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Diversity of *Picea omorika* (Pančić) Purk. Populations Based on Morpho-anatomical Needle Traits and Bioclimatic Parameters

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ABSTRACT

Picea omorika (Pančić) Purkyne is a well-known endemic conifer species. However, there have been few morpho-anatomical studies to date. Several years ago, preliminary population studies of *P. omorika* were published, but with the inclusion of additional populations, it became necessary to confirm whether the southernmost population from Mileševka Canyon, also known as var. *vukomanii*, (Pavlović and Matović 1994), remains distinct from the others. Two-year-old needles from the lower third of the crown were collected in late autumn from seven natural populations of *P. omorika* from Serbia and from the border with Bosnia and Herzegovina. The variability of ten morpho-anatomical traits and the cross-sectional anatomy of Serbian spruce needles were examined in seven natural populations in Serbia. Needle length was measured with a digital calliper, and the nine other needle traits, needle width, needle thickness, vascular bundle diameter, resin ducts diameter, number of resin ducts, distance between vascular bundle and resin duct, cuticle and epidermal thickness, hypodermal thickness, and hypodermal width, were examined under a light microscope. For most of the traits studied, the Štula population had the highest values, whereas the Bilo population had the lowest. Needles with triangular sections are most common in the southern part of Mt. Tara, while elliptical and rhomboidal sections are characteristic in populations at the edge of the distribution range in Serbia. Multivariate analyses separated populations into two main groups. Both principal component analysis and discriminant analysis indicated population overlap, while cluster analysis identified three main groups. Multivariate analysis of bioclimatic data and a correlation study of morpho-anatomical traits were also performed. Based on bioclimatic parameters, individuals in the populations from Mileševka and Štula showed the most distinct characteristics. Mantel and partial Mantel tests confirmed no correlation between the complete set of morphological traits and bioclimatic parameters, even after adjusting for regional differences.

Keywords: Serbian spruce; needles; morphology; anatomy; multivariate analyses, environment.

INTRODUCTION

Picea omorika (Pančić) Purk., commonly known as Serbian spruce, is a well-known, relict and endemic conifer species found naturally only in the high mountain regions in Serbia and Bosnia and Herzegovina. However, due to its ecological plasticity, this species is now widespread in artificial forests throughout the northern hemisphere, along with *Picea abies* (L.) H. Karst. and other *Picea* Mill. species (Vidaković 1982). It is a very common ornamental species in urban areas. Owing to its small natural range and endemo-relict character, it is listed on the IUCN Red List of Threatened Species (Nasri et al. 2008). Research in the 21st century has expanded knowledge of *P. omorika*'s natural populations, with studies covering genetics (Ballian et al. 2004, 2006, Nasri et al. 2008, Aleksić and Geburek 2010, 2014) and needle chemistry, including terpenes (Nikolić et al. 2008, 2009a, 2015) and *n*-alkanes (Nikolić et as. 2009b, 2013, 2023).

Research on the morpho-anatomical traits of various conifers in the Balkans and the surrounding regions remains active. Studies have covered the morpho-anatomy of species such as *Abies alba* Mill. (Pawlaczyk et al. 2005, Pawlaczyk and Bobowicz 2008, Ratknić et al. 2013, Popović et al. 2022a), *A. alba*, *Abies* × *borisii-regis* Mattf. and *Abies cephalonica* Loudon (Mitić et al. 2023), *Pinus heldreichii* H.Christ (Nikolić et al. 2014), *Pinus mugo* Turra (Bączkiewicz et al. 2005, Boratyńska and Boratyński 2007, Boratyńska et al. 2015), *Pinus nigra* J.F.Arnold (Macchioni et al. 2013, Mitić et al. 2021), *Pinus peuce* Griseb. (Nikolić et al. 2016), *Pinus sylvestris* L. (Boratyńska and Boratyński 2007, 2015), *Taxus baccata* L. (Stefanović et al. 2017), *Juniperus oxycedrus* L. and *J. deltoides* R.P.Adams (Vidaković et al. 2024), etc.

So far, the morpho-anatomical traits of *Picea* species have been examined by Vidaković (1982), Weng and Jackson (2000), Ishii et al. (2003), Bercu et al. (2010), Bercu and Popoviciu (2013), and Popović et al. (2022b), among others. Preliminary population studies of *P. omorika* needles were published about ten years ago (Radovanović 2014, Nikolić et al. 2015). However, it was necessary to confirm whether the southernmost population from Mileševka Canyon, known as var. *vukomanii* (Pavlović and Matović 1994), with the smallest needles, remains distinct from others when additional populations are included in the analysis.

The research objectives are obtaining morpho-anatomical properties of needles of seven populations of *Picea omorika* from Serbia, as well as their correlation with bioclimatic parameters.

The aim and novelty of this research lie in examining population diversity and the potential correlation between morpho-anatomical traits and the long-term environmental conditions at the studied localities of Serbian spruce, which is important for a better understanding of the ecology of this species and the future of this narrowly distributed taxon in the Balkans amid rapidly changing climate.

MATERIALS AND METHODS

Material Collection

Two-year-old needles from the lower third of the crown were collected in late autumn from seven natural populations of *Picea omorika* from Serbia and the border with Bosnia and Herzegovina: 1. BIL (Bilo), 2. CRST (Crvene Stene), 3. STU (Štula), 4. MIL (Mileševka Canyon), 5. VRA (Vranjak), 6. ZP (Zmajevački Potok) and 7. ZVE (Zvijezda). The spatial distribution of the analysed populations and their geographic information are presented in Table 1 and Figure 1.

Morphological and Anatomical Traits of the Needles

Needle length (NL) was measured with digital calliper Powerfix, while nine other needle traits, i.e. needle width (NW), needle thickness (NTH), vascular bundle diameter (VBD), resin ducts diameter (RDD), number of resin ducts (NRD), distance between vascular bundle and resin duct (VB-RD), cuticle and epidermal thickness (CT+EPI), hypodermal thickness (HYPO), and hypodermal width (HW) (Figure 2), were examined using a Leica Gallen III light microscope and a Topica TP/5001 (model CCD) camera. Needle cross-sections were made with a razor blade in the central part of the needles. Observations were carried out on 15 (six populations) or 10 (MIL population) trees per population and on 10 needles per tree. In total, approximately 1000 needles were measured.

Environmental and Geographical Data

Nineteen bioclimatic variables (BIO1-19) based on 30-year averages of temperature and rainfall, along with monthly precipitation, average temperatures, solar radiation and wind speed data (1970-2000) of all studied localities, were extracted from the WorldClim 2.0 set of global climate layers (Fick and Hijmans 2017), at 30 arc-second resolution (~1sq km) using QGIS 3.16 software (https://qgis.org/en/site/index.html). Inclination was recorded in the field and verified using Google Earth Pro (version 7.3.4.8248).

Statistical Analyses

Statistical analyses were performed by mean value (X), standard deviation (SD) and coefficient of variation (CV) calculations; ANOVA with homogenous groups identified using the LSD (least significant difference) test (P<0.05); CA (cluster analysis, Ward's method, Euclidean distance); PCA (principal component analysis), DA (discriminant analysis) and CLA (agglomerative hierarchical cluster analysis), using Statgraphics Plus (version 5.0; Statistical Graphics Corporation, U.S.A.), Statistica (version 10, Stat Soft Inc. 2011) and Addinsoft XLSTAT software (free trial version).

Table 1. Geographical parameters of analysed populations of *Picea omorika* in Serbia.

Population	**Population code**	**Latitude** (N)	**Longitude** (E)	**Mean elevation** (m)	**No. of analysed individuals**
Bilo	BIL	43° 55' 15''	19° 20' 20''	1200	15
Crvene Stene	CRST	43° 55' 15''	19° 22' 30''	950	15
Štula	STU	43° 55' 46''	19° 17' 11''	950	15
Mileševka Canyon	MIL	43° 20' 22''	19° 46' 32''	820	10
Vranjak	VRA	43° 51' 36''	19° 24' 05''	875	15
Zmajevački Potok	ZP	43° 51' 37''	19° 25' 29''	840	15
Zvijezda	ZVE	43° 58' 56''	19° 18' 29''	1220	15

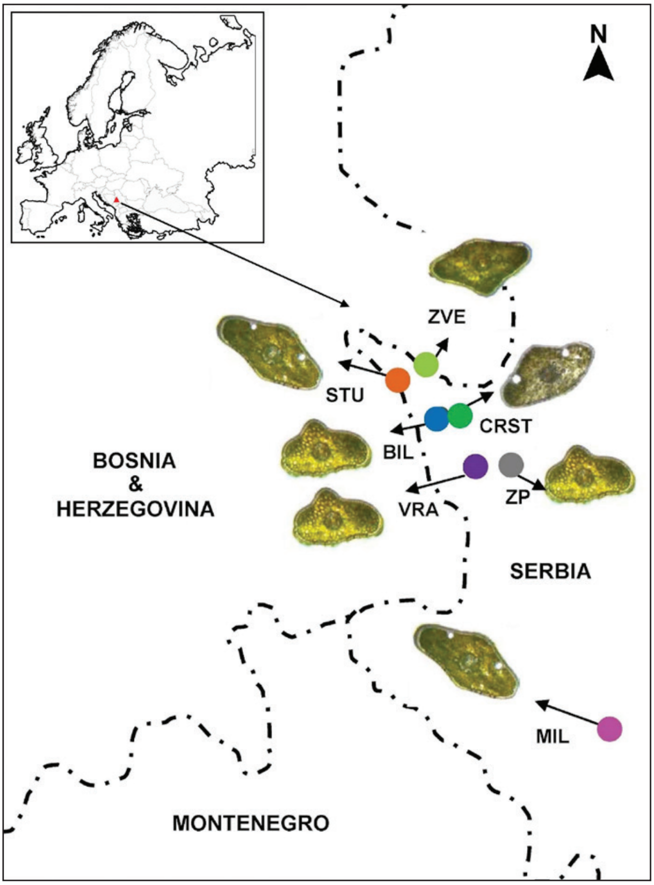

Figure 1. Locations of the analysed *Picea omorika* populations in Serbia, highlighting the predominant needle anatomy for each population.

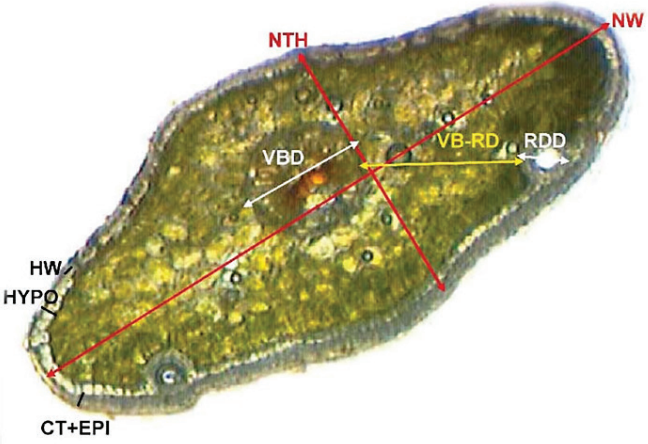

Figure 2. Cross-section of *Picea omorika* needles illustrating the measured traits: needle width (NW), needle thickness (NTH), vascular bundle diameter (VBD), resin ducts diameter (RDD), number of resin ducts (NRD), distance between vascular bundle and resin duct (VB-RD), cuticle and epidermal thickness (CT+EPI), hypodermal thickness (HYPO) and hypodermal width (HW).

For the multivariate statistical analysis, we included all nine studied morpho-anatomical traits. Multivariate statistical analysis involved principal component analysis (PCA), discriminant analysis (DA) and agglomerative hierarchical clustering (CA). PCA was carried out to determine the overall variation and relationships among individuals from the studied populations of *P. omorika*. Clustering-based discriminant analysis *(*DA) was performed to test the hypothesis that the analysed sample consists of distinct groups (studied populations) that are morpho-anatomically differentiated from one another. This analysis was done to determine the relative importance of morpho-anatomical traits as discriminators between *a priori* groups. Overall differences between the compared groups are presented by Mahalanobis distances. The calculated matrix distance was used for agglomerative hierarchical cluster analysis (CA), with the application of Ward's method.

Univariate and multivariate tests were used to check the correlation between environmental data and anatomical traits, including linear correlation, hierarchical cluster analysis, principal components analysis and Mantel and partial Mantel tests. Anatomical and bioclimatic data were log-transformed for multivariate analyses (ln(X+1)).

RESULTS

In cross-sections, the needles of *P. omorika* were elliptical, triangular or rhomboidal (Figure 1). There was no correlation between anatomical needle anatomy and the number of resin ducts (results were not presented). Needles with triangular cross-sections were the most common in the southern part of Mt. Tara, whereas elliptical and rhomboidal cross-sections were characteristic of the range edge in Serbia, i.e. the northern part of Mt. Tara (STU, ZVE) and Mt. Jadovnik (MIL) (Figure 1).

The measured traits of *P. omorika* needles were: needle width (NW), needle thickness (NTH), vascular bundle diameter (VBD), resin ducts diameter (RDD), number of resin ducts (NRD), distance between vascular bundle and resin duct (VB-RD), cuticle and epidermal thickness (CT+EPI), hypodermal thickness (HYPO) and hypodermal width (HW). These measured traits of *P. omorika* needles are also positioned in Figure 2, while their statistical parameters are given in Table 2. The highest mean values for the following traits were found in the STU population: NL, NW, NTH, VBD and HW. The ZP population exhibited the highest values for CT+EPI, while for the ZVE population the highest value was for HYPO. The lowest mean values for NW, NTH, VBD, CT+EPI, HYPO and HW were found in the BILO population; the ZP and VRA populations had the lowest values for RDD, NRD and VB-RD. Among all the measured traits, RDD and NRD exhibited the highest variability, while HYPO was the least variable trait.

For most of the analysed morpho-anatomical traits, the STU population showed the highest values and the BIL population the lowest. Needles with triangular cross-sections are most common in the southern part of Mt. Tara, whereas elliptical and rhomboidal cross-sections are characteristic for the range edge in Serbia, i.e. the northern part of Mt. Tara (STU, ZVE) and Mt. Jadovnik (MIL). No correlation was found between needle anatomy and the number of resin ducts.

Multivariate Statistical Analyses

The first two eigenvectors of PCA explain 72.34% of the total variability (Figure 3a). Morpho-anatomical traits that account for most of the variability in the entire data set are NTH, NW, VBD, NRD, and RDD (Figure 3b). Even though the first axis explains most of the variability (48%) and distinguishes the CRST, STU, ZP and ZVE populations from BIL, MIL and VRA, not all individuals within these populations are distinctly separated. There is a general impression of population overlap, as evidenced by the overlap of 95% confidence intervals of element affiliation within each population. Interestingly, the MIL population did not diverge from other populations, even though individuals from this population belong to a different variety (*P. omorika* var. *vukomanii*) and are situated in a different mountain range (Jadovnik vs. Tara).

Discriminant analysis (DA) with populations as predefined groups also showed population overlap (Figure 3c). The first discriminant axis also explains 48% of the total intrapopulation variability and it is highly influenced by VBD. DA was only able to differentiate BIL from ZP, while all the other populations exhibited an overlap. Unlike PCA and DA, cluster analysis (CA) revealed the formation of three clusters. The first cluster contains the MIL, VRA and BIL populations, the second ZP, ZVE and CRST, while the STU population forms the third cluster (Figure 3d).

Environmental and Geographical Data

To better understand the data variability, correlations with environmental and geographical data were analysed. The Mantel test did not show a correlation between anatomical data and geographic location (R=-0.31, *p*=0.98). Multivariate analysis revealed differentiation among localities based on bioclimatic data, as well as wind and solar radiation (Figure 4, Supplementary material 1).

A linear correlation test was used to assess the potential correlations among nineteen bioclimatic parameters, including temperature, precipitation, wind speed, solar radiation and inclination (Supplementary Material 2: Table S1). The analysed environmental factors showed different correlations with some of the anatomical characteristics. Bioclimatic parameters based on temperature (annual mean temperature, yearly temperature range, mean temperatures in the wettest and driest quarters) showed a moderate-to-strong negative correlation with leaf width and height (NL, NH). In other words, lower average temperatures correlated with thicker leaves (NW, NTH). Monthly mean temperatures were all negatively correlated with needle size (NL, NW, NTH). However, these correlations were somewhat higher for the winter months. Since altitude and temperatures are negatively correlated, the opposite holds true for altitude. While annual, average and spring solar radiation did not correlate with any of the anatomical traits, solar radiation in May showed a strong negative correlation with leaf size (NL, NW, NTH), central cylinder diameter (VBD) and hypodermal cell width (HW). Solar radiation in August and September was negatively associated with leaf size and thickness, particularly with needle thickness (NTH).

Table 2. Statistical parameters of the measured traits of seven *Picea omorika* populations.

No	POP		**NL** (mm)				**NW** (µm)		
		Min-Max	**X**	SD	**V** (%)	**Min-Max**	**X**	SD	**V** (%)
1	BIL	9.0 – 16.0	11.34^{a}	1.54	13.63	1079.3-1645.4	1355.6^{a}	111.9	8.25
2	CRST	7.0 – 16.5	11.50^{a}	1.85	16.11	1210.5-2103.1	1653.3^{c}	157.7	9.54
3	MIL	9.0 – 16.0	11.53^{a}	1.45	12.55	1059.7-1831.2	1523.9^{b}	153.1	10.05
4	STU	9.0 – 20.0	14.62^{e}	2.31	15.80	1279.5-2329.7	1825.6^{e}	218.4	11.96
5	VRA	10.0 – 16.0	13.10^{c}	1.30	9.93	1178.0-1962.9	1490.5^{b}	173.3	11.63
6	ZP	10.0 – 18.0	14.06^{d}	1.80	12.80	1352.3-2388.1	1700.9^{d}	159.9	9.40
7	ZVE	8.0 – 19.0	12.64^{b}	2.11	16.67	1146.9-2293.7	1669.8^{cd}	169.4	10.14
AVERAGE		7.0 – 20.0	12.74	2.18	17.11	1059.7-2388.1	1606.8	221.8	13.81
			NTH (µm)				**VBD** (µm)		
1	BIL	525.2-925.0	712.4^{a}	69.67	9.78	165.17-316.77	222.00^{a}	25.33	11.41
2	CRST	684.7-1192.8	914.8^{c}	95.35	10.42	229.45-392.14	291.99^{d}	33.20	11.37
3	MIL	616.4-1280.8	776.0^{b}	82.91	10.68	192.98-355.78	274.83^{c}	28.65	10.42
4	STU	739.8-1369.6	982.5^{d}	120.69	12.28	196.31-445.79	332.10^{f}	50.01	15.06
5	VRA	628.8-996.2	773.0^{b}	73.68	9.53	217.72-385.92	265.84^{b}	29.55	11.11
6	ZP	709.1-1078.3	910.5^{c}	64.51	7.08	203.87-388.96	313.75^{e}	28.10	8.96
7	ZVE	668.4-1338.9	917.8^{c}	111.58	12.16	210.20-414.09	292.10^{d}	39.44	13.50
AVERAGE		525.2-1369.8	859.3	130.11	15.14	165.17-445.79	285.15	48.25	16.92
			RDD (µm)				**NRD**		
1	BIL	0.00-119.06	36.07^{bc}	39.87	110.53	0.00-2.00	0.82^{c}	0.87	105.80
2	CRST	0.00-208.34	77.80^{d}	55.48	71.31	0.00-2.00	1.26^{d}	0.84	66.58
3	MIL	0.00-178.85	65.90^{d}	51.90	78.76	0.00-2.00	1.20^{d}	0.93	77.67
4	STU	0.00-186.74	69.89^{d}	51.16	73.20	0.00-2.00	1.23^{d}	0.84	68.01
5	VRA	0.00-122.43	24.31^{ab}	35.32	145.29	0.00-2.00	0.59^{ab}	0.84	140.94
6	ZP	0.00-136.26	23.89^{a}	42.49	177.86	0.00-2.00	0.43^{a}	0.74	172.06
7	ZVE	0.00-138.44	38.61^{c}	47.20	122.25	0.00-2.00	0.73^{bc}	0.85	118.13
AVERAGE		0.00-208.34	47.18	50.83	107.74	0.00-2.00	0.88	0.89	101.79
			VB-RD (µm)				**CT+EPI** (µm)		
1	BIL	0.00-449.7	135.90^{b}	136.1	100.2	12.50-31.62	18.44^{a}	3.73	20.23
2	CRST	0.00-588.1	239.80^{d}	148.5	61.9	14.58-35.67	22.31^{d}	3.95	17.70
3	MIL	0.00-448.2	187.20^{c}	150.3	80.3	10.80-31.62	21.34^{cd}	3.79	17.76
4	STU	0.00-534.0	242.80^{d}	151.4	62.4	12.50-34.43	22.03^{d}	4.52	20.52
5	VRA	0.00-510.1	100.50^{a}	142.6	141.9	10.61-35.35	20.22^{b}	4.71	23.29
6	ZP	0.00-484.0	89.30^{a}	146.5	164.0	12.75-35.23	23.55^{e}	4.03	17.11
7	ZVE	0.00-544.3	150.50^{bc}	171.6	114.0	10.61-35.35	20.87^{bc}	4.71	22.57
AVERAGE		0.00-588.1	162.50	160.5	98.8	10.61-35.67	21.25	4.51	21.25
			HYPO (µm)				**HW** (µm)		
1	BIL	17.68-46.10	29.01^{a}	4.86	16.74	19.04-51.29	31.39^{a}	5.98	19.06
2	CRST	19.04-49.24	31.38^{b}	5.09	16.36	18.03-46.10	35.49^{c}	5.61	15.81
3	MIL	20.15-48.54	31.80^{b}	5.67	17.84	23.05-53.55	35.20^{bc}	5.87	16.68
4	STU	18.20-48.02	31.59^{b}	6.01	19.02	23.05-62.10	36.96^{d}	7.39	19.99
5	VRA	21.36-42.72	31.41^{b}	4.49	14.31	10.31-55.90	33.57^{b}	7.04	20.97
6	ZP	17.68-44.23	31.42^{b}	4.80	15.30	20.16-50.31	35.43^{c}	5.50	15.52
7	ZVE	25.12-50.25	35.64^{c}	5.23	14.90	16.77-53.85	36.43^{cd}	6.86	18.83
AVERAGE		17.68-50.25	31.75	5.47	17.34	10.31-62.10	34.91	6.61	18.93

Conversely, only precipitation seasonality showed a strong negative correlation with needle length (NL), and a moderate negative correlation with leaf width and thickness (NW, NTH), central cylinder-diameter (VBD) and the number of resin ducts (NRD). The figures suggest that leaves tend to be smaller in environments where precipitation varies more during the year. When compared with average monthly temperatures, only January temperatures had a moderate-to-strong negative effect on needle size (NL, NW, NTH) and the width of hypodermal cells (HW). Temperature

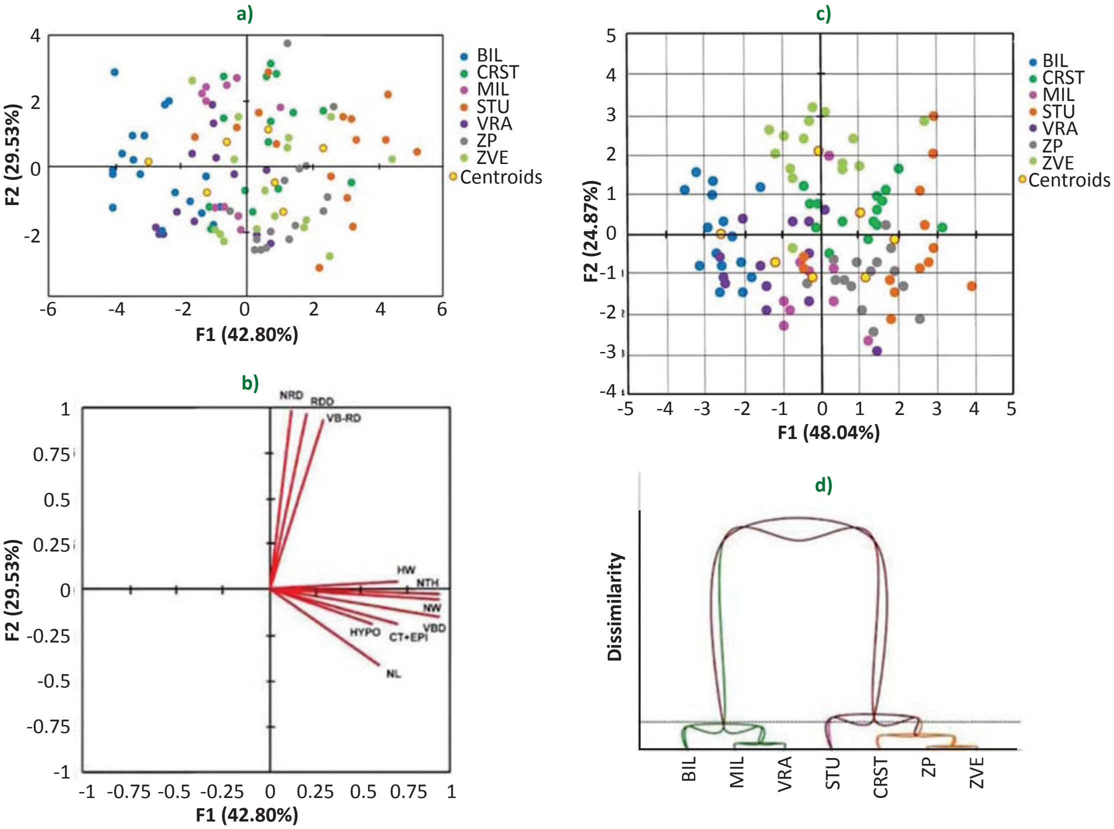

Figure 3. Multivariate statistical analyses: **a)** Principle component analysis (PCA); **b)** PCA – Morpho-anatomical traits; **c)** Discriminant analysis (DA); **d)** Cluster analysis (CA).

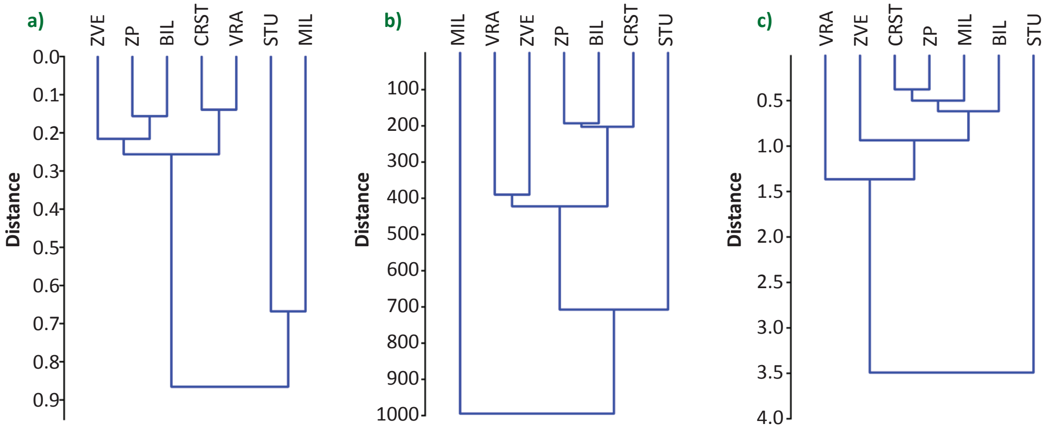

Figure 4. Dendrograms of seven *P. omorika* populations according to: **a)** precipitation and temperature; **b)** solar radiation, and; **c)** wind.

and precipitation did not correlate with resin duct size (RDD), number (NRD), position, and leaf epidermis and cuticular thickness (CT+EPI), underscoring the taxonomic significance of these characters. Since inclination and wind speeds contribute to local aridity, correlation with these environmental factors was also considered. With the exception of resin duct diameter (RDD) and thickness of cuticle and epidermis (CT+EPI), which had a moderately negative correlation with inclination, inclination did not have a strong correlation with other anatomical traits. This may be attributed to the varying inclinations across some localities. However, monthly, maximum and average annual wind speeds showed moderate correlations with needle thickness (NTH), suggesting that trees may develop more robust leaves to better withstand stronger winds.

Mantel and partial Mantel tests confirmed no correlation between the full set of morphological traits and bioclimatic parameters, even after adjusting for regional effects.

DISCUSSION

This study examined almost twice as many natural populations, including three newly sampled populations from Mt. Tara: Bilo, Crvene Stene and Zvijezda (BIL, CRST and ZVE, resp.), and assessed a higher number of morpho-anatomical needle traits, adding three new traits: central cylinder-diameter, distance between vascular bundle and resin duct and hypodermal width (VBD, VB-RD and HW, resp.) – compared to previous research by Nikolić et al. (2015). These new studies, as expected, significantly expanded the understanding of morpho-anatomical variability of *P. omorika* needles within its natural habitat. For most of the analysed morpho-anatomical traits, the STU population showed the highest values, and the BIL population the lowest. Needles with triangular cross-sections are most common in the southern part of Mt. Tara, whereas elliptical and rhomboidal cross-sections are characteristic for the range edge in Serbia, i.e. the northern part of Mt. Tara (STU, ZVE) and Mt. Jadovnik (MIL). No correlation was found between needle anatomy and the number of resin ducts.

In a previous study by Nikolić et al. (2015), which examined only four populations in Serbia, the mean NL value was a little higher but the range was somewhat lower. All the measured anatomical traits were significantly lower, even the NRD. Similar findings were observed when comparing our results with those by Milovanović et al. (2005) and Radovanović et al. (2014), except for RDD and NRD, which were much higher in Radovanović et al. (2014). This is likely due to the high natural variability of needles and differences in sampling methods. Additionally, since leaf anatomy and morphology can be influenced by local environmental conditions during leaf development, differing conditions between the two studies may have contributed to these differences. In cross-section, the needles are elliptical, triangular or rhomboidal, as previously reported in the literature (Radovanović et al. 2014, Nikolić et al. 2015). Most populations previously exhibited triangular cross-sections, either without resin ducts (BIL, VRA and ZP) or with two resin ducts (CRST). The STU and MIL populations previously had elliptical cross-sections with two resin ducts; however, in this study, the needle cross-sections were found to be either elliptical or rhomboidal. Rhomboidal cross-sections without resin ducts prevailed in the northernmost population (ZVE). According to Gebauer et al. (2019), sun-exposed needles in *Picea abies* were rhomboidal, whereas shade-exposed needles were more elliptical in cross-section. Based on this, sun-exposed needles were predominant in the ZVE population.

In the results presented herein, the average NL of the MIL population and its range of variation are significantly higher than those reported by Pavlović and Matović (1994). Popović et al. (2020) reported a larger NL for the MIL population, while the values of the other seven measured traits were lower than those found in the present study. The needles described by Isajev et al. (1999) were narrower but had a greater range of variation. The NTH in our study is slightly smaller than in *P. omorika* needles from an industrial zone (Ilijin-Jug 1995) and in artificial populations (Radotić and Topuzović 1988). The CT+EPI mean values presented here fall within the range of the results reported by Vilotić et al. (1994), but are higher than those found in artificial populations (Radotić and Topuzović 1988). HYPO has a larger range than that given by Vilotić et al. (1994). In the present study, NRD is zero to two. It seems that the third resin duct is formed only in polluted areas (Ilijin-Jug 1995).

In a previous analysis of four populations from Serbia (Nikolić et al. 2015), PCA indicated an overlap among all populations, even though the population from MIL diverged slightly from the others. However, the results of cluster analysis differ significantly. In the previous analysis, the VRA and ZP populations were the closest to each other, whereas in the current analysis, they were positioned in separate clades. This discrepancy may be attributed to the previous study using only five morpho-anatomical traits. In some other conifers, needle morphology, anatomy and population divergency have also been documented, including *Abies alba* and *Picea abies* (Ratknić et al. 2013), three *Abies* species (Mitić et al. 2023), *Pinus heldreichii* (Nikolić et al. 2014), *P. mugo* (Boratyńska et al. 2015), *P. nigra* (Mitić et al. 2021) *P. peuce* (Nikolić et al. 2016), *P. sylvestris* (Boratyńska and Boratyński 2015) and *Taxus baccata* (Stefanović et al. 2017), among others. Significant differentiation was observed in needle traits of *Abies alba* Mill. (Popović et al. 2022a, Popović et al. 2024) and *Picea abies* (L.) H. Karst. (Popović et al. 2022b) among mountain regions, among populations and within populations in relation to environmental factors.

Although there are differences in the environmental factors affecting *P. omorika* populations in Serbia, these factors correlated only with needle size and strength, showing no correlation with the thickness of epidermis, hypodermis or resin duct anatomy. Only inclination showed a moderate negative correlation with the thickness of the cuticle and epidermis. The lack of correlation with geographic locations may indicate that these traits have lower resolution than phytochemical traits (Judžentienė et al. 2006, Kupcinskiene et al. 2008, Rajčević et al. 2019), although no correlation was detected when analysing nuclear and mitochondrial DNA (Aleksić et al. 2014). There is some overlapping between molecular and morpho-anatomical markers. For example, the BIL and CRST populations, which are geographically close, clustered separately based on both morpho-anatomical and molecular markers. On the other hand, two geographically close populations, STU and Veliki Stolac (Aleksić et al. 2014), diverged from other populations based on morpho-anatomical and molecular markers. However, the overlapping is not absolute: the VRA and ZP populations were closely clustered in the morpho-anatomical analysis, but were strongly separated based on molecular markers. Mantel and partial Mantel tests confirmed that there was no correlation between the entire set of morphological traits and bioclimatic parameters. This lack of correlation persisted even after accounting for the influence of the geographic region and the distances between populations.

CONCLUSIONS

Although the morpho-anatomical features of the *P. omorika* needles partially distinguish two groups of populations (as indicated by PCA and DA), there is a general impression of population overlapping due to the high interpopulation variability of the analysed traits. Cluster analysis clearly differentiated two groups; however, the STU population was isolated from the other populations within its clade. In the previous analysis, which included fewer populations, the MIL population was the most distant and also the closest to the STU population. Based on bioclimatic parameters, individuals in the MIL and STU populations exhibited the most distinct characteristics.

Author Contributions

BN, ZM and PM conceived and designed the research, NČ and KM carried out the field measurements, SJ and MM performed laboratory analysis, ZM and NR processed the data and performed the statistical analysis, PM secured the research funding, supervised the research and helped to draft the manuscript, BN and NR wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary File 1 - To better understand the data variability, correlations with environmental and geographical data were analysed. All populations except MIL and STU populations formed two clusters: (1) VRA and CRST, and (2) ZVE, ZP and BIL, separated by differences in precipitation and temperatures in the driest and warmest quarters.

Supplementary File 2 - Linear correlation test was used to assess the possible correlation between nineteen bioclimatic parameters, temperature, precipitation, wind speed, solar radiation and inclination.

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