

# MORPHOLOGICAL VARIATION OF *BETULA PENDULA* AND *BETULA PUBESCENS* LEAVES IN SOUTH-EASTERN EUROPE

## MORFOLOŠKA VARIABILNOST LISTOV NAVADNE IN PUHASTE BREZE V JUGOVZHODNI EVROPI

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### IZVLEČEK

Navadna (*Betula pendula* Roth) in puhasta (*Betula pubescens* Ehrh.) breza sta ekološko pomembni v zmernih in borealnih gozdovih, in imata veliko morfološko variabilnost v okviru svojih arealov razširjenosti. V tej študiji smo želeli oceniti morfološko variabilnost listov med naravnimi populacijami breze v Bosni in Hercegovini (BiH) in v Sloveniji (SI) in raziskati odvisnost te variabilnosti od okoljskih in podnebnih dejavnikov.

Liste smo pridobili v 39 populacijah (31 v BiH in 8 v SI) v letih 2020–2022. Vzorčili smo 10 dreves na populacijo, z vsakega drevesa 30 listov. Skupno smo analizirali 11.700 listov. Morfometrične analize so vključevale izmerjene, ocenjene, preštete in izvedene znake. Uporabili smo opisno statistiko, gnezdeno analizo variance, koeficiente diferenciacije populacij, Pearsonovo korelacijo, analizo glavnih komponent (PCA) in analizo grozdov (UPGMA). Odnose med morfološki znaki in podnebnimi spremenljivkami smo ocenili z uporabo bioklimatskih podatkov iz baze WorldClim.

Rezultati so pokazali znatno fenotipsko variabilnost v morfologiji listov med preučevanimi populacijami. Prvi dve glavni komponenti sta pojasnili 73,2 % celotne variabilnosti, pri čemer parametri velikosti listov predstavljajo dominantno os diferenciacije. Analize grozdov in PCA so pokazale jasno regionalno strukturiranje, pri čemer so se slovenske populacije razlikovale od večine populacij v BiH. Kvalitativne lastnosti, kot so oblika listov, rob, dlakavost in tekstura, so pokazale znatno heterogenost med populacijami. Pri dveh populacijah, ki sta bili predhodno identificirani kot *B. pube-*

### ABSTRACT

Birch species (*Betula pendula* Roth and *Betula pubescens* Ehrh.) are ecologically important components of temperate and boreal forests, exhibiting considerable morphological variability across their distribution ranges. This study aimed to assess leaf morphological variation among natural *Betula* populations in Bosnia and Herzegovina and Slovenia and to examine its relationship with environmental and climatic factors.

Leaves were collected from 39 populations (31 in Bosnia and Herzegovina and eight in Slovenia) during 2020–2022. Ten trees per population were sampled, with 30 leaves collected from each tree. In total, 11,700 leaves were analyzed. Morphometric analyses included measured, assessed, counted, and derived leaf traits. Descriptive statistics, nested analysis of variance, population differentiation coefficients, Pearson correlations, principal component analysis (PCA), and cluster analysis (UPGMA) were applied. Relationships between morphological traits and climatic variables were evaluated using bioclimatic data from the WorldClim database.

The results revealed substantial phenotypic variability in leaf morphology among the studied populations. The first two principal components explained 73.2% of the total variation, with leaf size parameters representing the dominant axis of differentiation. Cluster and PCA analyses indicated clear regional structuring, with Slovenian populations forming a distinct group from most populations in Bosnia and Herzegovina. Qualitative traits such as leaf shape, margin, pubescence, and texture showed significant heterogeneity among populations. Leaf pubescence was confirmed in two populations previously

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*scens* (Han Kram in Mokro 2), smo potrdili dlakavost listov. Ugotovili smo močne korelacije med morfološkiimi lastnostmi listov in podnebnimi spremenljivkami, zlasti povprečno letno temperaturo, ki je pokazala močan vpliv na dimenzije listov.

Na splošno smo ugotovili izrazito morfološko raznolikost med populacijami brez v JV Evropi, pri kateri imajo ključno vlogo okoljski gradienti, zlasti temperatura.

*Ključne besede:* breza, morfologija listov, fenotipska variabilnost, diferenciacija populacij, klimatski gradienti, analiza glavnih komponent, gozdni genski viri

identified as *B. pubescens* (Han Kram and Mokro 2). Strong correlations were observed between leaf morphological traits and climatic variables, particularly mean annual temperature, which showed a strong influence on leaf dimensions.

Overall, the findings highlight pronounced morphological diversity in *Betula* populations and suggest that environmental gradients, particularly temperature, play a key role in shaping leaf morphological variation in south-eastern Europe.

*Keywords:* birch, leaf morphology, phenotypic variability, population differentiation, climatic gradients, principal component analysis, forest genetic resources

## INTRODUCTION

Trees of the genus *Betula* (birches) play important ecological, economic and cultural roles across Europe's temperate and boreal forests. *Betula pendula* Roth (silver birch) and *Betula pubescens* Ehrh. (downy birch) are two widely distributed species in the region, each with distinct ecological niches but also overlapping habitats. *B. pendula* is a fast growing, light demanding pioneer species found from the Mediterranean up to central Siberia, and is considered as an essential component of temperate and boreal forests due to its role as a pioneer, its associations with fungi and insects, and its broad ecological amplitude (VAKKARI 2009). *B. pubescens* is a fast-growing, light-demanding pioneer species found throughout northern and north-western Europe and extending into parts of Asia, and is considered an important component of boreal and temperate ecosystems due to its role as a pioneer species, its tolerance of cold and wet conditions, and its broad ecological amplitude (ATKINSON, 1992). Despite their broad distributions and occasional habitat overlap, the two species differ in their ecological preferences. *Betula pubescens* is typically associated with acidic, peaty, or waterlogged soils, whereas *B. pendula* most commonly occurs on well-drained, neutral to slightly acidic soils (ATKINSON 1992). These ecological differences contribute to their contrasting distribution patterns and may influence morphological variation among populations. *Betula pendula* exhibits strong environmental adaptability, colonising nutrient-poor, disturbed or early-successional sites, and thereby contributing to ecosystem regeneration and succession. For example, the species has been described as a pioneer anemochorous species with a significant role in river-valley forests of the Irtysh basin, demonstrating rapid growth rate and strong environmental adaptability (LI et al. 2024). In boreal/temperate forestry contexts, birches (including *B. pendula* and *B. pubescens*) are major components of forests, thus their management and conservation is relevant for climate change adaptation and multifunctional forestry

(VAKKARI 2009, SLEPETIENE et al. 2025). Additionally, many insect herbivore and symbiont communities are tightly associated with birch species. For example, in a *B. pendula* population the genotype of the tree explained substantial variation in the insect herbivore community structure (SILFVER et al. 2014). These observations highlight that the species are not just woody biomass carriers, but keystone components in forest biodiversity, nutrient cycling and ecosystem functioning.

Leaves serve as the primary interface between plants and their environment, playing essential roles in light capture, gas exchange, water regulation, and the integration of various developmental and physiological processes. Morphological variation in leaf traits reflects not only responses to environmental gradients but also developmental constraints, genetic variation, and evolutionary adaptations. For example, natural populations of *Quercus petraea* showed substantial variation in leaf morphological traits, with several traits significantly correlating with temperature- and precipitation-related climatic variables, suggesting patterns of local adaptation (MEMIŠEVIĆ HODŽIĆ et al. 2024, 2025). Such variation in leaf morphology can therefore serve as a reliable proxy for adaptive differentiation, phenotypic plasticity, and functional trait diversity within and among populations. Additionally, because leaf morphology is easier to quantify than many physiological traits, it provides a practical and accessible tool for studies of intraspecific variation and ecological adaptation.

Investigating morphological variation within species is increasingly recognized as essential for understanding how forest trees respond to environmental changes such as climate shifts, land-use transformation, and habitat fragmentation. This variation is particularly relevant for identifying adaptive traits, guiding the selection of suitable seed resources for ecological restoration, and conserving both functional and genetic diversity. Examining morphological traits across natural

populations of *B. pendula* and *B. pubescens* in diverse environments, such as those found in the south-eastern Europe, provides an opportunity to assess how environmental heterogeneity (e.g. differences in altitude, soil characteristics, moisture availability, and light conditions), along with possible genetic differentiation, influences leaf trait variation. Such insights are crucial for developing evidence-based conservation strategies, managing forest genetic resources, and selecting locally adapted material for future restoration efforts.

Although birch species are broadly distributed, there are relatively few detailed studies of morphological trait variation of birches in south-eastern Europe (the Balkans and adjoining Alpine/Pre-Alpine areas) (KOVAČIĆ and NIKOLIĆ 2005). By focusing on natural populations of *B. pendula* and *B. pubescens* in south-eastern Europe, the present study contributes novel data from this biogeographically interesting region characterised by complex topography, varied soils, and climatic gradients. The morphological variation documented may reflect unique local adaptation or plastic responses, which

have implications for both local ecosystem function and regional forest genetic resource management.

The present study aims to examine morphological variation of leaves in *B. pendula* and *B. pubescens* across natural populations in Bosnia and Herzegovina and Slovenia. Specifically, the objectives of this study were to:

- 1) quantify the morphological variation of key leaf traits within and among birch populations in Bosnia and Herzegovina and Slovenia
- 2) assess the extent to which observed variation corresponds to environmental and climatic gradients
- 3) discuss the implications of leaf morphological variation for functional ecology, forest management and conservation of birch genetic/trait diversity in the region.

By addressing these objectives, the study contributes to the trait based ecology of forest trees, enhances understanding of intraspecific variation in *Betula* species, and provides baseline information relevant for future provenance-based work and resource management of birches in south-eastern Europe.

## MATERIAL AND METHODS

During 2020-22, leaves of *Betula* sp. were collected from 31 locations (populations) in Bosnia and Herzegovina

and eight locations (populations) in Slovenia (Figure 1, Table 1).



Figure 1. Researched populations of *Betula* sp.

Four populations of birch in Bosnia and Herzegovina (Han Kram, Mokro 1, Mokro 2 - Triješanjan, Sokolac,

and Mrkonjić Grad) were previously described as *Betula pubescens* (FUKAREK 1957; STEFANOVIĆ 1961).

Table 1. Information on researched populations of *Betula* sp.

No	Population	Locality	Label	Altitude	Latitude	Longitude
1	BiH Bihać	Gata	BiH Bi	369	44.96308	15.83031
2	BiH Borike	Borike	BiH Bo	991	43.97831	19.22678
3	BiH Bosanski Petrovac	Šekovac	BiH BP	697	44.57231	16.51908
4	BiH Bosansko Grahovo	Resanovci	BiH BG	890	44.42389	16.37678
5	BiH Bužim	Baštra	BiH Bu	485	45.02912	16.08979
6	BiH Goražde	Hranjen	BiH Go	759	43.74917	19.03306
7	BiH Han Kram*	-	BiH HK	1095	44.09864	18.91961
8	BiH Jajce	Bavar	BiH Ja	935	44.37064	17.4375
9	BiH Kakanj	-	BiH Ka	798	44.19964	18.18628
10	BiH Kladanj	Budin potok	BiH Kld	764	44.37039	18.68597
11	BiH Ključ	Krasulje	BiH Klj	454	44.62947	16.76764
12	BiH Komar	Komar	BiH Ko	881	44.31747	17.61258
13	BiH Konjic	Jasenik	BiH Knj	1160	43.8375	17.83565
14	BiH Kreševo 1	Jelačke	BiH Kr 1	797	43.98411	18.05747
15	BiH Kreševo 2	Radava	BiH Kr 2	1308	43.89147	18.05928
16	BiH Kupres	Kupres	BiH Ku	1140	44.06372	17.26808
17	BiH Livno	Sajkovački lug	BiH Li	705	44.11075	16.72722
18	BiH Miljevina	Dobro polje	BiH Mi	951	43.58861	18.54606
19	BiH Mokro 1*	-	BiH Mo 1	991	43.99214	18.70242
20	BiH Mokro 2*	Triješanjan	BiH Mo 2	986	43.91901	18.62189
21	BiH Mrkonjić grad*		BiH MG	865	44.41424	16.99704
22	BiH Novi Travnik	Gornje Trenice	BiH NT 2	580	44.26094	17.7675
23	BiH Olovo	Bakići	BiH Ol	828	44.19872	18.65542
24	BiH Sanski Most 1	Dabar	BiH SM1	415	44.76014	16.61425
25	BiH Sanski Most 2	Hrustovo	BiH SM2	228	44.76206	16.84036
26	BiH Sokolac*		BiH So	872	43.92092	18.80037
27	BiH Srebrenik	Štalija	BiH Sr	427	44.75856	18.70758
28	BiH Tarčin	Osenik	BiH Ta	812	43.86367	18.25381
29	BiH Trnovo	Dejčići	BiH Tr	1001	43.71089	18.46456
30	BiH Žepa	Luka	BiH Že	1114	44.14378	19.31814
31	BiH Žepče	Donja Golubinja	BiH Žč	265	44.38827	17.98268
32	SLO Begunje	-	SLO Be	772	46.38163	14.21433
33	SLO Črnomelj	Semič	SLO Čr	203	45.70278	15.21667
34	SLO Ljubljansko Barje	Kozlerjeva Gošča	SLO LjB	278	46.08889	14.56944
35	SLO Novo Mesto	Koroška vas	SLO NM	334	45.79167	15.23889
36	SLO Pongrac	-	SLO Po	424	46.20183	15.15502
37	SLO Ponikva	Zagaj pri Ponikvi	SLO Pnk	405	46.27749	15.44412
38	SLO Šenčur	-	SLO Še	356	46.21111	14.45556
39	SLO Zgornje Gorje	-	SLO ZG	912	46.39444	14.05833

The geographic locations of the sampled populations were determined using GPS and consist of latitude, longitude, and altitude. The study used bioclimatic data obtained from the WorldClim portal (<https://www.worldclim.org/data/bioclimate.html>). The names of the bioclimatic variables are shown in Table 1.1.

worldclim.org/data/bioclimate.html). The names of the bioclimatic variables are shown in Table 1.1.

Ten trees per population were sampled, and a total of 30 leaves collected per tree. Birch leaves were collected from fertile shoots (short shoots) in the south-exposed parts of the crowns of adult trees growing in open areas or forest edges. Trees were selected based on standard functional trait sampling criteria, i.e., well-developed, healthy individuals growing in well-lit conditions to minimize the effects of shade-induced plasticity on leaf traits (CORNELISSEN et al. 2003). The leaves were then pressed, dried, and measured.

Table 1.1. List of bioclimatic variables (WorldClim database)

Bioclimatic variable	Variable Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

## Morphometric Analysis

The dried leaves were measured using a digital caliper (in mm) and a protractor. Six phenotypic traits were measured on each leaf (Table 2, Figure 2). Four traits

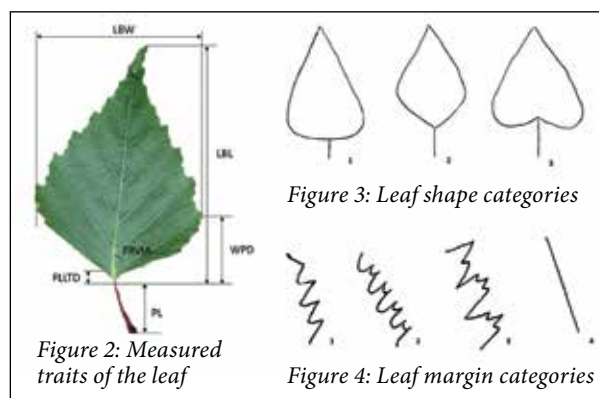


Table 2. Measured, assessed, counted, and derived traits of the birch leaf

No	Trait	Unit	Label	Trait type	Note
1.	Leaf blade length	mm	LBL	Measured	-
2.	Petiole length	mm	PL	Measured	-
3.	Leaf blade width at the widest point	mm	LBW	Measured	-
4.	Distance from leaf widest point to leaf base	mm	WPD	Measured	-
5.	Distance from petiole to first left leaf tooth	mm	FLLTD	Measured	-
6.	Angle of insertion of the first right vein	°	FRVIA	Measured	-
7.	Number of teeth between 2nd and 3rd vein		NT2-3	Counted	-
8.	Number of teeth between 3rd and 4th vein		NT3-4	Counted	-
9.	Leaf shape	-	LS	Assessed	Categories 1-3 (Fig. 3)
10.	Leaf margin	-	LM	Assessed	1 = coarsely serrate 2 = doubly serrate 3 = irregularly serrate 4 = entire margin (Fig. 4)
11.	Leaf pubescence on the abaxial side	-	LP	Assessed	1 = yes, 2 = no
12.	Leaf texture	-	LSR	Assessed	1 = smooth 2 = rough-textured leaf
13.	Total leaf length (blade + petiole)	-	TLL	Derived	-
14.	Blade width-to-length ratio	-	BW/BL	Derived	-

were assessed (Table 2, Figures 3 and 4), two were counted, and two were derived.

### Statistical Analysis

A logical data check was performed, and data indicating input errors were excluded.

Descriptive statistics included the maximum, minimum, and mean values, standard deviation (SD), and the coefficient of variation (CV) as a relative measure of variability, using all available data. The coefficient of variation was calculated as:

$$CV = \frac{SD}{\bar{x}} \cdot 100\%$$

The population differentiation coefficient (Vst) was calculated as:

$$Vst = \frac{\sigma_i^2}{\sigma_i^2 + \sigma_{j(i)}^2} \cdot 100\%$$

where  $\sigma_i^2$  denotes the variance among populations and  $\sigma_{j(i)}^2$  denotes the variance within populations (WANG et al. 2023). The variance components  $\sigma_i^2$  and  $\sigma_{j(i)}^2$  were obtained using a nested analysis of variance (ANOVA) (with trees nested within populations), according to the following linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + e_{(ij)k}$$

where:  $Y_{ijk}$  is the  $k$ -th observation on the  $j$ -th tree in the  $i$ -th population;  $\mu$  is the overall mean;  $\alpha_i$  is the

random effect of the  $i$ -th population;  $\beta_{j(i)}$  is the random effect of the  $j$ -th tree within the  $i$ -th population; and  $e_{(ij)k}$  is the experimental error associated with the  $ijk$ -th observation, representing the within-tree variation.

The homogeneity of distributions among populations for the assessed traits of leaf pubescence and leaf base shape was tested using the  $\chi^2$  test of homogeneity of distributions.

To investigate the correlation among phenotypic traits, the variables were standardized, and Pearson's correlation coefficients and their statistical significance were obtained using the `cor.test` function in R version 4.1.3 (R Core Team, 2016).

Principal component analysis (PCA) was used to reduce the phenotypic traits into a smaller number of principal components and to explore the continuum of trait variation. PCA was performed using the correlation matrix of standardized mean tree-level variables. The analysis was conducted using the MorphTools2 package in R version 4.1.3 (R Core Team, 2016), following the manual by KOUTECKÝ (2015).

Furthermore, a dendrogram of the nearest Euclidean distances was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) to examine the structure among the studied populations. Euclidean distances were calculated using the `dist` function in R based on population mean values standardized by the Z-score method, and subsequently subjected to cluster analysis (UPGMA method) using the `clust` function in the MorphTools2 package.

The analysis of correlations between the measured leaf traits and geographic and climatic factors was performed by Pearson's  $\chi^2$  Test.

## RESULTS

### Phenotypic variation of traits

Table 3 presents the results of descriptive statistics calculated for ten phenotypic leaf traits in order to assess phenotypic variability using the entire dataset. The coefficients of variation (CV) ranged widely, from 15.90% for the width of the widest part of the leaf blade to 45.70% for the distance from the petiole to the first tooth on the left side. The mean coefficient of variation was 27.42%.

### Phenotypic variation between and within populations

In Table 4, the proportion of variance components, population differentiation coefficients (Vst), and F-values

are presented for the ten studied leaf traits (measured, counted, and derived traits). The data are based on all observations from 39 natural populations of *Betula sp.* in Bosnia and Herzegovina and Slovenia, including variation between and within populations.

Analysis of the variance components showed the partitioning of variation in all traits among populations, among trees within populations, and within trees (residual) (Table 4). On average, the highest proportion of variation was observed among populations (mean = 45.70%), followed by within trees (mean = 32.85%), while the lowest proportion of variation occurred among trees within populations (mean = 21.45%).

Certain traits exhibited relatively high variation among populations. These traits included leaf blade length (69.71%), the distance from the widest point of

Table 3. Descriptive statistics of the studied leaf morphological traits for 39 natural populations of *Betula sp.* in Bosnia and Herzegovina and Slovenia

Variable code	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation (%)
LBL (mm)	34.60	77.20	50.20	12.00	23.90
PL (mm)	12.40	28.40	19.60	4.36	22.20
LBW (mm)	27.00	48.20	36.90	5.85	15.90
WPD (mm)	9.05	24.70	15.10	4.32	28.60
FLLTD (mm)	2.60	15.70	8.00	3.66	45.70
FRVIA (°)	25	63	43.70	10.30	23.70
NT2-3	2	7	4.30	1.43	33.30
NT3-4	1	7	4.05	1.76	43.40
TLL	48.90	101.0	69.90	14.50	20.70
BW/BL	0.52	0.95	0.76	0.13	16.80

Table 4. Proportion of variance components, population differentiation coefficient, and F-values for 10 traits based on all data from 39 natural populations of *Betula sp.* in Bosnia and Herzegovina and Slovenia.

Variable code	Proportion of variance components (%)			Population differentiation coefficient (%)	F - value	
	Among populations	Among trees within populations	Within trees (residual)		Among populations	Among trees within populations
LBL (mm)	69.71	15.06	12.23	53.50	1272.66***	28.82***
PL (mm)	20.36	34.19	45.46	11.33	137.73***	22.02***
LBW (mm)	20.36	37.69	41.95	11.33	161.26***	25.83***
WPD (mm)	50.64	21.39	27.97	33.90	519.37***	22.58***
FLLTD (mm)	38.21	29.85	31.93	23.62	360.55***	26.71***
FRVIA (°)	20.66	24.97	54.37	11.52	123.30***	14.01***
NT2-3	55.62	12.46	31.92	38.52	485.94***	11.55***
NT3-4	63.30	10.93	25.77	46.31	682.04***	12.53***
TLL	59.48	19.98	20.54	42.32	805.90***	28.18***
BW/BL	58.64	7.95	33.41	41.48	479.11***	7.69***
Mean	45.70	21.45	32.85	31.38	502.79***	19.99***

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

the blade to the base (50.64%), the distance from the petiole to the first tooth on the left side (38.21%), the number of teeth between the 2<sup>nd</sup>-3<sup>rd</sup> (55.62%) and 3<sup>rd</sup>-4<sup>th</sup> nerves (63.30%), total leaf length (59.48%), and the leaf blade length-to-width ratio (58.64%). In contrast, traits such as petiole length, leaf blade width, and the insertion angle of the first nerve on the right side showed lower variation among populations.

Regarding within-population variation among individual trees, high variability was observed for petiole length (45.46%), leaf blade width (41.95%), and the insertion angle of the first nerve on the right side (54.37%). The most stable traits within populations were the num-

ber of teeth between the 2<sup>nd</sup>-3<sup>rd</sup> (12.46%) and 3<sup>rd</sup>-4<sup>th</sup> nerves (10.93%), and the leaf blade length-to-width ratio (7.95%), while the least stable trait among individual trees within populations was leaf blade length (12.23%).

The population differentiation coefficient averaged 31.38%. The most pronounced differentiation was observed for leaf blade length (53.50%), followed by the number of teeth between the 3<sup>rd</sup> and 4<sup>th</sup> vein (46.31%), total leaf length (42.32%), and the ratio of leaf blade length to width (41.48%). In contrast, traits such as petiole length, leaf blade width, and the insertion angle of the first vein on the right side showed significantly lower values ( $\approx 11$ -12%).

## Correlations Between Leaf Traits

In Figure 5, the relationships among ten phenotypic leaf traits at the tree level are illustrated through a Pearson correlation matrix.

The strongest positive correlations were observed between leaf blade length and total leaf length, as well as between the number of teeth between the 2<sup>nd</sup>-3<sup>rd</sup> and 3<sup>rd</sup>-4<sup>th</sup> veins ( $r = 0.97-0.98$ ,  $p < 0.01$ ). Moderate positive correlations were detected between leaf blade length, leaf blade width, total leaf length, and the distance from the widest point of the blade to the base ( $r = 0.66-0.74$ ,  $p < 0.01$ ). Negative correlations were mainly found for the

leaf blade length-to-width ratio in relation to leaf blade length, total leaf length, and the distance to the base ( $r = -0.74--0.82$ ,  $p < 0.01$ ). The insertion angle of the first vein showed weak or negligible correlations with other traits.

## Principal components and cluster analysis

Principal Component Analysis (PCA) was conducted to identify and quantify variability among trees for the studied morphological traits and to identify the principal components explaining the largest portion of total variance (Table 5).

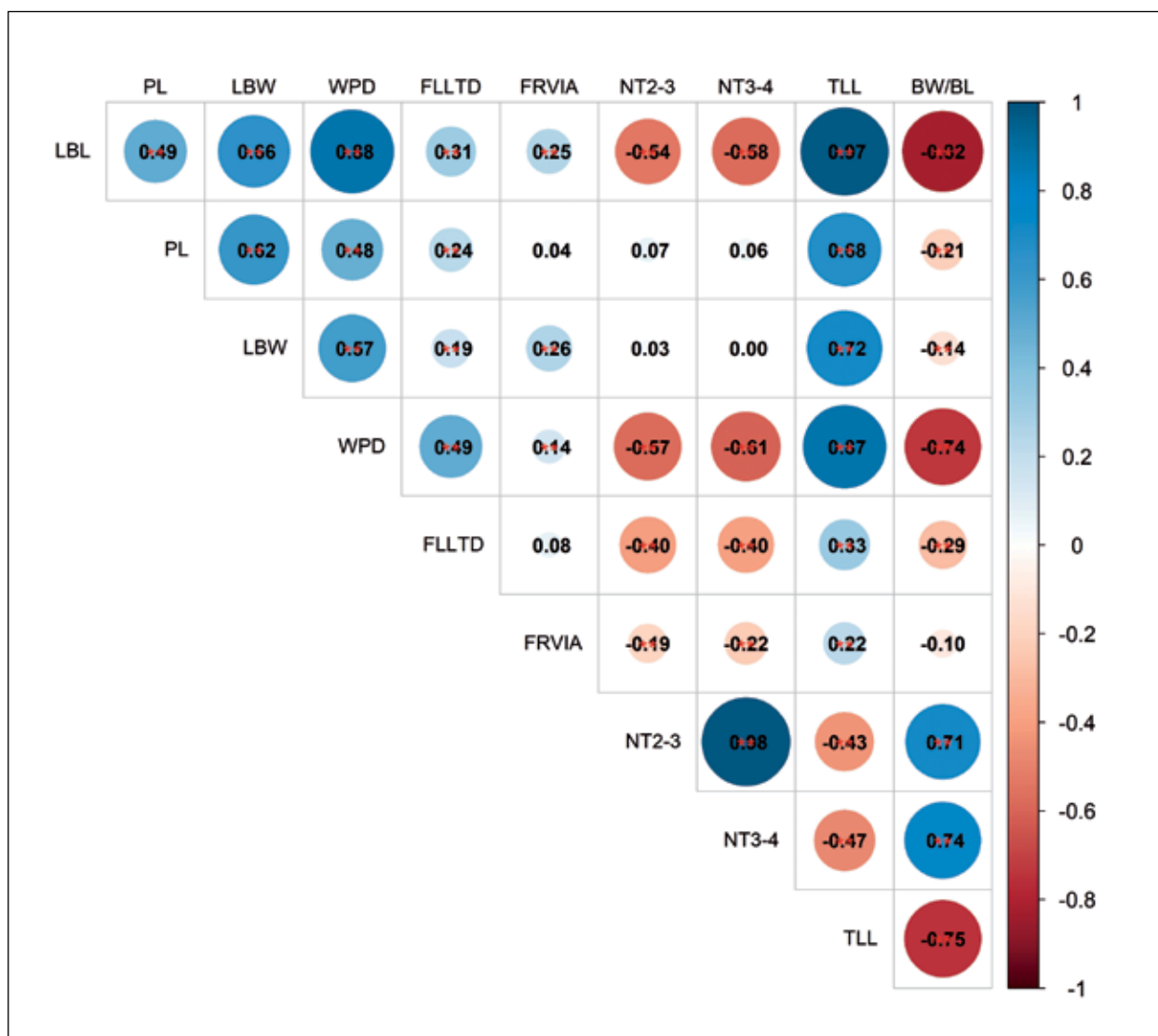


Figure 5: Correlation analysis of 10 phenotypic leaf traits (measured, counted and derived traits) at the tree level. Blue indicates positive correlations, while red indicates negative correlations. Larger circles and darker colors represent stronger correlations ( $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ).

Table 5. Eigenvalues, contribution rates, and cumulative proportion of the five principal components based on the mean tree values for the studied traits.

Variable code	PC1	PC2	PC3	PC4	PC5
LBL	0.415	0.104	0.026	0.216	-0.134
PL	0.217	0.484	-0.183	-0.100	0.693
LBW	0.246	0.490	0.168	-0.043	-0.572
WPD	0.406	0.052	-0.123	-0.035	-0.221
FLLTD	0.217	-0.083	-0.199	-0.891	-0.023
FRVIA	0.119	0.014	0.927	-0.163	0.248
NT2-3	-0.304	0.463	-0.033	0.023	-0.002
NT3-4	-0.317	0.447	-0.063	0.001	0.012
TLL	0.408	0.215	-0.026	0.155	0.061
BW/BL	-0.365	0.215	0.136	-0.309	-0.246
Eigenvalues	5.252	2.070	1.013	0.847	0.385
Variance contribution rate	0.525	0.207	0.101	0.085	0.038
Cumulative proportion	0.525	0.732	0.833	0.918	0.957

Principal component analysis (PCA) was conducted at both the tree and population levels and is shown in Figure 6.

The first two components explain a total of 73.20% of the variability in the analyzed leaf morphological

traits, confirming the stability of the model (Figure 6). The first component was mainly associated with leaf blade dimensions and total leaf length (LBW, PL, TLL, LBL, WPD), with these traits showing strong loadings on this axis. The second component was associated with

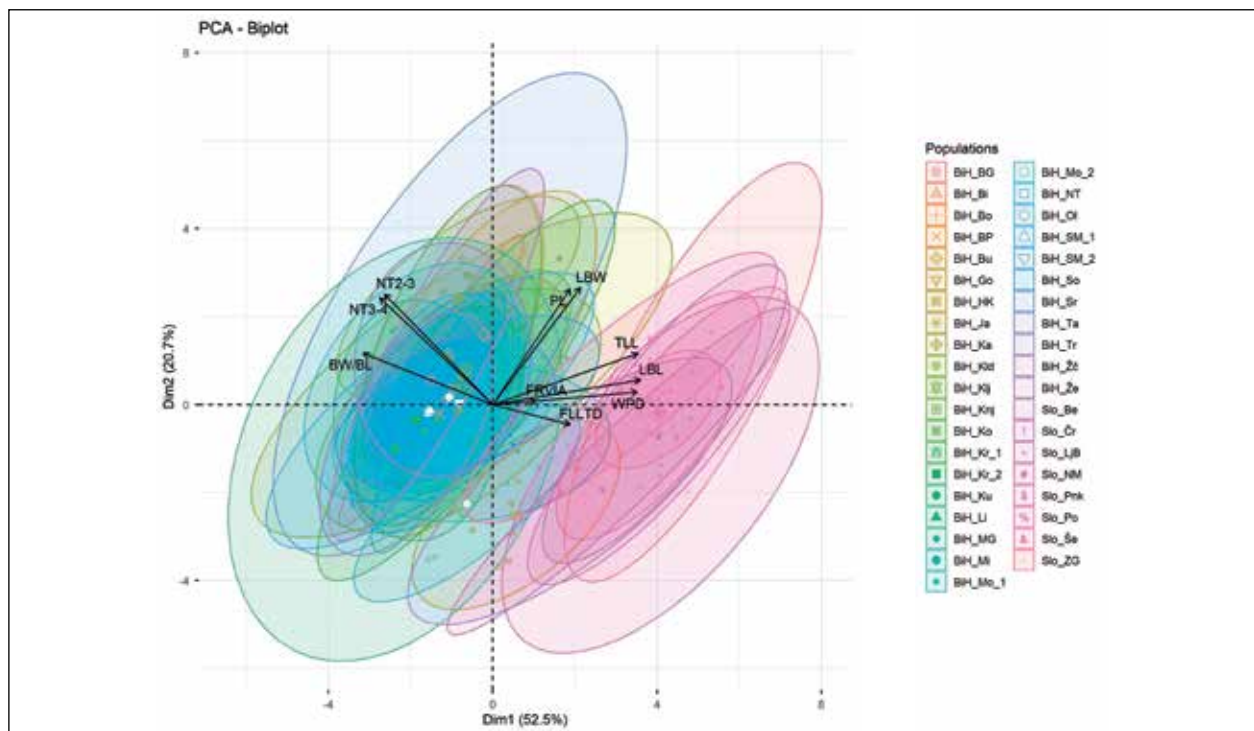


Figure 6: Biplot of the principal component analysis (PCA) based on tree mean values for 10 morphometric traits in 39 studied populations of *Betula* sp. in Bosnia and Herzegovina and Slovenia.

the number of teeth between the 2<sup>nd</sup>-3<sup>rd</sup> and 3<sup>rd</sup>-4<sup>th</sup> veins (NT2-3, NT3-4). In contrast, the distance from the petiole to the first left tooth (FLLTD) showed a lower loading on the main axes. The two-dimensional plot shows clear grouping of populations, with populations from Bosnia and Herzegovina overlapping among themselves, and populations from Slovenia forming a partially overlapping group. Populations previously identified as pubescent (*B. pubescens*) (BiH\_MG, BiH\_HK, BiH\_Mo2, BiH\_Mo1, and BiH\_So) are located within the main Bosnian and Herzegovinian cluster, with BiH\_MG, BiH\_HK, and BiH\_Mo2 positioned closer to the central part of this group, while BiH\_Mo1 and BiH\_So overlap with the remaining Bosnian and Herzegovinian populations.

The results of the cluster analysis of 39 birch populations from Bosnia and Herzegovina and Slovenia are shown in Figure 7.

Cluster analysis of 39 populations resulted in the formation of two clearly defined macro-clusters (Figure

7). The first macro-cluster includes all 8 populations from Slovenia and one population from Bosnia and Herzegovina (Žepče), while the second macro-cluster comprises the remaining 30 populations from Bosnia and Herzegovina. Populations previously identified as pubescent (*B. pubescens*), including Mrkonjić Grad (BiH\_MG), Han Kram (BiH\_HK), Mokro 2 (BiH\_Mo2), Mokro 1 (BiH\_Mo1), and Sokolac (BiH\_So), are distributed within the Bosnian and Herzegovinian cluster, with BiH\_MG, BiH\_HK, and BiH\_Mo2 forming a separate subcluster within this group.

### Homogeneity of evaluated leaf trait distributions among populations (Pearson's $\chi^2$ Test)

The homogeneity of distributions among populations for the evaluated leaf traits: shape, margin, pubescence, and texture was tested using Pearson's  $\chi^2$  test of homogeneity. The obtained  $\chi^2$  values were 994.35 (df = 38,  $p <$

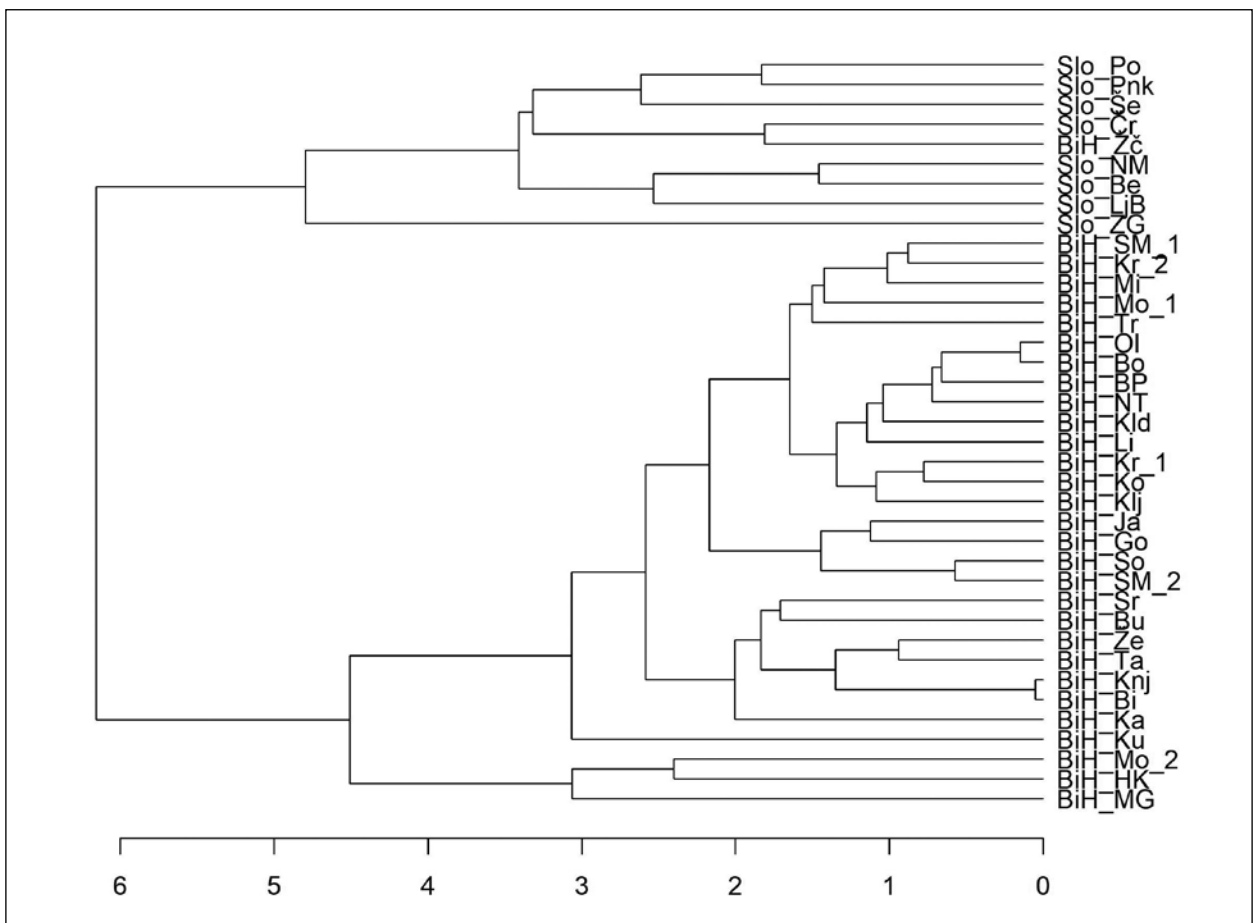


Figure 7. Dendrogram of *Betula* sp. populations in Bosnia and Herzegovina and Slovenia, constructed using the UPGMA method based on the analyzed morphological traits.

0.001) for leaf shape, 16,094.00 ( $df = 76$ ,  $p < 0.001$ ) for leaf margin, 4,552.70 ( $df = 38$ ,  $p < 0.001$ ) for leaf pubescence, and 994.35 ( $df = 114$ ,  $p < 0.001$ ) for leaf texture. All tests showed statistically significant differences in the distribution of categories among populations.

### Percentage distribution of leaf shape traits among *Betula* sp. populations

The maximum percentage representation of the leaf shape trait LS1 was recorded in the population Srebrenik (Bosnia and Hercegovina) with 59.5%. Other populations from Bosnia and Herzegovina also showed a notable representation of LS1: Kakanj (45.3%), Mokro 2 (42.3%), and Mrkonjić Grad (38.8%), whereas the populations Goražde and Kladanj showed much lower values, 7.4% and 7.0%, respectively. Among Slovenian populations, Begunje showed 48.0% representation of LS1, while in some other Slovenian population (Ljubljansko Barje, Ponikva, and Pongrac) this category was not recorded (0.0%).

The highest percentage representation of the leaf shape LS2 was recorded in the Slovenian populations Ljubljansko Barje and Pongrac (100.0%), followed by Ponikva (98.6%). High values among populations from Bosnia and Herzegovina was observed in Kladanj (92.7%) and Sokolac (92.2%). The lowest percentage representation of LS2 was recorded in the Bosnian-Herzegovinian population Srebrenik (40.5%), followed by Slovenian population Begunje (40.9%). All the populations previously described as pubescent birch (FUKAREK 1957, STEFANOVIĆ 1961) had the highest percentage of LS2 (Han Kram 76.9%, Mokro 1 64.0%, Mokro 2 57.0%, Mrkonjić Grad 57.0%, and Sokolac 89.8%).

The highest percentage of leaf shape LS3 was also recorded in the Slovenian population Begunje (11.2%), while this category was absent (0.0%) in the populations Ljubljansko Barje, Pongrac, and Zgornje Gorje. Among the populations from Bosnia and Herzegovina, the highest percentage was recorded in Mrkonjić Grad (9.7%). The leaf shape LS3 was not recorded in the Bosnian and Herzegovinian populations Jajce, Kakanj, Kreševo 1, Sanski Most 1, Srebrenik, and Tarčin.

### Percentage distribution of leaf margin traits among *Betula* sp. populations

The maximum representation of category LM1 (coarsely serrate) among the Slovenian populations was recorded in populations Ljubljansko Barje and Pongrac (100.0%), followed by Šenčur (90.0%), Zgornje Gorje (89.9%), Be-

gunje (75.8%), and Novo Mesto (40.3%). Populations Črnomelj and Ponikva had no LM1. Among the populations from Bosnia and Herzegovina the highest value of LM1 was observed in Žepče (93.3%), while other populations had small percentage or no LM1.

For category LM2 (doubly serrate), the highest representation among the Slovenian populations was recorded in Črnomelj and Ponikva (100.0%), followed by Novo Mesto (59.7%), Begunje (24.2%), and Šenčur (10.0%). In Bosnia and Herzegovina, LM2 was recorded in Mrkonjić Grad (25.5%) and Han Kram (9.7%), while 32 populations showed values within the interval 0.0%–2.0%.

In category LM3 (irregularly serrate), high representation was observed in Bosnia and Herzegovina, where 28 populations showed values above 90.0%. Among Slovenian populations, LM3 was recorded only in Zgornje Gorje (10.1%), while the remaining seven populations showed no representation (0.0%). All the populations previously described as pubescent birch (FUKAREK 1957, STEFANOVIĆ 1961) had the highest percentage of LM3 (Han Kram 73.6%, Mokro 1 100.0%, Mokro 2 100.0%, Mrkonjić Grad 69.4%, and Sokolac 99.3%).

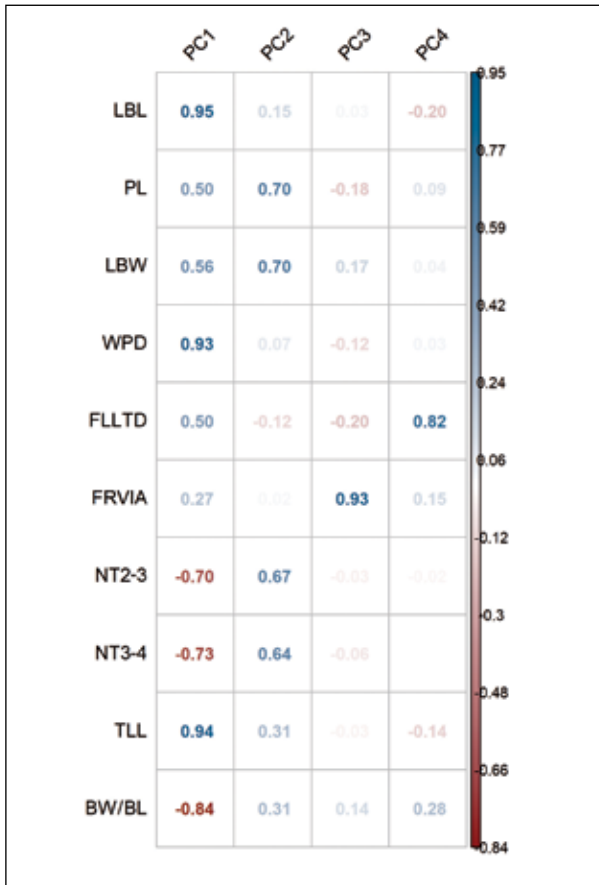
Leaf margin category LM4 (entire margin) was not recorded in any of the studied populations.

### Percentage distribution of leaf pubescence on the abaxial side traits among *Betula* sp. populations

The results of the assessment of the leaf pubescence (LP) showed that the highest percentage of pubescent leaves was recorded in the populations Han Kram and Mokro 2 (100.0%), which were previously described as pubescent (FUKAREK 1957; STEFANOVIĆ 1961). Other populations previously identified as pubescent (Mokro 1, and Mrkonjić Grad, and Sokolac) showed lower percentage of leaves with pubescence (1.4%, 6.8%, and 26.5%, respectively). The populations Ključ and Sanski Most 2 exhibited 56.7% and 53.8% of leaves with pubescence, respectively. Among the Slovenian populations, pubescence was not observed.

### Percentage distribution of leaf texture traits among *Betula* sp. populations

Among the Slovenian populations, high percentages of smooth leaves were recorded in the populations Novo Mesto (69.8%) and Zgornje Gorje (59.6%). Among the populations from Bosnia and Herzegovina, higher percentages of glabrous leaves were observed in the populations Jajce (70.3%) and Trnovo (45.3%).



### Correlation between the investigated morphological traits and climatic factors

Figure 8 shows the contribution of the analyzed morphological variables to the principal components.

The morphological variability of the analyzed leaf traits at the population level was reduced to five principal components, which cumulatively explain 95.7% of the total variance. The first two components explain (73.2%) of the total variance. The first component is mainly associated with leaf dimensions (LBL, WPD, TLL, LBW, and PL). The second principal component (PC2) is associated with length-width and numerical traits (LBW, PL, NT2-3, and NT3-4). The third principal component is mainly associated with the angle of insertion of the first vein on the right side (FRVIA), while the fourth principal component is associated with the distance from the petiole to the first left tooth (FLLTD) (Figure 8).

Figure 9 illustrates the correlation relationships between the first four principal components of leaf morphological traits and bioclimatic variables. PC1 shows a strong positive correlation with temperature variables BIO1 (mean annual temperature) and BIO9 (mean temperature of the driest quarter) ( $r = 0.97$ ). PC2 is associ-

Figure 8. Contribution of morphological variables to the principal components.

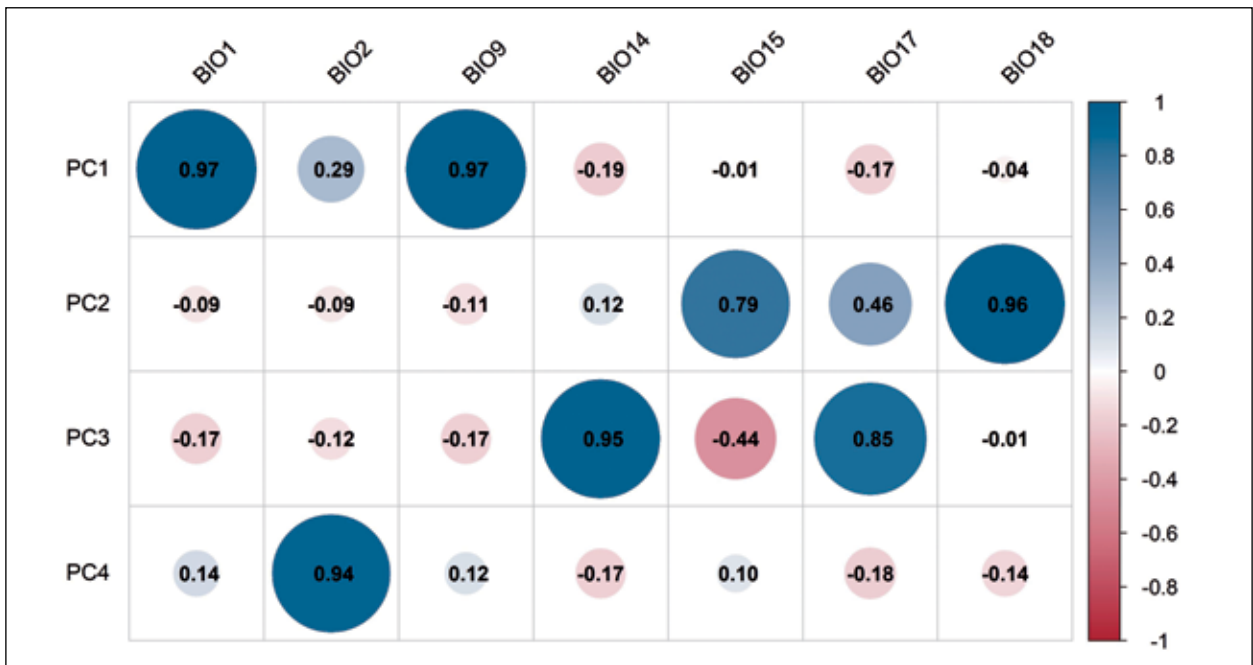


Figure 9. Correlation analysis between climatic variables and principal components. Blue indicates a positive correlation, while red indicates a negative correlation. Larger circles and darker colors represent stronger correlations ( $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ).

ated with seasonality and precipitation variables, particularly BIO15 (precipitation seasonality) and BIO18 (precipitation of the warmest quarter). PC3 is associated with extreme precipitation variables (BIO14 and BIO17), while PC4 is correlated with diurnal temperature range (BIO2).

Regression analysis was conducted to assess the relationship between the first principal component (PC1), representing leaf dimensions, and the climatic variables that showed high intercorrelation. Linear regression identified mean annual temperature (BIO1) as the best single predictor of the temperature gradient. The model

showed that BIO1 explains 94.0% of the total variability in PC1 ( $R^2 = 0.94$ ;  $p < 0.001$ ). The standardized regression coefficient ( $\beta = 0.97$ ) indicates a strong positive linear relationship between mean annual temperature and leaf dimensions. An increase of one standard deviation in mean annual temperature corresponds to an increase of approximately 0.97 standard deviations in PC1. Due to high collinearity between BIO1 and BIO9 ( $r = 0.996$ ), only BIO1 was retained in the interpretation to avoid multicollinearity between temperature variables. Both variables confirm that the variability in leaf dimensions is structured along a general temperature gradient.

## DISCUSSION

### Variation and differentiation of quantitative leaf traits

The present study highlights substantial phenotypic variability in leaf morphological traits of natural *Betula* sp. populations in Bosnia and Herzegovina and Slovenia, reflecting considerable heterogeneity consistent with patterns observed in other widespread woody species known for broad environmental tolerance and adaptive plasticity (SILFVER et al. 2007; CORNELISSEN et al. 2011).

Certain leaf traits appear highly variable, likely responding to microenvironmental conditions or local selective pressures, a phenomenon also reported in temperate trees such as oaks and poplars, where leaf shape and tooth characters vary with moisture, temperature, and light regimes (PEPPE et al. 2011; MEMIŠEVIĆ HODŽIĆ et al. 2024; BALLIAN et al. 2018; WANG et al. 2022). Conversely, traits like leaf width and shape tend to be more conserved, likely constrained by functional demands associated with photosynthesis, water-use efficiency, and heat dissipation (NICOTRA et al. 2011; LI et al. 2020). Our variance components analysis further indicates that the largest proportion of phenotypic variation occurs among populations, underscoring the importance of spatial structuring in leaf morphological traits across the landscape. This pattern suggests that populations occupy distinct phenotypic space, likely reflecting both local adaptation to environmental gradients and historical genetic structure. Similar among-population divergence has been observed in *Betula pendula* across Europe (SILFVER et al. 2007) and in *Quercus petraea* and *Q. robur* in Bosnia and Herzegovina along climatic and altitudinal gradients (MEMIŠEVIĆ HODŽIĆ et al. 2024; BALLIAN et al. 2018). In other forest tree species, including *Fagus syl-*

*vatica*, strong among-population variation in leaf traits has also been linked to adaptive differentiation along climatic gradients (BALLIAN et al. 2018; KARDOŠOVÁ et al. 2020). These observations collectively suggest that leaf morphological traits in long-lived deciduous trees reflect not only plastic responses but also inherited genetic variation structured by environmental heterogeneity.

Certain traits exhibiting high among-population variance, such as leaf size and tooth number likely carry strong adaptive signals. Leaf morphology influences key functional processes including light interception, transpiration, and thermoregulation (NICOTRA et al. 2011). Larger leaves are often associated with warmer, wetter environments, while smaller leaves and increased tooth frequency tend to occur in cooler or drier climates, reflecting adaptation to local thermal and water-use demands (PEPPE et al. 2011). Pronounced differentiation in these traits among *Betula* populations likely reflects divergent selection along climatic gradients in south-eastern Europe.

In contrast, traits such as petiole length, leaf width, and vein insertion angle showed greater variation within populations, suggesting that these traits are more homogeneous across populations and are influenced primarily by microenvironmental conditions and developmental plasticity. Similar patterns have been reported in other tree species, where fine-scale variations in light, nutrients, and ontogeny drive within-population trait diversity (VALLADARES et al. 2002; POORTER et al. 2009). Lower population differentiation in these traits implies they may be less subject to large-scale divergent selection or buffered by local plastic responses. The population differentiation observed indicates moderate genetic structuring among the studied populations, consistent with other temperate tree species with wind-dispersed

seeds and high gene flow, where moderate among-population divergence is common (ALBERTO et al. 2013; KREMER et al. 2012). Traits showing stronger differentiation may reflect adaptive divergence that overcomes gene flow, a pattern supported by studies combining common garden and landscape analyses (SAVOLAINEN et al. 2013).

Overall, the observed patterns of phenotypic variation and population differentiation in *Betula* leaf traits suggest the combined influence of environmental gradients, genetic differentiation, and phenotypic plasticity. Similar trends in *Quercus* and *Populus* species in the region indicate that leaf morphology often mirrors environmental heterogeneity, supporting the idea that adaptive differentiation in leaf traits is common among temperate broadleaf trees (MEMIŠEVIĆ HODŽIĆ et al. 2024; BALLIAN et al. 2018). Future work integrating genetic markers with common garden experiments could help to disentangle the contributions of genotype and environment and identify climatic drivers of adaptive variation.

### Variation in qualitative leaf morphological traits

Pearson's  $\chi^2$  test revealed significant heterogeneity in qualitative leaf traits among the studied populations, including leaf shape, margin, pubescence, and texture, indicating systematic differences rather than random variation. Such patterns likely reflect the combined effects of genetic variation, environmental influences, and phenotypic plasticity, consistent with observations in other widespread forest tree species (NICOTRA et al. 2011; CORNELISSEN et al. 2011). Pronounced differences in traits like leaf shape and margin suggest that birch populations in the studied region occupy distinct ecological niches, with leaf morphology corresponding to environmental drivers such as temperature, water availability, and light to optimize energy balance, transpiration, and photosynthetic efficiency (WRIGHT et al. 2017; PEPPE et al. 2011).

Leaf shape varied considerably among populations from both Bosnia and Herzegovina and Slovenia. Such variation aligns with previous studies showing that birch leaf form responds to environmental gradients, particularly temperature and moisture, which affect energy balance and water relations (NICOTRA et al. 2011). Populations previously identified as *B. pubescens* were dominated by the LS2 leaf shape category. Although *B. pendula* leaves are generally more triangular and *B. pubescens* leaves more ovate, the observed overlap and the occurrence of 100% LS2 in the Slovenian populations Ljubljansko Barje and Pongrac indicate that leaf shape alone is not sufficient for reliable species discrimination.

This supports the use of leaf shape as one of diagnostic traits, alongside other leaf characteristics and suggests that the dominance of specific shapes may reflect local adaptation or phenotypic plasticity.

Leaf margin categories showed clear geographic structuring, with LM3 dominant in most populations from Bosnia and Herzegovina, and LM1-LM2 prevailing in Slovenian populations. All populations previously identified as *B. pubescens* were consistently dominated by LM3. *B. pendula* typically has coarser serration, whereas *B. pubescens* exhibits finer, less sharp teeth (ATKINSON, 1992), supporting its use as a diagnostic trait alongside leaf shape and pubescence (FUKAREK 1957; STEFANOVIĆ 1961). However, our results only partially conform to the expected species-level pattern, as LM1-LM2 and LM3 categories showed overlap among populations and between species groups. This indicates that leaf margin morphology does not form discrete species-specific categories, but instead exhibits continuous variation influenced by both species identity and environmental conditions.

Leaf pubescence on the abaxial surface was most pronounced in the Han Kram and Mokro 2 populations, confirming their identification as *B. pubescens*, consistent with taxonomic descriptions where pubescence distinguishes it from the generally glabrous *B. pendula* (ATKINSON 1992). In *B. pubescens*, long shoots are hairy with glandular hairs and lack lenticels, whereas in *B. pendula*, shoots are smooth with lenticels (ATKINSON 1992). Other populations showed only minor pubescence, highlighting the trait's diagnostic value for species differentiation, especially in regions of overlapping distributions. Pubescence was absent in all Slovenian populations, suggesting they are predominantly *B. pendula*. However, reliable species identification should be based on a combination of morphological characters rather than a single trait, due to possible overlap and phenotypic plasticity. Given the potential for hybridization in Europe, genetic analyses would help clarify species identity and possible introgression (ATKINSON, 1992).

Leaf texture varied among populations, with smooth leaves recorded in certain populations from both countries. Surface characteristics can influence water retention, herbivory, and pathogen resistance, reflecting ecological strategies for balancing hydration and defense (VALLADARES et al. 2007).

Although geographic structuring was less pronounced for leaf texture than for other qualitative traits, these differences highlight overall morphological heterogeneity among populations.

Overall, the observed variation in qualitative leaf traits highlights substantial phenotypic diversity within natural *Betula* populations across Bosnia and Herzego-

vina and Slovenia. Similar patterns have been reported in other temperate tree species, where traits such as leaf shape, margin morphology, and pubescence respond to environmental gradients while also retaining signals of genetic differentiation (NICOTRA et al. 2011; PEPPE et al. 2011). Although several qualitative traits were useful for distinguishing groups of populations, particularly those previously identified as *B. pubescens*, considerable overlap among categories indicates that no single trait can reliably separate species. Instead, species identification and population differentiation are best interpreted using multiple morphological characters in combination with ecological and genetic information. The consistent differentiation of several populations previously identified as *B. pubescens* nevertheless demonstrates that qualitative leaf traits retain considerable taxonomic value when evaluated collectively.

A limitation of the present study is that species identification was evaluated primarily using leaf morphological characters. Although several diagnostic traits commonly used to distinguish *Betula pendula* and *B. pubescens* were assessed, not all taxonomically informative characters were systematically recorded during field sampling. In particular, bark characteristics, branching architecture, reproductive structures, and cytological traits were not examined. Consequently, species assignments should be interpreted with caution, especially for populations exhibiting intermediate morphological characteristics. Future studies integrating a broader set of diagnostic traits together with genetic analyses would provide more robust species identification and help clarify the extent of potential hybridization or introgression among populations.

### Trait correlations and functional integration of leaf morphology

Correlation analysis revealed strong positive relationships among leaf size traits, such as leaf blade length and total leaf length, and among tooth numbers, reflecting coordinated growth and shared developmental pathways. Similar patterns in *B. pendula* and other broad-leaved trees indicate functional integration of leaf morphology, which supports light interception and hydraulic balance (NICOTRA et al. 2011; PEPPE et al. 2011; CORNELISSEN et al. 2011). Moderate correlations among leaf length, width, and position of the widest part of the blade further suggest an integrated morphological syndrome influencing photosynthetic surface, transpiration, and thermal regulation, consistent with adaptations to temperature, moisture, and light conditions (NICOTRA et al. 2011; WRIGHT et al. 2017). In contrast,

the insertion angle of the first vein showed weak correlations with other traits, implying partial independence and regulation by biomechanical or hydraulic constraints rather than overall leaf allometry (SACK and SCOFFONI 2013).

### Multivariate patterns of leaf trait variation and population differentiation

Principal component analysis confirmed that most morphological variability among sampled trees can be explained by two major axes of variation. The first axis is associated with overall leaf size, reflecting its dominant role in individual differentiation and its ecological responsiveness in woody plants (NICOTRA et al. 2011; WRIGHT et al. 2017). The second axis corresponds to leaf tooth frequency, an independent trait often linked to climate, where populations in cooler regions exhibit more pronounced or numerous teeth, enhancing early-season photosynthesis and hydraulic efficiency (PEPPE et al. 2011). PCA also revealed regional structuring: populations from Bosnia and Herzegovina and Slovenia tended to cluster within their respective regions, reflecting environmental similarities and geographic proximity, while overlap within regions indicates substantial local variation and gene flow, as commonly observed in wind-pollinated, wind-dispersed species (KREMER et al. 2012; ALBERTO et al. 2013).

Cluster analysis further supported regional differentiation among populations, forming two major groups that broadly separated Slovenian from Bosnian-Herzegovinian populations, suggesting that geographic distance and environmental variation shape leaf morphology. Similar patterns have been reported in other European forest trees, where climatic gradients, historical migration, and limited long-distance gene flow drive population differentiation (KREMER et al. 2012; SAVOLAINEN et al. 2013). An exception was the Žepče population, which clustered with Slovenian populations, potentially reflecting historical connectivity, similar environmental conditions, or retained ancestral variation. In addition, cluster analysis showed clear differentiation of the Mrkonjić Grad, Han Kram, and Mokro 2 populations, consistent with their identification as *B. pubescens* (FUKAREK 1957; STEFANOVIĆ 1961). This pattern was less evident in the PCA, likely because PCA summarizes overall variation along a limited number of principal components, whereas cluster analysis is based on pairwise similarities among samples and can reveal groupings that are not strongly represented on the major axes of variation. Because this study relies on morphological data alone, integrating genetic markers in future

work would help clarify whether this pattern reflects shared ancestry, recent gene flow, or convergent adaptation.

For three populations from Bosnia and Herzegovina (Mrkonjić Grad, Han Kram, and Mokro 2) cluster analysis showed clear differentiation in leaf traits, consistent with their identification as *B. pubescens* (FUKAREK 1957; STEFANOVIĆ 1961). In contrast, two other populations previously described as pubescent birch (Mokro 1 and Sokolac) (FUKAREK 1957, STEFANOVIĆ 1961) clustered with *Betula pendula*, possibly reflecting intraspecific variation, phenotypic plasticity, or potential misidentification in earlier studies, highlighting the need for complementary genetic or cytological analyses to confirm species identity. Morphological differences between *B. pendula* and *B. pubescens*, including leaf shape, venation, and pubescence, are well documented (ATKINSON 1992), and the distinct clustering of three populations supports the reliability of leaf traits for species-level differentiation.

### Climatic drivers of morphological differentiation

Our results showed strong influence of temperature on leaf morphology across the studied populations, with larger leaves in warmer environments, consistent with reports indicating that temperature is a dominant driver shaping leaf traits (NICOTRA et al. 2011; WRIGHT et al. 2017). Precipitation and seasonal moisture were significantly correlated to leaf shape and tooth number of studied populations, reflecting plant water balance and growth dynamics (PEPPE et al., 2011). Certain traits, such as venation angles and tooth positioning, appear sensitive to climatic extremes and short-term fluctuations. Our results suggest that while climate is a major factor, historical and genetic effects also contribute,

particularly in populations with unusual trait combinations or high divergence from nearby populations. Compared with *B. pendula* populations in Croatia, where environmental correlations with leaf morphology were weak and morphology was mainly influenced by historical or genetic factors (KOVAČIĆ & NIKOLIĆ, 2005), our study, by including *B. pubescens*, indicates that species identity, reflected in traits, such as leaf pubescence, LS2 shape, LM3 margin is an important source of leaf morphological variation alongside environmental conditions.

### Implications for adaptation and forest genetic resources

Overall, the strong correlations between morphological traits and climatic variables indicate that leaf morphology in the studied *Betula* populations is structured along environmental gradients. Such patterns are commonly interpreted as evidence of local adaptation or environmentally induced phenotypic plasticity. In long-lived forest tree species with extensive gene flow, both processes often operate simultaneously to shape population-level trait variation (KREMER et al. 2012; SAVOLAINEN et al. 2013).

The pronounced differentiation observed among populations from Bosnia and Herzegovina and Slovenia therefore likely reflects the combined influence of regional climate variation, geographic distance, and historical population dynamics. Understanding these patterns is important for forest management and conservation, particularly in the context of ongoing climate change. Morphological traits associated with climatic adaptation may help identify populations with specific adaptive potential that could be valuable for future reforestation and restoration programs.

## CONCLUSIONS

This study revealed substantial morphological variability in leaf traits among natural populations of *B. pendula* and *B. pubescens* from Bosnia and Herzegovina and Slovenia. Both quantitative and qualitative traits showed significant differences among populations, indicating pronounced phenotypic differentiation across the studied region.

Multivariate analyses demonstrated that leaf size parameters represent the main axis of morphological variation, while traits related to leaf teeth form a secondary component of differentiation. Qualitative traits

such as leaf shape, margin, pubescence, and texture also varied significantly among populations. In particular, the presence of leaf pubescence was confirmed in two populations (Han Kram and Mokro 2), supporting their identification as *B. pubescens*.

The analyses further indicated regional structuring of populations and a strong relationship between leaf morphology and climatic variables. Leaf dimensions were strongly associated with temperature gradients, suggesting that climatic factors play an important role in shaping morphological differentiation.

Overall, the results highlight the importance of environmental gradients and potential genetic differentiation in structuring leaf morphological variation in birch

populations and provide useful baseline information for future ecological, genetic, and forest management studies in south-eastern Europe.

## VSEBINA

Navadna in puhasta breza (*Betula pendula* Roth in *Betula pubescens* Ehrh.) sta ekološko in ekonomsko pomembni gradnici zmernih in borealnih gozdnih ekosistemov po vsej Evropi. Za njiju sta značilni široka ekološka amplituda in velika morfološka variabilnost, kar jima omogoča, da zasedata raznolike habitate in se odzivata na okoljske gradiente. Zlasti morfologija listov odraža tako genetsko diferenciacijo kot fenotipsko plastičnost in zato omogoča dragocen vpogled v prilagoditvene procese v naravnih populacijah. Kljub široki razširjenosti obeh vrst brez so podrobne študije morfološke variabilnosti v JV Evropi še vedno relativno omejene. Cilj te študije je bil raziskati morfološko variabilnost listov v naravnih populacijah obeh vrst brez v Bosni in Hercegovini (BiH) ter Sloveniji (SI) ter oceniti povezave med morfološkimi lastnostmi in okoljskimi dejavniki.

V obdobju 2020–2022 smo zbrali vzorce listov iz 39 populacij, od tega 31 populacij v BiH ter 8 populacij v SI. V vsaki populaciji smo vzorčili deset odraslih dreves, z vsakega drevesa pa smo zbrali 30 listov s kratkih poganjkov v delih krošnje, izpostavljenih soncu. Skupno smo analizirali 11.700 listov. Geografske koordinate in nadmorske višine vseh lokacij vzorčenja smo zabeležili z GPS-om. Podnebne podatke smo pridobili iz baze podatkov WorldClim, vključevali pa so več bioklimatskih spremenljivk, ki opisujejo temperaturne in padavinske režime.

Morfometrične analize smo izvedli na posušenih vzorcih listov. Izmerili, ocenili, prešteli in izpeljali smo več fenotipskih lastnosti, da bi opredelili velikost, obliko listov, značilnosti žilnatosti in strukturo zobcev. Za vse lastnosti smo izračunali opisno statistiko, vključno s povprečnimi vrednostmi, standardnimi odkloni in koeficienti variabilnosti. Za oceno komponent variance in oceno porazdelitve fenotipske variabilnosti znotraj populacij in med njimi smo uporabili gnezdeno analizo variance. Izračunali smo tudi koeficiene diferenciacije populacij (Vst). Razmerja med lastnostmi smo ovrednotili s Pearsonovo korelacijsko analizo. Za identifikacijo glavnih osi morfološke variabilnosti smo uporabili analizo glavnih komponent (PCA), za raziskovanje strukture in združevanja populacij pa analizo grozdov na podlagi evklidskih razdalj (metoda UPGMA). Homogenost kategoričnih lastnosti listov med populacijami smo preizkusili s Pearsonovim  $\chi^2$  testom. Nazadnje smo preučili

korelacije med morfološkimi lastnostmi in podnebnimi spremenljivkami, da bi ugotovili potencialne okoljske dejavnike fenotipske variabilnosti.

Rezultati so pokazali znatno morfološko variabilnost med preučevanimi populacijami. Koeficienti variabilnosti so se za večino lastnosti listov gibali od zmernih do visokih, kar kaže na izrazito fenotipsko raznolikost znotraj preiskovane regije. Analiza komponent variabilnosti je pokazala, da je bil znaten delež celotne variabilnosti porazdeljen med populacijami, kar kaže na prostorsko strukturiranje morfoloških lastnosti po celotnem preučevanem območju. Močne korelacije smo opazili med več parametri velikosti listov, zlasti med dolžino listne ploskve in skupno dolžino lista, pa tudi med lastnostmi, povezanimi s številom listnih zobcev.

Analiza glavnih komponent je pokazala, da prvi dve glavni komponenti pojasnujeta več kot 70 % celotne morfološke variabilnosti. Prva glavna komponenta je bila povezana predvsem s parametri velikosti listov, kot so dolžina listne ploskve, širina listne ploskve, dolžina peclja in skupna dolžina listov, kar predstavlja prevladujočo morfometrično dimenzijo variabilnosti. Druga glavna komponenta je bila povezana predvsem z numeričnimi značilnostmi listnih zobcev. Ti rezultati kažejo, da velikost listov in značilnosti zobcev predstavljata dve ključni osi morfološke diferenciacije med preučevanimi populacijami.

Analiza grozdov je pokazala jasno združevanje populacij glede na geografsko poreklo. Večina populacij iz BiH je tvorila grozd, ločen od slovenskih populacij, kar kaže na regionalno diferenciacijo, na katero so verjetno vplivale okoljske razmere, geografska oddaljenost in zgodovinski populacijski procesi. Izjema je bila populacija iz Žepč (BiH), ki se je združila s slovenskimi populacijami, kar verjetno odraža podobne ekološke razmere ali zgodovinsko povezavo med populacijami.

Pomembna heterogenost je bila opažena tudi v porazdelitvi kvalitativnih listnih lastnosti, vključno z obliko listov, vrsto roba, dlakavostjo in teksturo. Rezultati Pearsonovega  $\chi^2$  testa so potrdili statistično značilne razlike med populacijami za vse ocenjene kategorije / lastnosti. Posebej pomembna je bila prisotnost listne dlakavosti v populacijah Han Kram in Mokro 2, kjer so bili vsi analizirani listi dlakavi. Ti populaciji sta bili prej opisani kot puhasta breza (*B. pubescens*), sedanji rezul-

tati pa potrjujejo diagnostično vrednost listne dlakavosti kot razlikovalne lastnosti med puhasto brezo in pretežno neodlakano navadno brezo. V slovenskih populacijah dlakavosti nismo opazili, kar kaže na to, da vzorce ni sestoji v Sloveniji sestojijo predvsem iz vrste *B. pendula*.

Analiza povezav med morfološki značilnostmi in podnebnimi spremenljivkami je pokazala močan vpliv okoljskih dejavnikov na morfologijo listov. Dimenzije listov so bile močno povezane s temperaturnimi spremenljivkami, zlasti s povprečno letno temperaturo, kar je pojasnilo velik delež variabilnosti v glavni komponenti, ki predstavlja velikost listov. Populacije iz območij s toplejšim podnebjem so imele običajno večje liste, kar je skladno objavljenimi globalnimi vzorci, ki povezujejo velikost listov s podnebnimi razmerami. Dodatne povezave med morfološki značilnostmi in spremenljivkami, povezanimi s padavinami, nadalje kažejo, da lahko razpoložljivost vlage in sezonska spremenljivost podne-

bja vplivata na nekatere vidike strukture listov.

Rezultati na splošno kažejo na izrazito morfološko raznolikost med naravnimi populacijami brez (dveh vrst iz rodu *Betula*) v Bosni in Hercegovini ter Sloveniji. Opazovana variabilnost najverjetneje odraža kombiniran vpliv okoljskih gradientov, fenotipske plastičnosti in potencialne genetske diferenciacije med populacijami. Te ugotovitve prispevajo k boljšemu razumevanju znotrajvrstne variabilnosti pri obeh vrstah brez in zagotavljajo dragocene izhodiščne informacije za prihodnje študije, ki bodo vključevale morfološke, ekološke in genetske pristope. S praktičnega vidika dokumentirana variabilnost poudarja pomen ohranjanja regionalne populacijske raznolikosti kot potencialnega vira prilagodljivosti. Takšne informacije so še posebej pomembne za upravljanje gozdnih genskih virov, načrtovanje ohranjanja in izbiro primerne reprodukcijekega materiala za pogozdovanje in obnovo v spreminjajočih se podnebnih razmerah v jugovzhodni Evropi.

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