Morphological variability of *Fraxinus angustifolia* Vahl in the north-western Balkans

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Abstract

Fraxinus angustifolia Vahl is a species native in the north-western Balkans, where it has a divided range. Most of its range is located in flood-prone riparian sites in the Pannonian plain but the species is also found on humid sites along some rivers in the Submediterranean region. We analysed morphologic variation within and between five populations (14 trees/population) located in the Subpannonian and Submediterranean region of Slovenia. Thirty two characters were assessed on ca. 80 leaves from each tree which were divided depending on exposure to sunlight (sun and shade leaves), and 8 traits on each fruit (ca. 50 fruits/tree). A hierarchically designed experiment using analysis of variance confirmed the significant contribution of all the analysed hierarchical levels of variation to the total variation. The results show that the differences between the trees in a single population are the greatest factor of variability of leaves, followed by differences between populations and differences which are the result of variation in exposure to sunlight. Leaves in shade positions are typically larger than those in sunny positions, and they have broader and shorter-pointed leaflets; in this feature they are very similar to the leaves of *F. excelsior*. Stepwise discriminant analysis showed that the traits which refer to the leaflet number and length are the best differentiating traits between individual populations. The Submediterranean population Dragonja stands out with the smallest leaves and the population Lijak from the same region is the most similar to Dragonja for most morphological traits. In general, phenotypic differences between the Subpannonian and the Submediterranean populations of *F. angustifolia* are minor and indistinct. Based on the differences ascertained by the study, we can only confirm the presence of *F. angustifolia* subsp. *oxycarpa* (Bieb. ex Willd.) Franco et Rocha Alfonso in this part of its natural range.

Keywords: plant variation, fruit, morphometric analysis, narrow-leaved ash, shade leaves, sun leaves

Introduction

Narrow-leaved ash (*Fraxinus angustifolia* Vahl) is a species native to Southern and Eastern Europe. Its range extends from Portugal and Spain in the west to Slovakia and south Moravia in the north and Turkey, Syria, Caucasus and southern Russia in the east. It also grows around the Black Sea and in North Africa [1-3]. *Fraxinus angustifolia* is considered a hydrophilic and moderately thermophilic and heliophilous species which thrives in low-lying riparian forests. It is also found on drier sites at higher altitudes <2000 m above sea level [1,2].

Fraxinus angustifolia is frequently sympatric with common ash (*Fraxinus excelsior* L.). The two species are very closely related, at the genetic [3-5] and morphological levels [6-8].

Consequently, it is very difficult to distinguish between them, and they are often misidentified. Most of the distinguishing traits (fruits, flowers, shoots) are in the upper canopy layers whereas the ground traits (epicormic shoots, bark, broken branches and leaves) are very variable and cannot be reliably used for identification [9]. Being so closely related, the two species often hybridize, which has been confirmed with controlled laboratory tests [5] and in natural sympatric habitats [6,8]. The occurrence of morphologically intermediate individuals in a sympatric area has also been confirmed by our observations.

Morphological differences are vital for practical field identification of plant species. Morphometric analysis is often used in the study of morphological traits and differences between taxa, for instance in oaks (*Quercus* ssp., [10,11]), junipers (*Juniperus* ssp., [12,13]) and poplars (*Populus* ssp., [14]). It has also been conducted on species from the genus *Fraxinus* [6-8], and in combination with phenologic and genetic research, it has proved to be a useful tool for the study of *F. angustifolia* and *F. excelsior* and their hybrids in a sympatric environment. Useful results have also been obtained with germination tests in the *Fraxinus* genus [7].

The division of *F. angustifolia* to lower taxonomic units varies depending on the author. The species is commonly divided into two or three subspecies: (*i*) *F. angustifolia* Vahl subsp.

angustifolia in the western Mediterranean; (***ii***) *F. angustifolia* subsp. *oxycarpa* (Bieb. ex Willd.) Franco et Rocha Alfonso [= *F. angustifolia* subsp. *pannonica* (Fuk.) Soó et Simon], which is found in Eastern and Central Europe as well as in Southern Europe and eastwards of north-eastern Spain; (***iii***) *F. angustifolia* subsp. *syriaca* (Boiss.), whose range extends from Turkey eastwards into Iran [1,2,15-17]. The divergence of *F. angustifolia* into geographic subspecies could be the consequence of smaller historical population sizes during glacial times and stronger drift [18]. Morphological traits described in identification keys [16,17,19] as distinguishing features between the subspecies, refer to size, shape and hairiness of leaves and leaflets, bark texture features and the shape and size of fruits. These are distinctly quantitative traits and their overlapping renders it impossible to divide them into discrete categories, especially in the case of morphologically variable species of which *F. angustifolia* is undoubtedly one [3,20]. A comprehensive morphometric analysis to clarify this issue has not been carried out to date.

Extant genetic analyses of *F. angustifolia* have dealt with its variability in natural populations [18] and differences compared to *F. excelsior* [5,7,21]. The species was also included in a study of phylogenetic relationships in the genus *Fraxinus* [3,4]. Analysis of chloroplast microsatellites [18] showed low genetic diversity of European populations of *F. angustifolia*, but a strong geographic structuring of haplotypes. Populations in north-western Balkans, and in northern Italy, southern France and north-eastern Spain, where the subspecies *F. a.* subsp. *oxycarpa* is thought to be present [1], have a different haplotype than populations in Spain [18], which is believed to be the distribution area of the subspecies *F. a.* subsp. *angustifolia* [1]. Smaller genetic differences among the lower taxa within the species *F. angustifolia* are also indicated by analysis of ITS regions [3]. The genetic differences, and the significant morphological variability, have led Wallander [3] to suggest a synonymization of taxa *F. oxycarpa*, *F. syriaca*, *F. pallisae*, *F. potamophila* and *F. sogdiana* under the taxon *F. angustifolia*. In identification keys [16,17] these taxa are occasionally treated as independent species.

In north-western Balkans *F. angustifolia* grows in two regions which are separated by the Dinaric Alps mountain chain. The major part of the range is located in flood-prone riparian sites on the plain, where it grows alongside penduculate oak, black alder, common hornbeam, willow and poplar. A smaller part of the range is in the Submediterranean region, where it is found in smaller and more isolated populations along the downstream of certain rivers and streams [2,9,22-24]. Most *F. angustifolia* populations are remnants of once extensive forests which had been cleared for agricultural use. Due to the proximity of settlements, strong economic impacts and constant human interventions, these remnants are often severely altered. Some authors [2,19] claim that north-western Balkans is home to two subspecies of *F. angustifolia*: *F. angustifolia* subsp. *angustifolia* in the Submediterranean region and *F. angustifolia* subsp. *oxycarpa* in the Subpannonian region. Populations of *F. angustifolia* from these two regions have been included in genetic analyses [18] as well as provenance tests [22], which did not detect any essential differences between the populations from either region. This analysis aims to establish the morphological variability of *F. angustifolia* in this part of its range, to determine whether there may be morphological differences between the Submediterranean and Subpannonian populations, as well as to specify which subspecies of *F. angustifolia* occur in NW Balkans.

Material and methods

The analysis was carried out in the Subpannonian ecological region of Slovenia, where three populations (Črni log, Orlovšček and Kostanjevica) were selected, and in the Submediterranean region of Slovenia, where two populations (Dragonja and Lijak) were chosen (Tab. 1). The main criterion for population selection was density of *F. angustifolia* in the stands (high abundance) and tree social status: trees in the stand canopy in particular were selected for analysis. Variability of *F. angustifolia* was studied with morphometric analysis of leaves and fruits.

The sampling concept and methods were similar for each population. Fourteen individuals at least 30 m apart were randomly chosen in each population. From each tree we randomly selected approx. 10 branches from the outer part (light subsample) and approx. 10 branches from the inner part of the crown (shade subsample). From each shoot 3-4 fully developed leaves were chosen in the laboratory. The leaves were selected from the middle part of the shoots. Up to 50 fruits were picked from each of the 48 trees on which the fruits were developed at the time of sampling. Fruits were not developed in the population Kostanjevica at the time of the sampling. All leaves and fruits were dried and herbarized. After herbarization they were scanned and measured with WinFolia and WinSeedle Régent Instruments Inc. software. In total 4930 leaves and 2099 fruits were measured.

Eight morphological traits were measured on fruits and 32 on leaves (Fig. 1, Tab. 2). Leaves were also analyzed for hairiness to determine presence/absence of hairs on leaflets.

Tab. 1 Main characteristics of the study populations.

| Label	Site	Ecological region	Latitude	Longitude	Average number of sun/shade leaves per tree	Number of trees in fruit analysis	Average number of fruits per tree	Average annual rainfall	Average annual temp.
KO	Kostanjevica	Subpannon.	45°51'32"N	15°24'09"E	33.00/30.79	0	0	987	11.1
CL	Črni log	Subpannon.	46°35'59"N	16°22'38"E	38.93/36.71	10	44.20	694	11.2
OR	Orlovšček	Subpannon.	46°32'12"N	16°19'56"E	36.93/34.07	14	47.14	799	11.2
LI	Lijak	Submediterra.	45°55'56"N	13°42'02"E	35.21/36.36	10	46.50	1257	12.9
DR	Dragonja	Submediterra.	45°26'59"N	13°40'26"E	37.14/33.00	14	38.00	945	13.7
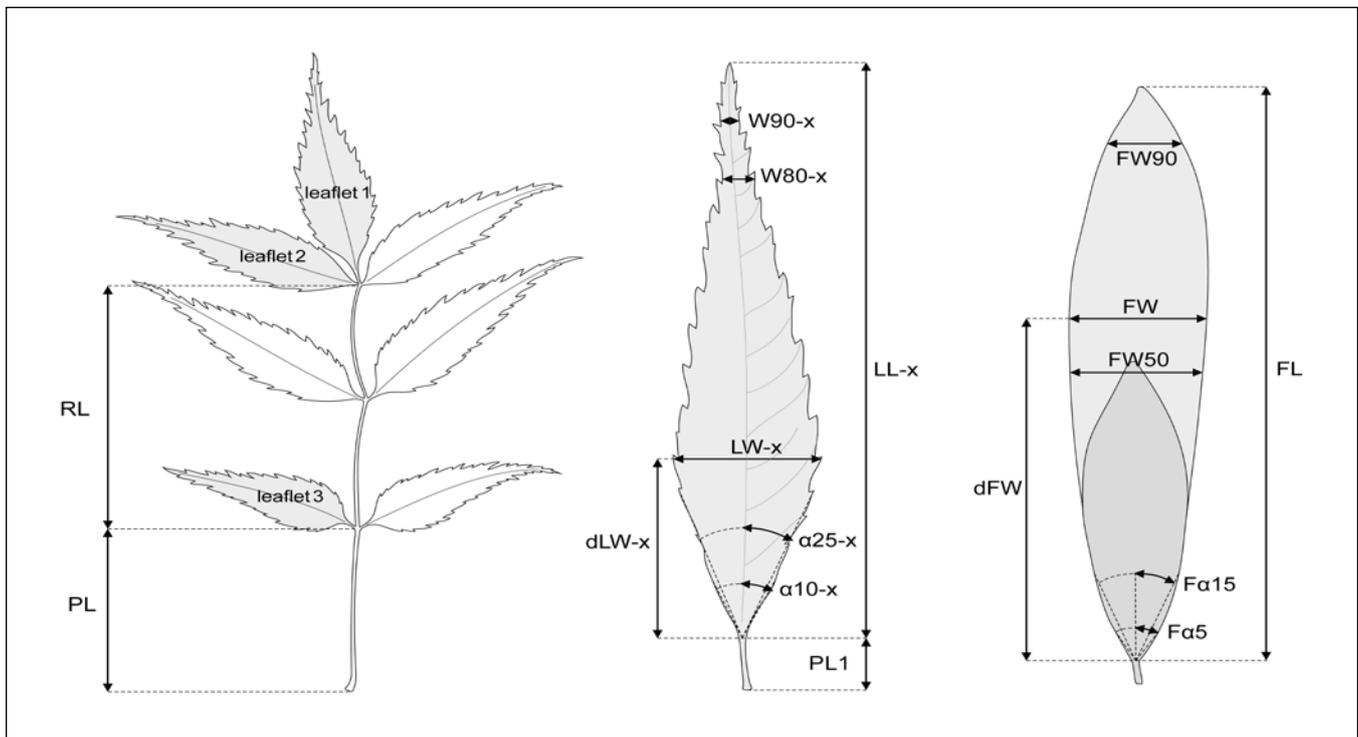
Fig. 1   Schematic illustration of the measured traits on the whole leaf (left), single leaflet (middle) and fruit (right). Sign x replaces the number of leaflet (1, 2 or 3).

From the leaf measurements – separately for the shade and light subsamples – the arithmetic mean was calculated for each tree. This produced two values for each trait of each tree, which were used as input data for analysis. The arithmetic mean was also calculated for all fruits at the level of individual trees.

Assumptions of normality were checked with Shapiro-Wilk's test. Normality for traits W901, PL1, LW2, LL2, LW3, LW3/LL3, W903, Fα15 was obtained after logarithmic transformation, for traits LL1, LW2/LL2, α251, α252 after reciprocal transformation and for trait dLW1 after square-root transformation. For traits α101, dLW2, α102, W902, dLW3 and W803 normality could not be obtained with transformations so they could not be included in the analysis where normality was a criterion.

Analysis of variance was used to verify the differences among individual populations, among trees in a single population, and between leaves from parts of the canopy that have different exposure to sunlight. For this purpose a nested (hierarchical) analysis of variance was designed where trees were nested within populations.

The nested design was carried out with the model: Y = P + E + T(P) + ε to test the main effects of population (P), exposure to sunlight (sun/shade; E) and the nested effects of tree within population T(P) on all measured traits on leaves. The model had two fixed factors (P and E) and one random effect (T). The contribution of a hierarchical level to total variance was presented as a share of total variance.

Forward stepwise discriminant analysis with Wilks' lambda method was used to determine the differences between the individual populations of *F. angustifolia*. By using a stepwise selection procedure, only the most significant of the 32 possible discriminant variables (Tab. 2) were identified. The scatterplot of discriminant scores corresponding to each tree of each population/exposure to sunlight combination in the multivariate space defined by the first two discriminant functions was introduced to present multivariate phenotypic variation (based

Tab. 2   List of morphological characters examined.

Leaf

A (cm²)	Leaf area
PL (cm)	Petiole length
N	Number of leaflets
RL (cm)	Rachis length
A1 (cm²)	Area of leaflet No. 1
A2 (cm²)	Area of leaflet No. 2
A3 (cm²)	Area of leaflet No. 3
LW1 (cm)	Lamina width of leaflet No. 1
LW2 (cm)	Lamina width of leaflet No. 2
LW3 (cm)	Lamina width of leaflet No. 3
PL1 (cm)	Petiole length of leaflet No. 1
dLW1 (%)	Distance from the lamina's base to the point where LW1 was measured
dLW2 (%)	Distance from the lamina's base to the point where LW2 was measured
dLW3 (%)	Distance from the lamina's base to the point where LW3 was measured
LL1 (cm)	Lamina length of leaflet No. 1
LL2 (cm)	Lamina length of leaflet No. 2
LL3 (cm)	Lamina length of leaflet No. 3
LW1/LL1	Lamina width/length ratio of leaflet No. 1
LW2/LL2	Lamina width/length ratio of leaflet No. 2
LW3/LL3	Lamina width/length ratio of leaflet No. 3
W801 (cm)	Width of lamina of leaflet No. 1 on 80% of lamina's length from lamina's base up
W802 (cm)	Width of lamina of leaflet No. 2 on 80% of lamina's length from lamina's base up
W803 (cm)	Width of lamina of leaflet No. 3 on 80% of lamina's length from lamina's base up
W901 (cm)	Width of lamina of leaflet No. 1 on 90% of lamina's length from lamina's base up

Tab. 2 *(continued)*

W902 (cm)	Width of lamina of leaflet No. 2 on 90% of lamina's length from lamina's base up
W903 (cm)	Width of lamina of leaflet No. 3 on 90% of lamina's length from lamina's base up
α101 (°)	Angle at 10% of lamina's length from lamina's base up of leaflet No. 1
α102 (°)	Angle at 10% of lamina's length from lamina's base up of leaflet No. 2
α103 (°)	Angle at 10% of lamina's length from lamina's base up of leaflet No. 3
α251 (°)	Angle at 25% of lamina's length from lamina's base up of leaflet No. 1
α252 (°)	Angle at 25% of lamina's length from lamina's base up of leaflet No. 2
α253 (°)	Angle at 25% of lamina's length from lamina's base up of leaflet No. 3

Fruit

FA (cm²)	Fruit area
FL (cm)	Fruit length
FW (cm)	Fruit width
dFW (%)	Distance from the fruit's base to the point where fruit width was measured
FW50 (cm)	Width of fruit on 50% of fruit's length from fruit's base up
FW90 (cm)	Width of fruit on 90% of fruit's length from fruit's base up
Fα5 (°)	Angle at 5% of fruit's length from fruit's base up
Fα15 (°)	Angle at 15% of fruit's length from fruit's base up

on leaves) between examined groups. To verify the actual differences among groups of *F. angustifolia*, 14 trees of *F. excelsior* were also included in the analysis. SPSS for Windows and Statistica for Windows software were used for statistical analysis.

Results

The results showed that all the studied levels (population, exposure to sunlight, trees within the population) had a significant impact on the variability of *F. angustifolia* leaves.

The mean values of all studied morphological traits of leaves were significantly different between the 14 trees within populations (Tab. 3). The hypothesis that the mean values of studied traits across five populations of *F. angustifolia* would be the same was rejected for 20 morphological traits. The light/shade effect is statistically significant in 24 studied traits (Tab. 3).

Differences among trees in a single population accounted for the greatest part of the variability, followed by differences among populations and differences that are the result of exposure to sunlight (Tab. 4). Variability among trees in a single population accounted for 35% to 88% of total variation; the impact is slightly larger in traits which describe leaf form than in traits which describe leaf size. In terms of leaf sections, most of the differences among trees in a single population are explained by the variability of leaflet No. 3 (71%), leaflet No. 1 (67%) and leaflet No. 2 (58%). Differences among trees explain the least variability of traits that refer to the entire leaf. The impact of differences among populations was smaller, between 2% and 47%. However, even though they are responsible for a smaller share of total variability, differences among populations explained a significant part of variability of leaf

Tab. 3 *F*–statistic for the analyzed traits from the nested analysis of variance.

Variance component	A	PL	N	RL	A1	LW1	LW1/LL1
Population	20.76***	5.66***	17.91***	18.71***	7.72***	3.82**	3.30*
Sunlight exposure	52.52***	30.61***	80.79***	86.88***	6.32*	40.45***	103.85***
Trees within population	3.63***	6.26***	9.86***	6.40***	8.83***	5.59***	8.68***

Variance component	LL1	dLW1	W801	W901	α251	PL1	A2
Population	8.36***	4.13**	4.12**	1.82 n.s.	0.35 n.s.	9.12***	12.00***
Sunlight exposure	16.87***	55.48***	52.37***	25.30***	60.54***	16.01***	25.30***
Trees within population	14.77***	11.18***	5.35***	5.02***	17.70***	13.43***	6.71***

Variance component	LW2	LW2/LL2	LL2	W802	α252	A3	LW3
Population	4.61**	3.72**	14.96***	4.89**	3.29*	5.79***	2.16 n.s.
Sunlight exposure	63.77***	117.67***	2.23 n.s.	65.45***	92.04***	1.25 n.s.	6.05*
Trees within population	5.34***	9.95***	10.72***	4.73***	10.10***	7.35***	6.37***

Variance component	LW3/LL3	LL3	W903	α103	α253		
Population	3.09*	6.95***	1.02 n.s.	0.57 n.s.	2.49 n.s.		
Sunlight exposure	128.70***	31.81***	8.71**	16.96***	87.93***		
Trees within population	11.95***	9.66***	4.02***	8.96***	9.30***		

n.s. $p > 0.05$; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$.

Tab. 4 Partitioning of variation by hierarchical component in all morphological traits of *F. angustifolia* leaves.

Trait	Percentage of variation			
	P	E	T(P)	Error
A	45.7	8.0	35.8	10.5
PL	21.9	4.7	62.8	10.6
N	47.2	5.4	42.8	4.6
RL	45.5	8.3	39.6	6.6
A1	29.4	0.7	61.8	8.1
LW1	15.3	7.3	65.0	12.4
LW1/LL1	13.4	12.2	66.3	8.1
LL1	32.1	1.1	62.3	4.5
dLW1	19.8	5.2	68.5	6.5
W801	15.8	9.4	62.4	12.4
W901	8.0	5.5	71.4	15.1
α251	1.9	4.6	88.2	5.3
PL1	33.8	1.1	60.3	4.8
A2	37.8	3.0	51.1	8.1
LW2	17.0	11.1	60.0	11.9
LW2/LL2	15.1	12.0	65.9	7.0
LL2	45.5	0.2	49.4	4.9
W802	17.3	12.2	57.6	12.9
α252	14.0	9.7	69.0	7.3
A3	23.7	0.2	66.5	9.6
LW3	10.1	1.1	76.1	12.7
LW3/LL3	13.2	11.5	69.2	6.1
LL3	26.9	3.2	63.0	6.9
W903	4.6	2.5	73.5	19.4
α103	3.0	2.5	84.5	10.0
α253	10.8	10.3	70.8	8.1
Average	21.9	5.9	63.2	9.0

P – population; E – exposure to sunlight; T(P) – tree within population.

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size traits (Tab. 4). Variability among populations explains the most variability of traits of the entire leaf (40%), followed by traits of leaflet No. 2 (24%), leaflet No. 1 (19%) and leaflet No. 3 (13%). The contribution of exposure to sunlight to total variability is smaller, between 0.2% and 12%, yet it has a larger impact on leaf form than leaf size.

Discriminant analysis of leaves

The analysis included 5 discriminant functions, of which 4 have a significant contribution to group differentiation.

Function 1, which accounts for 70.5% of the variance explained by the model, is weighted most heavily by leaf area (Tab. 5). Thus, function 1 separates all groups of *F. angustifolia* with small leaves from *F. excelsior*, which has much bigger leaves (Fig. 2). The second function accounts for another 15.7% of the explained variance and seems to be associated mostly with the lamina width of leaflet No. 2 and to a lesser extent by the number of leaflets and the leaf area. Hence function 2 partially separates population Dragonja from the other populations of *F. angustifolia*. But this separation is not as clear as the separation between both species (Fig. 2). The subsamples within population are different but the light/shade differences are smaller than the differences among populations. Functions 3 and 4 significantly contribute to group differentiation (data

Tab. 5 Standardized canonical discriminant function coefficients for the first three functions.

Trait	Function 1	Function 2	Function 3
A	.942	-1.809	-.304
PL	.557	.025	.321
N	.337	1.800	-.143
LW2/LL2	-.295	1.045	-.128
LL2	-.286	2.384	.726
Eigenvalue	5.10	1.14	0.72
% of explained variance	70.5	15.7	10.0

not shown). Their contribution to the explained variance is 10.0% and 3.5% respectively, but the low values of standardized coefficient show weak discriminant ability, so their interpretation is less important.

Thirty-eight percent of the trees were classified in the correct subsample within population, whereas the share of correct classifications to populations averaged 61% (Tab. 6). Most incorrect classifications were due to placement in the other subsample within population (41%). The most unique population is Dragonja, where the share of correct classifications was 78%, followed by Črni log and Kostanjevica (both 64%). Populations Orlovšček and Lijak had the highest share of misidentification and incorrect classification (Tab. 6). In 20% of cases where the model grouped samples in an incorrect population, the samples were also misidentified in terms of their light and shade position (e.g. in shade samples from Kostanjevica one of the nine incorrectly classified samples were grouped under sun samples of population Orlovšček). This indicates that light-exposed leaves of one population can have very similar morphological traits than shade leaves of another population – and vice versa.
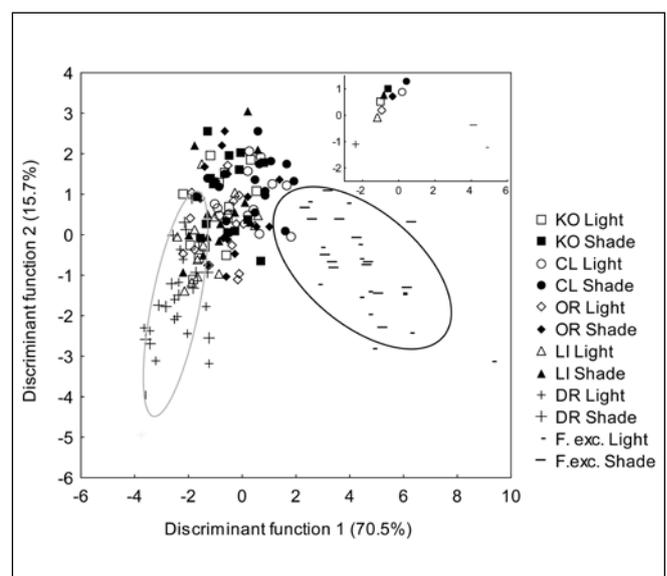

Fig. 2 Distribution of the first two discriminant functions according to the stepwise discriminant analysis of leaf morphological traits in *F. angustifolia*. The ellipses delimit the space that includes the samples of Dragonja population (gray) and *F. excelsior* (black) with $p_{0.95}$. The small graph on the above right indicates centroids for individual subsamples of each population.

Tab. 6 Classification of cases (cross-validation sample).

	% of correct cases	KO Sun	KO Shade	CL Sun	CL Shade	OR Sun	OR Shade	LI Sun	LI Shade	DR Sun	DR Shade	Fr. exc. Sun	Fr. exc. Shade
KO Sun	36	5	4	0	0	5	0	0	0	0	0	0	0
KO Shade	36	4	5	0	0	1	3	0	0	0	1	0	0
CL Sun	57	1	0	8	3	1	0	1	0	0	0	0	0
CL Shade	29	0	0	3	4	0	3	1	3	0	0	0	0
OR Sun	14	4	0	2	0	2	3	3	0	0	0	0	0
OR Shade	21	0	4	1	0	1	3	3	1	0	1	0	0
LI Sun	29	1	0	2	0	3	0	4	2	0	2	0	0
LI Shade	21	0	1	1	3	0	3	2	3	1	0	0	0
DR Sun	64	0	0	0	0	0	0	2	0	9	3	0	0
DR Shade	36	0	0	0	0	1	0	1	2	5	5	0	0
F. exc. Sun	64	0	0	1	0	0	0	0	0	0	0	9	4
F. exc. Shade	57	0	0	1	1	0	0	0	0	0	0	4	8
Total	38.70	15	14	19	11	14	15	17	11	15	12	13	12

Fruits

Differences among populations were statistically significant only for the trait which describes distance from the fruit's base to the maximum width of fruit (dFW; Tab. 7). This trait is the shortest in population Črni log (on 52% from fruit's base up) and the longest in population Dragonja (on 59% from fruit's base up). Significant differences among populations were not found for any other fruit traits. The comparison between *F. angustifolia* and *F. excelsior* shows that the fruits of the former are typically larger (traits FA and FL) and more sharply pointed at the base (traits Fα5 and Fα15). All values of traits Fα5 and Fα15 in all populations of *F. angustifolia* are smaller than the corresponding values for *F. excelsior* (Tab. 7). This trait of *F. angustifolia* fruits earned the species one of its synonyms, "oxycarpus", which means having a sharp-pointed fruit.

Discussion

Comparison among and within populations of individual morphological traits of *F. angustifolia* leaves in north-western

Tab. 7 Arithmetic means of morphological traits on fruits.

Trait	Population	CL	OR	LI	DR	DO
	Species	*F. a.*	*F. a.*	*F. a.*	*F. a.*	*F. e.*
FA (cm²)		2.49a	2.41a	2.06a	2.16a	1.90
FL (cm)		4.06a	3.94a	3.68a	3.85a	3.38
FW (cm)		0.79a	0.80a	0.76a	0.77a	0.75
dFW (%)		0.52a	0.57ab	0.58ab	0.59b	0.63
FW50 (cm)		0.77a	0.77a	0.73a	0.72a	0.71
FW90 (cm)		0.53a	0.54a	0.48a	0.49a	0.52
Fα5 (°)		32.60a	31.36a	29.14a	26.30a	35.86
Fα15 (°)		21.98a	21.35a	20.18a	18.93a	22.83

Means followed by the same letter in the same row are not significantly different at $p > 0.05$ according to Tukey's test (*F. excelsior* is excluded from tests). *F. a.* – *F. angustifolia*; *F. e.* – *F. excelsior*.

Balkans shows that the species is highly variable. On most traits variability within a population exceeds variability among populations, which is characteristic of most plant species in general. This proves that the species is indeed highly variable, but it also aggravates the detection of population differences that could be the result of different factors – including the possibility that populations do belong to different taxa, *F. angustifolia* subsp. *angustifolia* and *F. angustifolia* subsp. *oxycarpa*. Discriminant analysis of leaves shows that leaf "size" traits are the strongest differentiating traits among populations. The analysis produced a fairly clear distinction between *F. angustifolia* and *F. excelsior*. The latter has longer, bigger, broader and more elliptical leaflets. However, discriminant analysis is less successful in distinguishing between *F. angustifolia* populations, of which the Dragonja population indicates uniqueness. The leaves in the Dragonja population are slightly smaller; they have fewer and smaller leaflets.

In this research all the studied traits are quantitative traits. Such traits are frequently affected by alleles at a large number of loci and the influence of each allele on the trait is minor [25]. Consequently, quantitative traits show continuous variation, which makes it very difficult to set clear boundaries between studied groups. Indeed, this is even more difficult in morphologically highly variable species, of which *F. angustifolia* is one according to our findings as well as research by other authors [3,20].

Variation of leaf size may also be a result of environmental, in particular abiotic, factors. It is a well-known fact that plants react to water stress by reducing their leaf area [26]. However, the *F. angustifolia* included in this study were not water stressed, as they were all selected on humid riparian sites. Additionally, the annual rainfall in the Subpannonian ecological region is significantly lower than in the Submediterranean region (Tab. 1) [27,28]. Populations in the former would thus be expected to have the smallest leaves while in fact the results show the opposite.

A second ecological factor that has a strong impact on leaf size is temperature and the associated leaf overheating [29-31]. According to weather station data, the temperatures in the Submediterranean region are higher than in the Subpannonian region (Tab. 1), which could have a significant impact on leaf traits. Smaller leaves in Submediterranean populations

may also be a result of evolutionary adaptation to wind [31], which is particularly strong in the Mediterranean [6]. Because we could not make any conclusions about the impact of environmental factors, they should be dealt with more comprehensively, but this exceeds the purpose of this study.

The analysis also dealt with morphological traits that refer to leaflet shape (LW/LL, dLW, α10, α25). Generally, these traits are very variable, they are significantly affected by light/shade position and none indicate differences between Submediterranean and Subpannonian populations. The population Kostanjevica has leaflets that, on average, are the narrowest and have the longest pointed tips and bases, whereas leaflets in the population Lijak are the broadest and have the shortest tips and bases. The identification keys [17,19] for leaflet shape in subspecies of *F. angustifolia* are somewhat contradictory. Nevertheless, the distinguishing power of traits in the identification keys is too weak to distinguish between the miniscule differences between populations.

In the identification keys [16,17] one of the differentiating traits setting apart the subspecies *F. a.* subsp. *angustifolia* and *F. a.* subsp. *oxycarpa* is the hairiness of leaves. However, no differences in leaf hairiness were established in the populations included in this study. In all analysed trees the bottom sides of the leaves were very hairy, at least along the main vein. The studied populations were compared for hairiness with the population Motovun along the River Mirna in the Istria peninsula in Croatia for which Fukarek [2] reports that it belongs to the subspecies *F. a.* subsp. *angustifolia*. The leaves of all observed trees in that population were very hairy on the bottom side along the main vein, which is in contradiction to identification keys that attribute hairiness primarily to the subspecies *F. a.* subsp. *oxycarpa*. However, based on leaf hairiness, we came to the conclusion that the subspecies *F. a.* subsp. *oxycarpa* thrives in the north-western Balkans; because not a single analysed and observed tree was found to have leaves with smooth bottom sides, the presence of the subspecies *F. a.* subsp. *angustifolia* in the region cannot be confirmed.

Many studies show that light leaves compared with shade leaves are typically smaller (e.g. [29,32]). Our study confirmed the influence of light in the majority (24 of 26) of morphological traits of the leaves of *F. angustifolia*. Shade leaves are bigger than light leaves, they are longer and have more leaflets (data not shown). Light leaves have longer stalks, which is the opposite of what research [32] has found for sessile oak, the reason being that ash has compound leaves with multiple pairs of leaflets on the rachis and consequently a shorter stalk. The leaflets of shade leaves are broader and more short-pointed, and the location of the maximum width is higher than in light leaflets. In general shade leaflets are more rounded, but it is precisely the roundness of shade leaves that makes it difficult to identify species in stands. Only shade leaves in the bottom layers of the canopy can be observed considering the height of the trees, but shade leaves are bigger and have broader and shorter-pointed leaflets, a feature they share with the leaves of *F. excelsior*. This is one of the main reasons why it is difficult to tell them apart in the forest.

The shape of the fruits of *F. angustifolia* is very variable, which had in the past led authors to exclude numerous lower taxa [33]. In identification keys the morphological traits of fruits have weak distinguishing powers in classifying *F. angustifolia* to lower taxonomic units. However, the subspecies *F. a.* subsp. *oxycarpa* is attributed greater heterogeneity in fruit length compared to *F. a.* subsp. *angustifolia* [17]. In our

analysis, Submediterranean populations (LI and DR) show higher variability than Subpannonian populations (ČL and OR), but the differences were not statistically confirmed. On most of the measured traits Submediterranean populations (DR and LI) are morphologically closer and Subpannonian populations (OR and CL) closer, but the differences between the pairs are not big enough to be statistically significant.

The results indicate certain differences between populations from the Submediterranean and Subpannonian ecological regions of Slovenia, but they are not sufficiently significant to confirm the existence of two separate subspecies of *F. angustifolia*. Based on the morphometric analysis of leaves and fruits, we can claim with great certainty that the subspecies *Fraxinus angustifolia* subsp. *oxycarpa* thrives in NW Balkans but there is no convincing evidence for the presence of the subspecies *Fraxinus angustifolia* subsp. *angustifolia*. Finally, populations of *F. angustifolia* included in this analysis are part of a broader genetic analysis, which is ongoing. This analysis will provide additional insight into the variability of the species, and it is expected to confirm the findings of our morphometric analysis.

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