Leaf Morphological Variation in *Betula medwediewii* Regel Populations from Türkiye

Arzu Ergül Bozkurt *

Leaf variations of Betula medwediewii in Türkiye with respect to its distribution pattern were studied because this plant taxon is a Euxine element and a distinct relict species in Türkiye. In this context, the aim of this study is to investigate variations in leaf morphological characteristics within and among *B. medwediewii* populations in natural distribution areas in Türkiye. Thus, the morphological leaf characteristics of four populations of B. medwediewii growing at different elevations spanning from 1472 to 2065 m.a.s.l in two cities (Artvin and Rize) and four boroughs (Arhavi, Borçka, Murgul, and Çamlıhemşin) of Türkiye were analyzed. The four populations were selected based on their natural distribution and 1,200 leaf samples belonging to 40 individuals were measured with ImageJ. According to the results of correlation analysis, statistically significant relationships were determined among morphological leaf characteristics. Mean values for petiole length (1.16 cm), leaf width (5.11 cm), leaf length (7.96 cm), length of lamina (6.79 cm), leaf area (26.62 cm²), leaf vein angle (54.21°), and number of primary leaf veins (20.03) were determined in all populations. Because B. medwediewii contributes to biodiversity and can preserve the ecological stability of the Turkish forest area, its conservation is crucial.

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Keywords: Betula medwediewii; Leaf morphology; Relict species

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INTRODUCTION

Betula medwediewii Regel (family Betulaceae), which is known as Transcaucasian birch, is naturally distributed in four provinces: Rize and Artvin cities in north-eastern Türkiye, Adjara in south-western Georgia, and on Mount Jvari in western Georgia. This species usually occurs in subalpine mixed forests of *Picea orientalis* (L.) and in *Rhododendron* scrubs at an elevation of 1300 to 2160 m.a.s.l. It is a Euxine species, which means Western Black Sea Subregion (Euxine) of the Euro-Siberian Flora Area, and a distinct relict species in Türkiye (Browicz 1972; Davis 1988). This plant taxon is also rare in Georgia, where populations are very small, fragmented, and unstable. Its presence in Iran, Azerbaijan, and Armenia has not yet been confirmed (Shaw *et al.* 2014). On the Red List of Endangered Species, *B. medwediewii* is categorized as DD (Data Deficient) based on IUCN (International union for the conservation of nature) criteria (Wilson *et al.* 2018). According to Davis (1988) Artvin-Hatila valley is one of the natural distribution areas of *B. medwediewii* in Türkiye. However, during extensive field work between 2019 and 2020 years, this species was not encountered in the Artvin-Hatila valley.

Morphological differences in leaves can be associated with geographical region, climatic condition, and elevation (Gratani *et al.* 2012). Artvin is surrounded by high mountains and forests. In this region, the Eastern Black Sea oceanic rainfall regime is seen in the Arhavi district, and Arhavi is 30 km from Artvin. The elevation of the area ranges between 70 and 3000 asl. Camili is in the Borçka district, 30 km from Artvin, with an elevation ranging from 350 to 3440 asl, and it is under the influence of the Eastern Black Sea climate. This research area borders Georgia. Murgul is 48 km away from Artvin at an elevation of 250 to 3977 asl, and with abundant rainfall. Çamlıhemşin is 61 km away from Rize at an elevation of 295 to 3200 asl (Buser and Cvetic 1972; Akman 1999; Batan and Özdemir 2013; Yener *et al.* 2017), and the climate is characterized by abundant rain in each season.

Various research studies have indicated that a broad variety of plants' ability to grow and survive in competition depends on leaf variation (Raschke 1960; Parkhurst and Loucks 1972; Givnish 1979; Hinckley *et al.* 1989; Gurevitch 1992; Calagari *et al.* 2006). Many studies on leaf morphology have shown that the shapes of leaves are of great importance for plant growth and are highly influenced by environmental conditions (Coleman *et al.* 1994; Pyakurel and Wang 2013). The main aim of this study was to analyze the leaf morphological characteristics of four populations of *B. medwediewii* growing at different elevations with different climatic conditions in two cities (Artvin and Rize) and four boroughs (Arhavi, Borçka, Murgul, and Çamlıhemşin) of Türkiye. The results are expected to show whether elevational and regional variations in these traits indicate future adaptive potential. At the same time, the results of the study will be instructive in terms of individuals that will outperform in the productive generation.

EXPERIMENTAL

Study Area

The four populations of *B. medwediewii* in Artvin-Arhavi (AA), Artvin-Borçka (Camili) (ABC), Artvin-Murgul (AMU), and Rize-Çamlıhemşin (RÇA) distributed in Türkiye were selected based on their natural distribution.

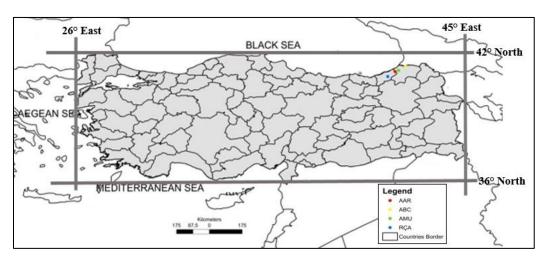


Fig. 1. Geographical locations of *B. medwediewii* populations (AAR (Artvin-Arhavi), ABC (Artvin-Borçka (Camili), AMU (Artvin-Murgul), and RÇA (Rize-Çamlıhemşin))

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Leaf samples were taken from a limited number of random individuals, in the other natural distribution areas (Artvin-Arhavi, Artvin-Borçka (Camili), Artvin-Murgul, and Rize-Çamlıhemşin). *B. medwediewii* is in A8 according to the grid system adopted in the Flora of Turkey (Browicz 1972; Davis 1988) Türkiye is located between 26°- 45° east longitudes and 36°- 42° north latitudes (Fig. 1).

The climate types of the study area were determined according to the Thornthwaite Climate Classification (Thornthwaite 1948), which provides detailed results by considering the water storage capacity of the soil (Akman 2011). Long-term meteorological data were evaluated (2008 to 2020 year), and climate diagrams were created by using Thornthwaite's system.

The city of Artvin is a semi-moist area with medium temperatures (mesothermal); water deficiency is moderate and mainly occurs in summer between July and September, whereas January, February, March, and December have excessive rainfall. The city of Rize is a very moist area with medium temperatures (mesothermal) with little or no water deficit and an ocean climate (Fig. 2).

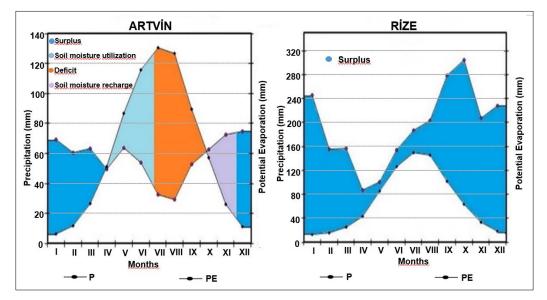


Fig. 2. Climate diagrams of Artvin and Rize according to the Thornthwaite method

Table 1. Information on the Four Betula medwediewii Populations (Artvin-Arhavi
(AAR), Artvin-Borçka (Camili) (ABC), Artvin-Murgul (AMU), Rize-Çamlıhemşin
(RÇA) North (N), Northeast (NE), Southwest (SW))

Population			Total	Coord			
No.	Province	Label	Number of Individuals	x	У	Altitude	
1	Artvin-Arhavi	AAR-1	5	697613	4562454	2034	
2	Altvill-Alliavi	AAR-2	5	697612	4562454	2065	
3	Artvin-Borçka	ABC-1	5	738379	4589423	1753	
4	(Camili)	ABC-2	5	738379	4589424	1792	
5	Artvin Muraul	AMU-1	5	710399	4570308	1472	
6	Artvin-Murgul	AMU-2	5	710400	4570308	1498	
7	Rize-	RÇA-1	5	668174	4536983	1818	
8	Çamlıhemşin	RÇA-2	5	668176	4536979	1827	

Morphological Leaf Measurements

The morphological leaf characteristics petiole length (PL) (cm), leaf width (LW) (cm), leaf length (LL) (cm), length of lamina (LLA) (cm), leaf area (LA) (cm²), leaf vein angle (LVA) (°), and number of primary leaf veins (NLV) were determined using ImageJ (Image Analysis Software). From the four selected populations, a total of 1,200 leaf samples, belonging to 40 individuals, were measured (Table 1). Leaf vein angle was obtained by measuring the middle part of each leaf (Fig. 3).

In total, 40 individuals and 1,200 leaf samples (300 from AA, 300 from ABC, 300 from AMU, and 300 from RÇA) were selected (Table 1) in 2019 and 2020. The crown was visually divided into four different parts. Mature leaves were collected in the middle of the upper crown of the tree. Solely fully developed leaves of trees, without signs of damage from pest, disease or wilting were measured (Kozlov *et al.* 2021). Leaves were subjected to a preservation treatment that included pressing and drying (Tomaszewski and Górzkowska 2016). After that, leaf images were taken using a scanner and a ruler. Leaf traits were measured using ImageJ analysis software package (Schneider *et al.* 2012), calibrated by the ruler for accuracy (Mhamdi *et al.* 2013; Yildirim and Turna 2021) (Fig. 3).

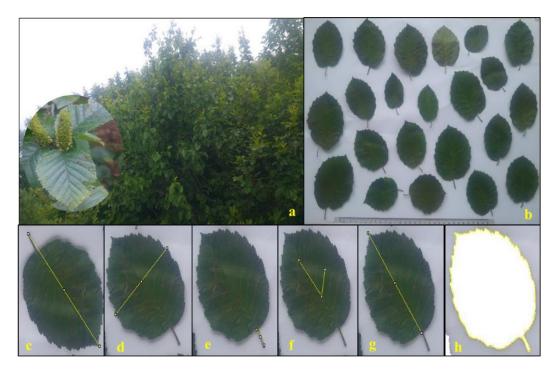


Fig. 3. (a) General view of *Betula medwediewii,* (b) leaf samples, (c) measurement of leaf length, (d) leaf width, (e) length of petiole, (f) leaf vein angle, (g) length of lamina, and (h) leaf area, using ImageJ

Statistical Analysis

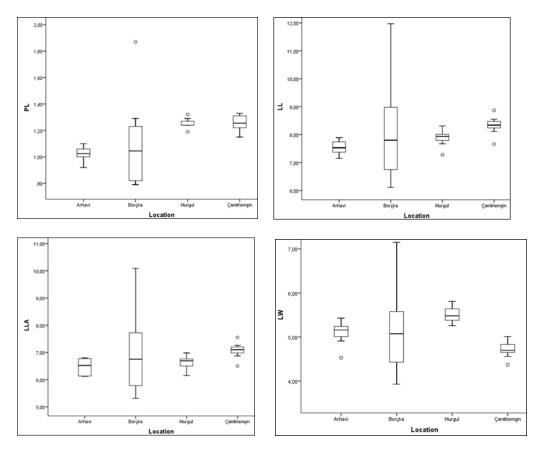
Data were analyzed using the statistical software package SPSS 20.0 software package (IBM, Armonk, NY, USA). Data were submitted to ANOVA (Analysis of Variance), P < 0.05, Duncan's test, and cluster analysis. A variance analysis (one-way ANOVA) was used to determine variations among and within populations. Duncan's test was performed to determine the groups that were found in terms of populations for all leaf traits. A cluster analysis using the Euclidean method was performed to examine leaf

variation within and between populations. Pearson's correlation was carried out to test for correlations between leaf morphological characteristics and altitude.

RESULTS

Variations in leaf morphology were observed among the populations. Figure 4 shows the mean values and standard deviations for the measured parameters. There were statistically significant differences (P < 0.01) among populations for all the measured characteristics of the leaves.

Based on the Duncan's test, there were three groups in terms of petiole length, leaf length, leaf width, leaf area, and leaf vein angle; two groups in terms of length of lamina; and four groups in terms of the number of primary leaf veins. Among the four populations, the highest values of petiole length, leaf length, and length of lamina were obtained in the population of Rize-Çamlıhemşin, while the lowest values were obtained in the population of Artvin-Arhavi. The Artvin-Murgul population had the highest values of leaf width, leaf area, and leaf vein angle, while the lowest value was obtained in the populations of Rize-Çamlıhemşin and Artvin-Arhavi. The Artvin-Borçka (Camili) population had the highest number of primary leaf veins, while the lowest value was obtained in the Artvin-Arhavi population (Fig. 4).



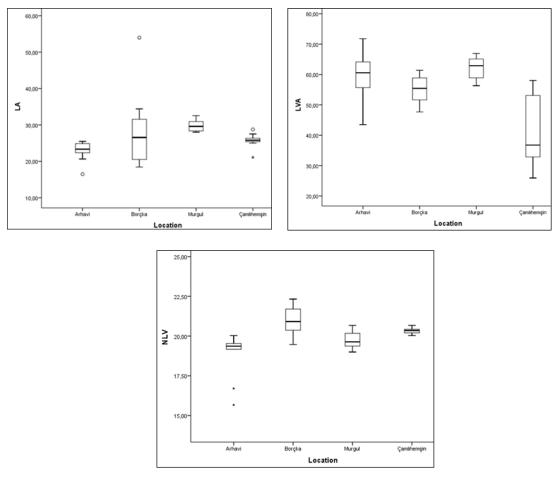


Fig. 4. ANOVA and Duncan test results of the average petiole length (PL), leaf length (LL), length of lamina (LLA), leaf width (LW), leaf area (LA), leaf vein angle (LVA), and number of primary leaf veins (NLV for the four *Betula medwediewii* populations

There were two groups based on PL, LA, and NLV and three groups based on LVA in the Artvin-Arhavi population. There were six groups in terms of LW, LA, and NLV, five groups in terms of PL, LL, and LLA, and four groups in terms of LVA in the Artvin-Borçka (Camili) population. There were three groups based on LVA and NLV in the Artvin-Murgul population. There were three groups based on LL, LLA, and LVA and two groups based on LA in the Rize-Çamlıhemşin population (Table 2).

As a result of the variance analysis of the Artvin-Arhavi population, there were significant differences in terms of petiole length, leaf area, leaf vein angle, and number of primary leaf veins ($\alpha < 0.05$), while there was no significant difference in leaf length, length of lamina, and leaf width. In the Artvin-Borcka (Camili) population, there was a significant difference based on all leaf characteristics. In the Artvin-Murgul population, there was a significant difference in terms of leaf vein angle and number of primary leaf veins. As a result of the ANOVA in the Rize-Çamlıhemşin populations, there was a significant difference in leaf length, length of lamina, leaf area, and leaf vein angle, while there was no significant difference in terms of petiole length, leaf width, and number of primary leaf veins (Table 2).

Table 2. Average Values and ANOVA Results for Petiole Length (PL), Leaf Width (LW), Leaf Length (LL), Length of Iamina (LLA), Leaf Area (LA), Leaf Vein Angle (LVA) and Number of Primary Leaf Veins (NLV) Values within Populations

Individual		Artvin-Arhavi										
No	PL (cm)	LL (cm)	LLA (cm)	LW (cm)	LA (cm²)	LVA(°)	NLV					
1	0.95±0.20a,b	7.74±1.33	6.80±1.17	5.24±1.11	22.71±7.96b	60.92±18.42b	19.53±2.51b					
2	1.02±0.24a,b	7.15±1.52	6.12±1.37	5.22±1.16	24.13±10.98b	60.55±13.75b	20.03±1.52b					
3	1.03±0.28a,b	7.17±1.18	6.13±1.01	4.91±0.97	16.48±6.55a	57.31±13.21b	15.67±2.18a					
4	1.33±0.28c	7.47±1.07	6.13±0.92	4.53±0.92	24.99±8.82b	71.78±18.95c	16.70±2.12a					
5	1.03±0.28	7.58±1.54	6.56±1.40	5.22±1.13	24.87±10.36b	65.14±17.70a,b	19.67±2.24b					
	a,b											
6	1.06±0.27	7.83±1.54	6.77±1.36	5.32±1.26	23.43±9.39b	60.56±13.80b	19.30±2.71b					
	a,b											
7	1.00±0.27	7.57±1.25	6.60±1.17	5.03±1.11	23.27±8.56b	64.13±16.55a,b	19.17±2.30b					
	a,b											
8	1.02±0.25	7.49±1.61	6.48±1.46	5.10±1.18	22.38±11.17b	55.67±14.16b	19.43±2.02b					
	a,b											
9	1.10±0.30b	7.89±1.25	6.78±1.11	5.43±1.02	25.50±7.43b	43.48±27.40a	19.43±2.66b					
10	0.92±0.22a	7.37±1.35	6.44±1.23	5.01±1.07	20.65±7.80a,b	55.51±12.17b	19.17±2.56b					
Avg.	1.05	7.53	6.48	5.10	22.84	59.51	18.81					
F	5.277	1.002	1.453	1.579	2.595	5.675	11.453					
S	0.000*	0.438	0.165	0.121	0.007*	0.000*	0.000*					
Individual				Artvin-Borçka (Camili)							
No	PL (cm)	LL (cm) LLA (cm) LW (cm) LA (cm ²) LVA (°)										
1	1.87±0.29e	11.97±2.01e	10.09±1.82e	7.15±1.57f	53.98±21.24f	51.46±10.50a,b	25.20±4.68f					
2	1.16±0.28c,d	8.98±1.64d	7.81±1.47d	5.58 ±1.20e	34.41±12.31e	59.70±14.60c,d	22.33±3.24d					
3	1.02±0.20b,c	7.68±0.95c	6.65±0.81c	4.99±0.72c,d	25.39±6.28b,c	55.57±8.54b,c	21.10±1.26b					
							c,d					
4	1.29±0.27d	9.02±1.73d	7.72±1.51d	5.16±0.93d,e	29.15±10.56c,d,	51.62±6.24a,b	20.67±2.29a					
					е		b,c					
5	0.96±0.24b	6.75±1.14a,b	5.78±0.94a,	4.09±0.64a,b	18.44±5.77a	54.18±8.18b,c	20.93±1.87b					
			b				С					
6	0.82±0.20a	6.30±1.11a	5.47±0.98a	4.58±0.82b,c	20.51±7.43a,b	61.38±9.85d	20.37±1.67a					
							b,c					
7	1.07±0.30b,c	7.92±1.51c	6.85±1.27c	3.93±0.77a	21.08±6.77a,b	47.67±8.93a	20.07±1.46a					
							b					
8	0.79±0.24a	6.11±1.19a	5.31±1.01a	4.43±0.87a,b	19.11±8.67a	55.28±9.80b,c	20.90±1.49b					
							С					
9	1.23±0.43d	8.75±2.35d	7.52±1.96d	5.56±1.28e	31.56±15.42d,e	57.12±8.12b,c,d	19.47±1.75a					
10	0.80±0.14a	7.14±0.77b,c	6.34±0.68b,	5.70±0.82e	27.73±5.76c,d	58.87±12.10c,d	21.70±2.30c					
	-	- 1 -	C		,-	, -	d					
Avg.	1.10	8.06	6.95	5.12	28.13	55.28	21.27					
F	41.587	39.119	35.112	26.758	27.299	5.404	13.079					
S	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*					
Individual												
No	PL (cm)	LL (cm)				LVA(°)						

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PEER-REVIEWED ARTICLE

1	1.32±0.27	8.31±1.06	6.98±0.85	5.81±0.89	32.56±9.00	64.28±14.53a,b,	19.00±1.74a
						С	
2	1.29±0.31	8.19±1.44	6.89±1.19	5.26±0.94	29.84±9.62	65.45 ±	19.60±2.72a
2 4 40 0 40						14.11b,c	b,c
3	1.12±0.18	7.28±0.93	6.15±0.86	5.56±0.75	28.02±7.11	56.29 ± 12.76a	20.67±1.440
4	1.24±0.23	7.83±1.29	6.47±1.24	5.30±1.05	28.43±9.99	58.90±8.72a,b,c	19.37±2.45
							a,b
5	1.27±0.28	8.01±1.43	6.73±1.23	5.64±0.86	30.94±8.86	61.68±13.48a,b,	19.67±2.12a
						С	b,c
6	1.19±0.25	7.67±1.10	6.50±0.91	5.40±0.93	28.32±8.80	64.07±15.36a,b,	19.47±1.92a
						С	b,c
7	1.24±0.28	7.91±1.24	6.67±1.04	5.39±1.04	29.36±9.91	59.75±14.13a,b,	19.07±2.65
						С	a,b
8	1.25±0.28	7.96±1.20	6.71±0.98	5.61±0.89	30.69±9.34	65.10±14.79b,c	20.00±1.93
							b,c
9	1.24±0.23	7.79±1.21	6.52±0.98	5.38±0.74	28.37±6.82	57.60±15.77a,b	20.30±1.87
							b,c
10	1.24±0.24	8.01±1.06	6.76±0.91	5.70±0.84	31.25±8.26	66.94±16.90 c	20.17±1.98
							b,c
Avg.	1.24	7.90	6.64	5.51	29.78	62.01	19.73
F	1.325	1.671	1.593	1.249	0. 907	2.015	1.971
•							
S	0.223	0.096	0.117	0.265	0.520	0.038*	0.043*
		0.096	0.117	0.265 Rize-Çamlıh		0.038*	0.043*
S	0.223			Rize-Çamlıh			0.043*
S ndividual		0.096 LL (cm) 8.87±1.06c	0.117 LLA (cm) 7.55±0.94c		emşin	0.038*	
S ndividual No	0.223 PL (cm) 1.32±0.26	LL (cm)	LLA (cm) 7.55±0.94c	Rize-Çamlıh LW (cm) 5.01±0.74	emşin LA (cm²) 28.78±7.74b	LVA (°) 54.56±10.48c	NLV 20.40±1.94
S ndividual No 1 2	0.223 PL (cm) 1.32±0.26 1.15±0.22	LL (cm) 8.87±1.06c 7.66±1.12a	LLA (cm) 7.55±0.94c 6.50±0.95a	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74	emşin LA (cm²) 28.78±7.74b 21.10±6.48a	LVA (°) 54.56±10.48c 58.02±12.03c	NLV 20.40±1.94 20.50±1.69
S ndividual No 1	0.223 PL (cm) 1.32±0.26	LL (cm) 8.87±1.06c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b,	Rize-Çamlıh LW (cm) 5.01±0.74	emşin LA (cm²) 28.78±7.74b	LVA (°) 54.56±10.48c	NLV 20.40±1.94
S ndividual No 1 2 3	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c	NLV 20.40±1.94 20.50±1.69 20.43±1.47
S ndividual No 1 2	0.223 PL (cm) 1.32±0.26 1.15±0.22	LL (cm) 8.87±1.06c 7.66±1.12a	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74	emşin LA (cm²) 28.78±7.74b 21.10±6.48a	LVA (°) 54.56±10.48c 58.02±12.03c	NLV 20.40±1.94 20.50±1.69 20.43±1.47
S ndividual No 1 2 3 4	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56
S ndividual No 1 2 3	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56
S ndividual No 1 2 3 4 4 5	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76
S ndividual No 1 2 3 4	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56
S ndividual No 1 2 3 4 5 6	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91
S ndividual No 1 2 3 4 4 5	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91
S ndividual No 1 2 3 4 5 6 7	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.11±1.10a,b	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66
S ndividual No 1 2 3 4 5 6	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69	emşin LA (cm²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76
S ndividual No 1 2 3 4 5 6 7 7 8	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22 1.22±0.20	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71 4.68±0.81	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b 25.66±8.92b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b 38.95±23.17b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66 20.40±2.02
S ndividual No 1 2 3 4 5 6 7	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c 8.24±1.18a,b,	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c 6.98±1.02a,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66
S ndividual No 1 2 3 4 5 6 7 8 8 9	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22 1.22±0.20 1.27±0.26	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c 8.24±1.18a,b, c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c 6.98±1.02a, b,c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71 4.68±0.81 4.65±0.73	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b 25.66±8.92b 25.42±8.10b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b 38.95±23.17b 32.83±22.51a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.76 20.30±1.66 20.40±2.02 20.03±1.71
S ndividual No 1 2 3 4 5 6 7 7 8	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22 1.22±0.20	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c 8.24±1.18a,b,	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c 6.98±1.02a,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71 4.68±0.81	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b 25.66±8.92b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b 38.95±23.17b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66 20.40±2.02
S ndividual No 1 2 3 4 5 6 7 8 8 9	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22 1.22±0.20 1.27±0.26	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c 8.24±1.18a,b, c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c 6.98±1.02a, b,c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71 4.68±0.81 4.65±0.73	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b 25.66±8.92b 25.42±8.10b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b 38.95±23.17b 32.83±22.51a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66 20.40±2.02 20.03±1.71
S ndividual No 1 2 3 4 5 6 7 8 8 9	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22 1.22±0.20 1.27±0.26	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c 8.24±1.18a,b, c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c 6.98±1.02a, b,c 7.19±1.22b,	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71 4.68±0.81 4.65±0.73	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b 25.66±8.92b 25.42±8.10b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b 38.95±23.17b 32.83±22.51a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66 20.40±2.02 20.03±1.71
S ndividual No 1 2 3 4 5 6 7 8 8 9 9	0.223 PL (cm) 1.32±0.26 1.15±0.22 1.33±0.23 1.22±0.28 1.24±0.25 1.25±0.24 1.31±0.22 1.22±0.20 1.27±0.26 1.26±0.23	LL (cm) 8.87±1.06c 7.66±1.12a 8.55±1.14b,c 8.32±0.97b,c 8.34±1.10b,c 8.34±1.12b,c 8.34±1.12b,c 8.11±1.10a,b 8.47±1.39b,c 8.24±1.18a,b,c 8.44±1.35b,c	LLA (cm) 7.55±0.94c 6.50±0.95a 7.20±1.01b, c 7.09±0.79b, c 7.06±0.99a, b,c 7.11±0.99b, c 6.87±0.96a, b 7.26±1.20b, c 6.98±1.02a, b,c 7.19±1.22b, c	Rize-Çamlıh LW (cm) 5.01±0.74 4.37±0.74 4.90±0.81 4.72±0.65 4.66±0.80 4.83±0.69 4.56±0.71 4.68±0.81 4.65±0.73 4.73±0.82	emşin LA (cm ²) 28.78±7.74b 21.10±6.48a 26.36±7.59b 25.02±6.04a,b 25.93±8.41b 26.26±6.81b 25.39±7.93b 25.66±8.92b 25.42±8.10b 27.52±9.14b	LVA (°) 54.56±10.48c 58.02±12.03c 53.09±11.77c 25.89±13.60a 34.64±17.56a,b 35.66±21.02a,b 37.82±21.16b 38.95±23.17b 32.83±22.51a,b 29.17±14.77a,b	NLV 20.40±1.94 20.50±1.69 20.43±1.47 20.20±1.56 20.20±1.76 20.30±1.91 20.30±1.66 20.40±2.02 20.03±1.71 20.67±1.64

* Significance level (P) < 0.05 statistically difference

As a result of the study, the mean values according to the measurements made in all populations were petiole length (1.16 cm), leaf width (5.11 cm), leaf length (7.96 cm), length of lamina (6.79 cm), leaf area (26.62 cm²), leaf vein angle (54.21°), and number of primary leaf veins (20.03).

Variables	PL	LL	LLA	LW	LA	LVA	NLV	Altitude
PL	1	0.707	0.482	0.081	0.523	-0.471	0.206	-0.671
LL		1	0.960*	-0.525	0.411	-0.802	0.750	-0.397
LLA			1	-0.640	0.277	-0.825	0.834	-0.205
LW				1	0.553	0.910	-0.226	-0.565
LA					1	0.209	0.562	-0.968*
LVA						1	-0.387	-0.387
NLV							1	-0.393
Altitude								1

Table 3. Correlations Among Leaf Variables and Altitude

* Significance level (P) < 0.05 statistically difference (2-tailed)

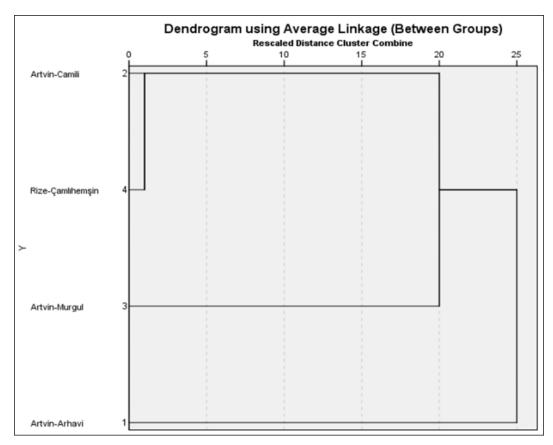


Fig. 5. Dendrogram obtained by hierarchical cluster analysis including all leaf characteristics. Group one is Artvin-Arhavi, group two is Artvin-Murgul, and group three are Artvin-Camili and Rize-Çamlıhemşin

According to the ANOVA test, no significant correlation between annual temperature (*while the annual temperature average value is 13.1* °C *in Artvin-Arhavi, Borçka (Camili) and Murgul regions, it is 15.5* °C *in Rize Çamlıhemşin region)*, annual precipitation (*while annual precipitation total value is 685.5 mm in Artvin-Arhavi, Borçka (Camili) and Murgul regions, it is 2307.3 mm in Rize Çamlıhemşin region)* and leaf traits was noted in this study; this could be because the leaf samples came from the same area (the Eastern Black Sea region). Regarding the variance analysis for all populations, there were meaningful results based on leaf length, length of lamina, leaf area, and altitude. The results of the correlation analysis for the measured seven leaf characteristics and altitude are provided in Table 3. According to the results of the correlation analysis, there were statistically significant positive and negative correlations between leaf characteristics, while the correlations for PL, LL, LW, LA, LVA, and NLV were not statistically significant. Both LLA and LL had a positive correlation, and LA and altitude had a negative correlation. Hierarchical clustering analysis was performed to determine how the populations were graphically grouped according to the measured leaf parameters (Fig. 5).

The significance of the groupings formed because of the hierarchical cluster analysis was tested *via* separation analysis, resulting in two groups. According to all measured leaf characteristics, the population from Artvin-Arhavi was in one group, while the populations from Artvin-Murgul, Rize-Çamlıhemşin, and Artvin-Borcka (Camili) formed another group.

DISCUSSION

The present study showed that morphological traits and geoclimatic parameters (combination of geographical and climatic conditions) were closely related to the distribution of *Betula medwediewii* populations, as this species occurred in a restricted area of Türkiye (Artvin and Rize, only in the Eastern Black Sea Region).

When the regions of Artvin-Arhavi (1772 to 2065 asl), Artvin-Borcka, Camili (1753 to 1792 asl), Artvin-Murgul (1472 to 1498 asl), and Rize-Çamlıhemşin (1818 to 1827 asl) were evaluated in terms of the natural distribution area of *Betula medwediewii*, this species was found at different elevations. However, this situation did not result in statistically significant differences in terms of leaf characteristics. The population from Artvin-Borcka, in the Camili region, provided the best results in terms of many morphological features (Table 2), most likely because of the other ecological features (microclimate, soil) in this region.

Regarding leaf morphological variation in relation to habitat conditions, *Betula* L. often shows significant differences in leaf morphological characteristics, such as leaf size and shape (Dancik *et al.* 1974; Sharik and Barnes 1979; Joel *et al.* 1994; Aspelmeier and Leuschner 2006; Uribe-Salas *et al.* 2008; Pyakurel and Wang 2013). Here, noticeable differences were also found in morphological leaf traits for *B. medwediewii*. The results of present study are in line with the findings of Kundu and Tigerstedt (1997), Bruschi *et al.* (2003), and Calagari *et al.* (2006) for other species (*Azadirachta indica* A. Juss., *Quercus petraea* (Matt.) Liebl., *Quercus pubescens* Willd., and *Populus euphratica* Oliv., respectively). Kleinschmit *et al.* (1995) and Bruschi *et al.* (2000) measured several morphological leaf parameters in *Quercus petraea* (Matt.) Liebl and found differences among the populations. Hiura *et al.* (1996) found differences in leaf size among various provenances of *Fagus crenata* Blume.

Information on leaf morphological traits, widely available in flora, could be used to strengthen predictive models of species distribution and vegetation function. In addition, leaf morphological trait variations could be exploited in models to assess the consequences of climate change for species and ecosystems (Wang *et al.* 2022). Through examining leaf morphological traits, it will be possible to obtain information about the climate, flora, and vegetation, which make up the ecosystem, using less time and less cost. Populations may persist in their current areas and withstand environmental changes if they have adaptive capacity (Gratani *et al.* 2012). However, this adaptation capacity of *B. medwediewii* seems quite low, considering its limited distribution areas. For that reason, *B. medwediewii* occasionally grows in arboreta and a few large gardens in Europe. Further research, including long-term monitoring, phenology studies, and seed germination capability studies, is needed to establish the competitive capacity of *B. medwediewii*. This plant taxon was assessed as category DD according to the IUCN criteria (Wilson *et al.* 2018). In this context, determining the efficiency of *in situ* conservation and development of propagation techniques of this taxon is very important.

The essential oil of leaves of *B. medwediewii* has antifungal properties against plant pathogenic fungi (Demirci *et al.* 2000). Taking into this account, the cultivation of this species can generate economic income. In this context, it is important to know the natural distribution areas of this species. To determine individuals that will outperform in the productive generation, leaf characteristics of individuals showing optimum and maximum development need to be examined. In addition, it is recommended that *B. medwediewii* individuals, which are thought to show superior performance in generative progeny, should be obtained from Artvin-Borçka and Artvin-Murgul regions, which have the healthiest and optimum leaf characteristics (according to the highest and optimum measurement values) (Table 2). Individuals from Rize-Çamlıhemşin can also be selected, but individuals in the Artvin-Arhavi region have very low values in terms of both optimum and maximum value of leaf characteristics. In this sense, determining the efficiency of *in situ* conservation and production of this taxon is important.

Betula medwediewii is categorized as vulnerable (VU) under criterion B1ab (iii,v) in the "Red List of the Endemic Plants of the Caucasus", based on a provisional assessment undertaken during a Global Tree Specialist Group workshop in Tbilisi in 2005. *Betula medwediewii* nearly meets the threshold for qualifying as Vulnerable under criterion B1ab (Eristavi *et al.* 2014; Shaw *et al.* 2014). This taxon is heavily overgrazed by wild and domestic animals, especially in the Artvin-Borçka (Camili) region. Grazing causes damage to both the trees and the seeds, thereby reducing natural reproduction. In consequence, this taxon must be protected *in situ* (closing some areas to prevent grazing), or *ex situ* (greenhouse propagation of seedlings for reforestation strategies).

Betula medwediewii, which is a kind of relict plant taxon, has a narrow natural distribution in Türkiye. Although a very extensive field study was conducted in the Artvin-Hatila valley, which is one of the places specified as its natural distribution area in the flora of Turkey (Browicz 1972; Davis 1988), this plant taxon has not been encountered between 2019 and 2020. In addition, in other natural distribution areas of this plant taxon, a limited number of individuals were encountered. While plant individuals were determined, the distribution of plant taxa were too small in the research areas, and this plant taxon did not form a stand. The raised decline natural distribution of *B. medwediewii*, is concerned that this plant taxon would disappear in the natural distribution areas, in the future.

This manuscript analyzed leaf morphological characteristics of four populations, which have only restricted distribution in Türkiye, growing at different elevations and

climatic conditions. Through researching the leaf characteristics of a particular location, ecological information about this location can be obtained. Thus, leaf morphology studies can facilitate the development of more economic and time-efficient cultivation practices. Depending on the plant taxa, the obtained data can be used to select places for plant production, determining the appropriate ecological conditions (*e.g.*, climate, elevation, and aspect groups) and thereby reducing the mistake rate.

CONCLUSIONS

Determining variation within and among populations of *Betula medwediewii* was completed according to morphological leaf traits.

- The population from Artvin-Borcka, in the Camili region, provided the best results in terms of many morphological features. The small habitat range of this endemic and rare species often leads to a high risk of extinction (Rodrigues *et al.* 2006; Mhamdi *et al.* 2013; Yildirim and Turna 2021). For that reason, biodiversity conservation and the preservation of variation are important for the conservation of genetic resources. In situ and ex situ conservation activities should be applied to this plant taxon.
- 2. Significant relationships between population size or rarity and measures of genetic variation have been found in several rare plant species (Karron *et al.* 1988; Billington 1991; Treuren *et al.* 1991; Oostermeijer *et al.* 1994). In this sense, the possibility of selecting individuals with superior performance in generative progeny is an important issue.
- 3. In terms of leaf morphology, the individuals who have had the healthiest and optimum leaf characteristics, will likely be individuals that will outperform in the productive generation.
- 4. As a result of the present study and many studies (Joel *et al.* 1994; Bruschi *et al.* 2003; Calagari *et al.* 2006; Aspelmeier and Leuschner 2006; Uribe-Salas *et al.* 2008; Pyakurel and Wang 2013; Yildirim and Turna 2021), it has been seen that elevation and climate types (especially microclimatic characteristics) affect the morphological characteristics of the leaves, so this should be taken into account especially when the using activities of leaf morphology as a bioindicator against environmental factors are carried out.

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