greater than 1.00 explained 67.6% of the total variation of anatomical characters (Table 3). The first principal component (PC1), explaining 21.1% of the total variation, primarily described differences in the size of needles, as well as the width of mesophyll and central bundle diameter. ANOVA

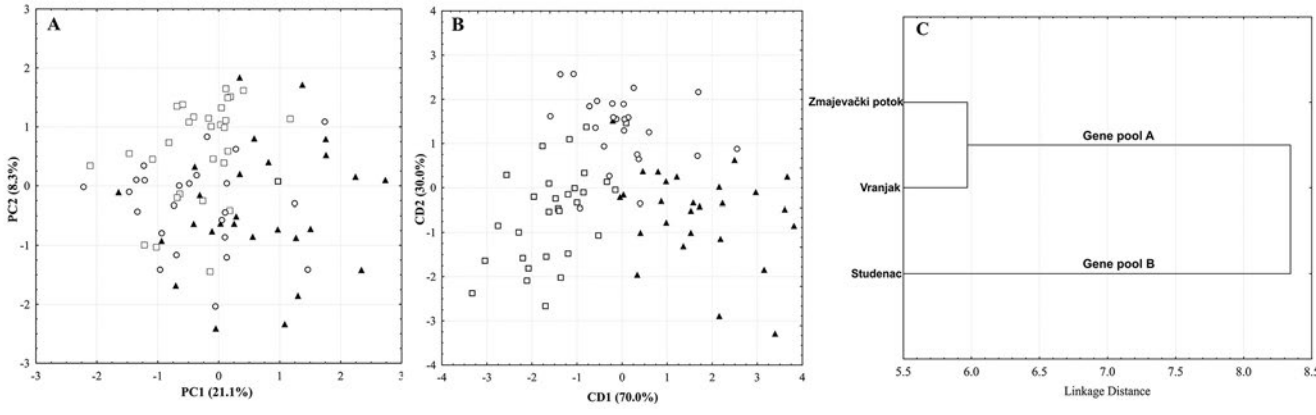


**Fig. 4.** Principal Component Analysis (PCA) (A) and Canonical Discriminant Analysis (CDA) (B) plotted along the first two axes (□ - Zmajevački stream (gene pool A); ○ - Vranjak (gene pool A); ▲- Studenac (gene pool B)); Cluster analysis (UPGMA) for populations of *Picea omorika* (C)

**Table 3.** Anatomical characteristics of *Picea omorika* needles within each population, PCA factor loadings, eigenvalue and % of variance for the first three principal components (see Table 2 for acronyms of anatomical characters).

| | Gene pool A | | | | | | | | Gene pool B | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zmajevački stream | | | | Vranjak | | | | Studenac | | | | | | |
| | N | Mean | Std | SE | N | Mean | Std | SE | N | Mean | Std | SE | PC1 | PC2 | PC3 |
| Nt | 30 | 776.3[a] | 56.94 | 10.40 | 24 | 761.5[a] | 70.10 | 14.31 | 27 | 800.8[a] | 71.73 | 13.80 | **0.79** | 0.12 | -0.46 |
| Nw * | 30 | 1384[a] | 118.8 | 21.70 | 24 | 1393[ab] | 141.1 | 28.81 | 27 | 1492[b] | 126.1 | 24.26 | **0.88** | -0.06 | 0.00 |
| AdEt | 30 | 21.98[a] | 5.33 | 0.97 | 24 | 22.77[a] | 5.16 | 1.05 | 27 | 21.51[a] | 7.73 | 1.49 | 0.25 | -0.01 | -0.37 |
| AbEt | 30 | 19.07[a] | 3.53 | 0.64 | 24 | 20.87[a] | 4.88 | 1.00 | 27 | 19.43[a] | 4.66 | 0.90 | -0.06 | -0.03 | 0.00 |
| LlEw | 30 | 18.96[a] | 5.26 | 0.96 | 24 | 18.81[a] | 3.26 | 0.67 | 27 | 20.30[a] | 6.16 | 1.19 | 0.35 | 0.16 | -0.12 |
| RlEw | 30 | 21.38[a] | 4.84 | 0.88 | 24 | 22.55[a] | 3.31 | 0.67 | 27 | 22.52[a] | 4.75 | 0.91 | 0.35 | 0.10 | 0.02 |
| AdSt * | 30 | 25.73[a] | 8.37 | 1.53 | 24 | 19.06[b] | 5.25 | 1.07 | 27 | 23.37[ab] | 5.17 | 0.99 | 0.20 | 0.31 | -0.10 |
| AbSt | 30 | 18.73[a] | 6.33 | 1.16 | 24 | 16.76[a] | 5.45 | 1.11 | 27 | 17.67[a] | 4.53 | 0.87 | 0.18 | -0.09 | -0.30 |
| LlSw | 30 | 20.19[a] | 6.12 | 1.12 | 24 | 21.07[ab] | 4.93 | 1.01 | 27 | 23.10[b] | 5.14 | 0.99 | 0.17 | -0.01 | 0.30 |
| RlSw | 30 | 21.78[a] | 5.51 | 1.01 | 24 | 19.53[a] | 7.09 | 1.45 | 27 | 23.19[a] | 5.78 | 1.11 | 0.18 | -0.03 | -0.42 |
| AdMt | 30 | 265.3[a] | 30.14 | 5.50 | 24 | 268.4[a] | 37.32 | 7.62 | 27 | 275.2[a] | 34.84 | 6.70 | 0.36 | -0.05 | -0.45 |
| AbMt | 30 | 169.8[a] | 22.44 | 4.10 | 24 | 174.7[a] | 24.03 | 4.90 | 27 | 185.1[a] | 31.28 | 6.02 | 0.51 | 0.11 | -0.33 |
| Mw * | 30 | 1301[a] | 118.2 | 21.58 | 24 | 1311[ab] | 136.8 | 27.93 | 27 | 1402[b] | 122.1 | 23.49 | **0.86** | -0.06 | 0.01 |
| Ent * | 30 | 22.14[a] | 4.49 | 0.82 | 24 | 17.67[b] | 4.40 | 0.90 | 27 | 21.64[a] | 5.56 | 1.07 | 0.26 | 0.39 | 0.11 |
| Enw * | 30 | 21.39[a] | 3.81 | 0.70 | 24 | 17.35[b] | 4.66 | 0.95 | 27 | 19.42[ab] | 3.88 | 0.75 | 0.14 | 0.34 | 0.15 |
| Cbd | 30 | 212.1[a] | 19.75 | 3.61 | 24 | 203.9[a] | 26.55 | 5.42 | 27 | 217.5[a] | 31.37 | 6.04 | **0.84** | 0.06 | -0.13 |
| LStb | 30 | 253.9[a] | 61.13 | 11.16 | 24 | 265.4[a] | 82.99 | 16.94 | 27 | 304.6[a] | 68.69 | 13.22 | 0.55 | 0.07 | 0.46 |
| RStb | 30 | 271.6[a] | 93.23 | 17.02 | 24 | 250.0[a] | 82.74 | 16.89 | 27 | 272.1[a] | 68.78 | 13.24 | 0.58 | 0.31 | 0.27 |
| Rdd * | 28 | 68.15[a] | 16.18 | 3.06 | 24 | 73.88[a] | 17.3 | 3.54 | 27 | 102.1[b] | 26.87 | 5.17 | 0.51 | -0.37 | 0.31 |
| NoS | 30 | 1.00[a] | 0.50 | 0.09 | 24 | 1.00[a] | 0.44 | 0.09 | 27 | 1.00[a] | 0.47 | 0.09 | 0.07 | 0.31 | -0.25 |
| NoEn * | 30 | 15.00[a] | 0.87 | 0.16 | 24 | 15.00[a] | 1.05 | 0.21 | 27 | 16.00[b] | 1.87 | 0.36 | **0.70** | -0.02 | 0.00 |
| NoSt | 30 | 8.00[a] | 1.74 | 0.32 | 24 | 8.00[a] | 1.97 | 0.40 | 27 | 9.00[a] | 1.67 | 0.32 | 0.59 | 0.22 | 0.51 |
| NoRd | 30 | 2.00[a] | 0.57 | 0.10 | 24 | 2.00[a] | 0.38 | 0.08 | 27 | 2.00[a] | 0.42 | 0.08 | -0.09 | -0.12 | 0.39 |
| Rdl | 22 | 844.5[a] | 696.4 | 148.5 | 18 | 831.6[a] | 616.9 | 145.4 | 24 | 801.3[a] | 691.6 | 141.2 | -0.04 | -0.30 | 0.29 |
| Rdmd * | 22 | 61.70[a] | 15.0 | 3.19 | 18 | 62.8[a] | 15.22 | 3.59 | 24 | 84.24[b] | 24.73 | 5.05 | 0.44 | -0.35 | 0.27 |
| AIn | 30 | 0.12[a] | 0.04 | 0.01 | 24 | 0.14[a] | 0.05 | 0.01 | 27 | 0.15[a] | 0.05 | 0.01 | 0.14 | **-0.70** | -0.31 |
| ALS * | 30 | 2.07[a] | 0.36 | 0.07 | 24 | 2.39[b] | 0.40 | 0.08 | 27 | 2.56[b] | 0.44 | 0.08 | 0.43 | -0.57 | 0.23 |
| NoIn | 30 | 49.00[a] | 11.18 | 2.04 | 24 | 53.00[a] | 11.97 | 2.44 | 27 | 55.00[a] | 13.82 | 2.66 | 0.07 | -0.65 | -0.16 |
| Eigenvalue | | | | | | | | | | | | | 5.92 | 2.31 | 2.28 |
| % of variance | | | | | | | | | | | | | 21.1 | 8.3 | 8.2 |

**Abbreviations:** N – number of plant samples, Mean – mean value, Std – standard deviation, SE - standard error of mean, PC1-PC3: first three principal components

Characters that are significantly different among populations according to ANOVA are marked with an asterisk (*). Means separated by Bonferroni test; for each anatomical character means indicated by the same letter(s) are not significantly different at $P < 0.05$

Factor loadings >0.700000 are indicated in bold

**Table 4.** Standardized coefficients and correlations (r) with anatomical variables for canonical discriminant functions (CD1 & CD2), eigenvalues, % of variance and canonical correlation (R) for the CDA of *Picea omorika* from three populations. The highest correlations for each variable are indicated in bold (see Table 2 for acronyms of anatomical characters).

| | CD1 | r | CD2 | r |
|---|---|---|---|---|
| Rdd | 0.54 | **0.50** | -0.23 | -0.28 |
| AdSt | -0.29 | -0.11 | -0.39 | **-0.44** |
| ALS | 0.34 | **0.38** | 0.48 | 0.12 |
| Enw | -0.39 | -0.15 | -0.60 | -0.40 |
| AbMt | 0.54 | **0.18** | 0.05 | -0.05 |
| Cbd | -1.26 | 0.06 | 0.26 | **-0.22** |
| NoEn | 0.75 | 0.32 | -0.37 | **-0.35** |
| Ent | 0.12 | -0.03 | -0.50 | **-0.46** |
| RlSw | -0.06 | 0.07 | -0.49 | **-0.25** |
| Rdl | -0.17 | **-0.02** | -0.41 | 0.01 |
| LlSw | 0.25 | **0.17** | 0.18 | -0.05 |
| Rdmd | 0.17 | **0.33** | -0.28 | -0.24 |
| Nw | 0.44 | **0.26** | -0.03 | -0.17 |
| NoIn | 0.24 | **0.15** | -0.16 | 0.06 |
| Eigenvalue | 1.86 | | 0.80 | |
| % of variance | 70.0 | | 30.0 | |
| Canonical R | 0.806 | | 0.666 | |

showed the populations to differ significantly in PC1 score means ($F_{2,78}$=9.83, P<0.001). Individuals that belong to the gene pool B (Studenac) had higher PC1 scores and were significantly different from individuals that belong to the gene pool A (Zmajevački sream and Vranjak) (Fig. 4A). Namely, needles of individuals from Studenac were thicker and wider than those from Vranjak and Zmajevački potok. They had a wider mesophyll and central bundle, respectively. The second principal component (PC2) explained 8.3% of the total variation, and was due mainly to differences in the size and number of intercellular spaces. ANOVA confirmed that differences between PC2 scores were significant ($F_{2,78}$=10.86, P<0.001). PC2 separated individuals from Zmajevački potok that were characterized by larger surface area, as well as by larger number of intercellular spaces.

Stepwise canonical discriminant analysis used 14 anatomical characters and their differences among populations of *P. omorika* were described by two canonical discriminant functions (Wilk's Lambda = 0.195, $\chi^2$= 116.9, df=28, P<0·001) (Table 4). The first canonical discriminant function (CD1) explained 70.0% of the total variation and was mainly correlated with dimensions of resin ducts. The second canonical discriminant function (CD2), that described the remaining 30.0% of the variation, was principally correlated with endodermis. Consequently, CD1 divided individuals from gene pool B (Studenac) that were characterized by shorter and larger resin ducts, from individuals belonging to the gene pool A (Zmajevački sream and Vranjak). CD2 also separated individuals from Zmajevački potok that were characterized by slightly larger endodermal cells (Fig. 4B). CDA classified correctly 90.00% of individuals from the Zmajevački potok population, 85.19% from the Studenac population and 83.33% from the Vranjak population (overall 86.42% correctly classified individuals).

In accordance with previous results, cluster analysis based on the Mahalanobis distances indicated differentiation of the three *P. omorika* populations into two groups. The first one consisted of populations that belong to gene pool A (Zmajevački sream and Vranjak), while the second included from gene pool B (Studenac) (Fig. 4C).

## DISCUSSION

Our results on the anatomy of needles in Serbian spruce from three populations on Mt Tara were mostly in accordance with the recently published results by Nikolić *et al.* (2013). The only significant differences were the number and size of resin ducts and the number of schlerenchyma layers. Namely, we found that the average number of resin ducts was 1.8 per needle, whereas Nikolić *et al.* (2013) reported an average number of 0.7. Similarly, our study found that the average diameter of resin ducts was 81.5 mm, whereas it was 53.5 mm in the samples analyzed by Nikolić *et al.* (2013). Larges differences were detected in resin duct diameter in needles from plants grown in Vranjak (the same population that was analyzed in our study). Specifically, Nikolić *et al.* (2013) reported a diameter of 47.9 mm, which was almost the half the diameter we found (Table 3). Moreover, in all needles they detected a single hypodermal schlerenchyma layer, whereas we found that schlerenchyma tissue in some individuals was composed of two continuous cell layers.

In relation to previous anatomical investigations of Serbian spruce that included a relatively small number of anatomical characters explored only on transverse sections, our morphometric study included 28 characters measured on transverse, sagittal and longitudinal sections, which provided very detailed insights into the fine anatomical structure of *P. omorika* needles. In addition, anatomical characteristics of needles that grow oriented toward N, SE and SW, as well as dimensions of various tissues on adaxial, abaxial and lateral sides of needles were analyzed for the first time. Our study described here also provides the first data on dimensions of the endodermis, vascular bundle and stomata band, and the first record of very broad intercellular spaces in needles of *P. omorika*.

Although transverse sections of needles showed a very compact plicate mesophyll tissue, without larger intercellular spaces, the sagittal and longitudinal sections showed a completely different leaf mesophyll organization. Namely, on sagittal cross sections, mesophyll cells formed distinct compact layers, each separated by regular and broad intercellular spaces, as already described for other species within the genus *Picea* (Kozlowski *et al.* 1999; Lhotakova 2008). The latter form long canals of communication among mesophyll cells as well as among mesophyll and numerous stomata along the needle. This kind of leaf structural organization, represented by *(a)* sunken stomata, *(b)* specific spatial distribution of mesophyll cells within the leaf in both transverse and longitudinal planes, *(c)* a prominent leaf internal surface area and *(d)* consequently a large mesophyll surface that is in contact with intercellular air spaces, is a characteristic of needles that is directly related to the high efficiency of light absorption, gas exchange and control of water loss in this conifer species (Slaton & Smith 2002).

The presence and distribution of resin ducts in needles can be an important taxonomic character in conifers (Weng & Jackson 2000). In *P. omorika* plants from the three populations from Tara Mt., they vary in dimensions and position within the leaf. Leaf resin ducts were present mostly in the distal and basal parts of the needles. In some transverse sections only one or both resin ducts could also be detected in the middle part of the needle. However, more detailed investigation of the resin ducts, their position and length within the needle with respect to the age and position of the leaf in the crown of *P. omorika* would be needed to obtain information on whether this character could be used as a taxonomically-valuable trait.

Although the anatomy of spruce needles has been analyzed and described in detail by several authors, most of their results referred to plants growing in urban areas or artificial plantations. On the basis of the available literature, anatomical investigations were conducted in only four natural populations (Nikolić *et al.* 2013). These authors emphasized the pronounced variability of morpho-anatomical properties of Serbian spruce needles, explaining it by adaptations to different environmental factors such as climate, soil and especially air pollution (Ilijin-Jug 1995; Isajev *et al.* 1999) or by possible genetic specificities of different phenogroups (Milovanović *et al.* 2005; Nikolić *et al.* 2013). For the first time, our data provide an insight into variation of the anatomy of needles from populations that have been found to belong to two gene pools characterized by different history and levels of genetic diversity. Plants originating from gene pool A (Zmajevački potok and Vranjak) and gene pool B (Studenac) showed significant anatomical differences, with the main being related to needle thickness and width, mesophyll dimensions, vascular bundle and endodermis, volume and number of intercellular spaces and dimensions of resin ducts.

Although variations in leaf anatomical characteristics are affected by different environmental factors, in some groups of plants, including conifers, they could have taxonomic significance (Vidaković 1982; Weng & Jackson 2000; Lakušić & Lakušić 2011). Nikolić *et al.* (2013) showed that, at the anatomical level, the population from Mileševka Canyon, which has previously been described as variety *P. omorika* var. *vukomanii* Pavlović & Matović (Pavlović & Matović 1994), is finely differentiated from Mt Tara populations and suggested that some characters in the needle anatomy of Serbian spruce may also have taxonomic significance. Our results showed that anatomical differentiation of populations of Serbian spruce on Tara Mt. corresponded to their genetic differentiation indicating the potential taxonomic significance of leaf anatomy. However, bearing in mind that extensive anatomical investigations of needles have included only five of more than 30 natural populations (Ostojić 2005; Aleksić 2008), of which four are spatially very closely positioned on Mt Tara (Štula, Vranjak, Zmajevački potok, Studenac), whereas one is distant (Mileševka Canyon), we must be cautious in making general conclusions. Therefore, further research involving a large and representative number of natural populations is required.

## CONCLUSIONS

Transverse sections of needles showed a compact plicate mesophyll tissue. However, longitudinal and sagittal sections showed the presence of wide intercellular spaces between regularly organized layers of mesophyll cells. This large mesophyll surface area is in the contact with wide intercellular spaces which indicate very efficient gas exchange and light absorption. Resin ducts were mostly represented by two canals that occurred in the basal and distal parts of the needle and were of variable length. Schlerenchyma s usually single-layered, rarely in places two-layered. Our results showed that anatomical differentiation of populations of Serbian spruce on Tara Mt. corresponds to their genetic differentiation previously decribed by Aleksić & Geburek (2010), indicating the potential taxonomic significance of leaf anatomy.

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## REFERENCES

Aleksić JM. 2008. Genetic structure of natural populations of Serbian spruce [*Picea omorika* (Panč.) Purk.]. Dissertation, University of Natural Resources and Applied Life Sciences, Vienna, Austria.

Aleksić JM & Geburek T. 2010. Mitochondrial DNA reveals complex genetic structuring in a stenoendemic conifer

*Picea omorica* [(Panč.) Purk.] caused by its long persistence within the refugial Balkan region. Plant Syst. Evol. **285**: 1-11.

Gajić M. (ed.) 1994. Omorika-(*Picea omorika* (Pančić) Purkyne) na teritoriji Nacionalnog parka Tara (Monografska studija). Nacionalni park Tara, Bajina Bašta, Šumarski fakultet, Beograd.

Ilijin-Jug M. 1995. Histološko-citološke i morfološke promene kod vrste *Picea omorica* na području Pančeva u hortikulturi, izazvane aerozagadenjem [dissertation]. Beograd: Biološki fakultet. p.153.

Isajev V, Šijačić M & Vilotić D. 1999. Promenljivost nekih svojstava dvogodišnjih četina omorike iz šest populacija sa različitim stepenom aerozagadenja. Šumarstvo (Beograd) **2–3**: 147–157.

Kozlowski TT, Kramer PJ & Pallardy SG. 1999. The Physiological Ecology of Woody Plants. Academic Press, Inc., San Diego.

Lakušić B & Lakušić D. 2011. Anatomy of four taxa of the Genus *Juniperus* sect. *Juniperus* (Cupressaceae) from the Balkan peninsula. Botanica Serbica, **35**: 145-156.

Lhotakova Z. 2008. Study of coniferous needles in relation to environmental factors using approaches of quantitative anatomy and laboratory spectroscopy. Faculty of Science, Prague, PhD Thesis

Milovanović J, Ivetić V, Vilotić D & Šijačić-Nikolić M. 2005. Morfo-anatomske karakteristike četina različitih fenogrupa omorike. Acta herbologica **14**: 41-50.

Nikolić B, Bojović S & Marin DP. 2013. Variability of morpho-anatomical characteristics of the needles of *Picea omorika* from natural populations in Serbia. Plant Biosyst. DOI: 10.1080/11263504.2013.810180.

Ostojić D. 2005. Ekološki činioci prirodnog održavanja i obnove cenopopulacija Pančićeve omorike u NP Tara. Doktorska disertacija, Šumarski fakultet Univerziteta u Beogradu.

Pavlović B & Matović M. 1994. Vukoman's spruce - new variety of Serbian spruce in the Mileševka kanyon - *Picea omorika* var. *vukomanii*. Arch. Biol. Sci. **46**: 27–28.

Slaton MR & Smith WK. 2002. Mesophyll architecture and cell exposure to intercellular air space in alpine, desert, and forest species. Int. J. of Plant Sci. **163**: 937–948.

StatSoft 1996. STATISTICA (data analysis software system), version 5.1. StatSoft Inc, Tulsa.

Thiers, B. 2014. (continuously updated). Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. The New York Botanical Garden. Available from: http://sweetgum.nybg.org/ih/ (accessed 10 July 2014).

Vidaković M. 1982. Četinjače. Morfologija i varijabilnost. Zagreb: JAZU i Sveučilišna naklada Liber. pp.710.

Weng C & Jackson TS. 2000. Species differentiation of north American spruce (*Picea*) based on morphological and anatomical characteristics of needles. Can. J. Botany **78**: 1367-1383.

**REZIME**

# Anatomska varijabilnost četina *Picea omorika* (Pinaceae) iz genetički diferenciranih prirodnih populacija sa planine Tare u Srbiji

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*Picea omorika* (omorika) je stenoendemična vrsta čiji je današnji areal ograničen na refugijalna staništa u zapadnoj Srbiji i istočnoj Bosni i Hercegovini. U ovom radu analizirana je anatomska varijabilnost četina u populacijama sa planine Tare za koje je utvrđeno da pripadaju genetički diferenciranim populacijama. Anatomija četina je analizirana na poprečnim, sagitalnim i uzdužnim presecima na kojima je praćeno 28 karaktera. U cilju opisivanja opšte anatomske varijabilnosti, kao i odnosa među individuama iz različitih populacija urađene su deskriptivna statistika, jednofaktorska i dvofaktorska analiza varijanse (ANOVA), analiza osnovnih komponenti (PCA), kanonijska diskriminantna analiza (CDA) i klaster analiza (UPGMA). Utvrđeno je da biljke koje potiču iz genetički diferenciranih populacija pokazuju statistički značajne razlike u nekim anatomskim odlikama četine. Osnovne razlike između analiziranih populacija odnose se na debljinu četina, širinu mezofila, provodnog snopića i endodermisa, kao i zapreminu i broj intercelulara i veličinu smonih kanala.

**Ključne reči:** *Picea omorika,* stenoendemit, relikt, anatomija četina, smoni kanal, varijabilnost