

## SEXUAL DIMORPHISM AND DISTRIBUTION OF *Daphne laureola* L. IN THE BOHOR AREA

### SPOLNI DIMORFIZEM IN RAZŠIRJENOST LOVOROLISTNEGA VOLČINA (*Daphne laureola* L.) NA OBMOČJU BOHORJA

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#### IZVLEČEK

Gynodioecious *Daphne laureola* L. is one of six species of the genus *Daphne* L. native to Slovenia. The main goals of this research were to determine the distribution of *Daphne laureola* in the Bohor area, the sexual structure of populations and its correlation with site conditions, and whether morphological differences exist between females and hermaphrodites beyond those of flower structure. Four research plots located on the north side of Bohor were selected – two in each altitude range. A total of 277 plants were studied to determine the sexual structure of the populations, 90 of which were additionally studied for sexual dimorphism. The species was recorded for the first time in quadrant 9959/1 of the Central European flora mapping grid, extending its previously known distribution. The share of female plants was between 3.17% and 47.17% and was lower at higher altitudes. The frequency of plants, morphological traits, and the proportion of female individuals are highly correlated with environmental factors. Plants bore more flowers at higher altitudes, and leaves were longer, with rounder tips and sharper bases. The majority of morphological differences between females and hermaphrodites were minor and not statistically significant.

**Ključne besede:** *Daphne laureola* L., spurge laurel, sexual dimorphism, species distribution, Bohor, morphometric analysis, sexual structure

#### ABSTRACT

Ginodiecijna vrsta lovorolistni volčin (*Daphne laureola* L.) je ena od šestih samoniklih vrst iz rodu volčin (*Daphne*) v Sloveniji. Cilji raziskave so bili: ugotoviti razširjenost lovorolistnega volčina na Bohorju, spolno strukturo populacij na izbranih ploskvah lovorolistnega volčina na Bohorju in njeno povezavo z rastiščnimi razmerami ter ali poleg razlike v cvetovih obstajajo še druge značilne morfološke razlike med obojespolnimi in ženskimi rastlinami. V raziskavo so bile vključene štiri ploskve na severnem delu Bohorskega pogorja, in sicer po dve v vsakem višinskem pasu. Skupno je bilo za preučevanje spolne strukture populacij popisanih 277 osebkov, med njimi pa še 90 osebkov za preučevanje spolnega dimorfizma. Poleg že prej znane razširjenosti smo vrsto prvič doslej zabeležili v kvadrantu 9959/1 kartiranja srednjeevropske flore. Delež ženskih rastlin po ploskvah se je gibal med 3,17 % in 47,17 % in je bil obratno sorazmeren z naraščanjem nadmorske višine. Pogostnost pojavljanja, deleži ženskih rastlin in morfološke lastnosti rastlin so bile odvisni od dejavnikov okolja. Grmi so imeli na višji nadmorski višini več cvetov, listi pa so bili daljši, z bolj topim vrhom listne ploskve in ostrejšim dnom listne ploskve. Morfološke razlike med obojespolnimi in ženskimi osebki so bile majhne in večinoma niso bile statistično značilne.

**Key words:** *Daphne laureola* L., lovorolistni volčin, spolni dimorfizem, razširjenost vrste, Bohor, spolna struktura, morfometrijska analiza

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#### 1 INTRODUCTION

##### 1 UVOD

Sexual dimorphism in angiosperms is exhibited not only in the reproductive organs, but also in traits not directly related to reproduction. Gender differences may occur in the allocation and acquisition of resources, in interactions with other members of the community, and in the size, colour, and longevity of vegetative structures (Delph, 1999). However, Ashman

(2005) observed that sexual dimorphism in gynodioecious species is expressed in reproductive traits to a much greater extent than in vegetative traits. This may suggest that sexual dimorphism in vegetative traits evolved after the formation of different genders in response to selection stemming from reproductive differences.

Evolution has resulted in many different types of flowers, which may be distributed on plants in various

ways (Brus, 2005): bisexual (hermaphrodite) flowers evolved stamens and pistils in the same flower, unisexual flowers contain only stamens (male flowers) or pistils and ovules (female flowers), and sterile flowers which have evolved perianth, but no pistils or stamens. Gynodioecy is a breeding system in which hermaphrodites and female individuals coexist in the same population and is considered to be the most important evolutionary pathway from hermaphroditism to dioecy (Webb, 1999; Weiblen *et al.*, 2000). In gynodioecious species, the sexual structure of populations can be highly variable. If only hermaphrodites are present in a population, their functional gender is 50% female, but as unisexual individuals increase in frequency, the functional gender of hermaphrodites becomes skewed towards the function that unisexual individuals lack – towards maleness in gynodioecious populations (Delph and Wolf, 2005).

The plant reproductive system is not necessarily a species-specific trait, but can be a variable, labile feature subject to coevolutionary feedbacks (Kokko and L’Opez-Sepulcre, 2007). This also applies to *Daphne laureola*, where hermaphroditism and selfing are positively selected at the low individual frequencies characteristic of newly colonized populations. Male-sterile mutants are able to spread in more dense (older) populations if they reach some reproductive advantage through quantitative seed production or inbreeding avoidance (Medrano *et al.*, 2005).

In gynodioecious *Daphne laureola* there are differences between hermaphrodites and female plants in the morphological structure of the flowers. Female flowers have vestigial stamens that do not produce pollen and have shorter corolla tubes than the perfect flowers of hermaphrodites (Alonso and Herrera, 2001). The differences between different genders rarely appeared in vegetative traits; however, there are populations where female plants appeared to be larger and bore more flowers than hermaphrodites (Alonso *et al.*, 2007).

Gender differences were also discovered in chemical defences and pollination success. It was discovered that female *Daphne laureola* tend to have greater concentrations of coumarins in the leaves. This is consistent with optimal theory and supports the idea that male reproductive function is costly for hermaphrodites. It has also been shown that there is a positive correlation between concentrations of coumarins in female leaves and increasing altitude (Alonso *et al.*, 2005). Alonso (2005) studied pollination success according to altitude and sex ratio. Pollination success, estimated as stigmatic pollen loads, the number of pollen tubes per style, and the percentage of fertilized flowers, was hi-

gher for hermaphrodites than females in populations with 20–56% females.

It was also shown that the frequency of females was highly variable and negatively related to plot elevation. This suggests that establishment and persistence of female plants depends on ecological factors (Alonso and Herrera, 2001). Hermaphroditic populations were found where temperatures were higher and annual precipitation lower. It was discovered that various mechanisms including abiotic conditions and pollinator service may account for breeding system variation within a species distribution range (Alonso *et al.*, 2007) and that populations vary widely in the frequency of females; the range of variation is 10–60% (Medrano *et al.*, 2005).

*Daphne laureola* L. (Thymelaeaceae) is one of six species from the genus *Daphne* L. native to Slovenia. The plant is an evergreen shrub with glabrous, dark green, glossy leaves. Inconspicuous axial flowers are yellow-green and hidden among the leaf bases. Variation among plants in the number and length of stems and the degree of branching generates a broad spectrum of architectural types in this species. All plant parts are poisonous. The species is shade tolerant and calcareous. In Slovenia, *Daphne laureola* preferentially occupies the undergrowth of shady mountain forests (Brus, 2005; Alonso and Herrera, 1996). Its known distribution in Slovenia is shown in Figure 1 (after Jogan *et al.*, 2001).

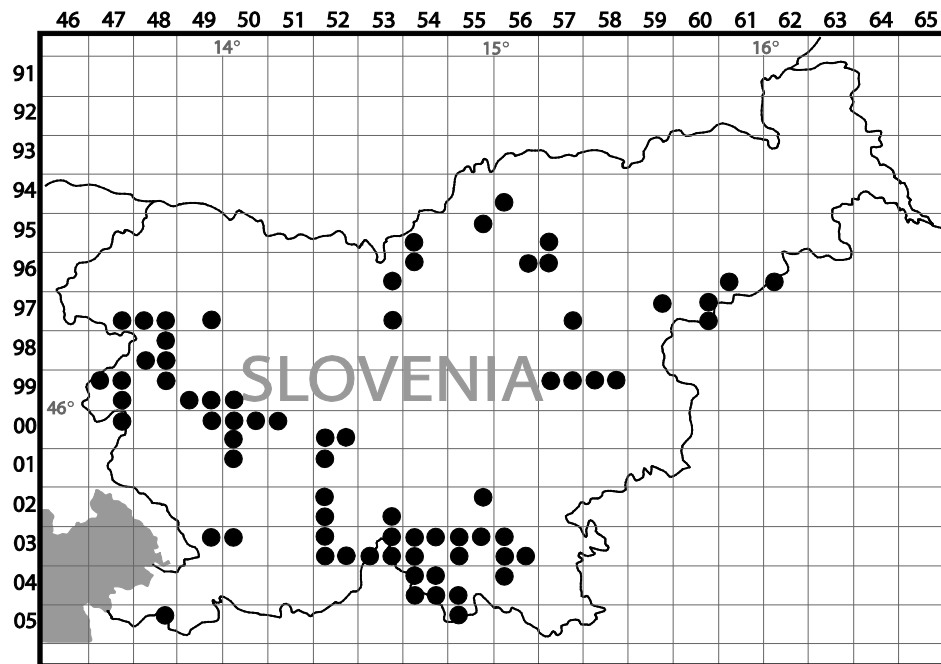
Although the species is common in Slovenian forests, it has not been extensively studied in our country. Alonso and Herrera (2011) studied sexual dimorphism in this species using samples from populations in Vrhnika and Kočevje. Only hermaphrodites were found in the Vrhnika population, while in Kočevje 15% of plants were female.

In this study we focused on *Daphne laureola* populations in the Bohor district to determine the i) precise distribution of the species, ii) sexual structure of populations, and iii) potential morphological differences in vegetative traits between females and hermaphrodites.

## 2 METHODS

### 2 METODE

Research plots were selected on the northern side of Bohor (Figure 2, Table 1). The Bohor Mountain range is known for its diverse terrain with steep ridges and trenches with numerous minor streams. The vegetation is influenced by climate as pre-Dinaric phytoclimate type meets the Subpannonian type in this area. The dominant forest associations are *Lamio orvale* – *Fagetum* var. geogr. *Dentaria polyphillos*, *Car-*



**Fig. 1:** Distribution of *Daphne laureola* in Slovenia (after JOGAN 2001)

**Slika 1:** Razširjenost lovorolistnega volčina v Sloveniji (po JOGAN 2001)

*damine savensi* – *Fagetum* and *Arunco* – *Fagetum*. The highest proportion in growing stocks has a species *Fagus sylvatica* (43.4%) followed by *Picea abies* (21.7%) and *Abies alba* (11.6%). There is also a high share of valuable broadleaves (*Acer pseudoplatanus*, *Acer platanoides*, *Fraxinus excelsior*, *Ulmus glabra*, *Tilia platyphyllos* and *Tilia cordata*). Out of four research plots, two were from the altitude range of 450 to 650 m, and two from the altitude range of 650 to 850 m. Plots were selected in the area where the density of the studied plants was the highest. Basic data for each plot were collected (GPS coordinates, altitude, slope, approximate size). Plots were not limited in size since more attention was paid to include as many plants as possible in the particular population. With the MapSource program, the centre of the plot was determined such that the coordinates of the plant nearest to the centre of the plot were taken. GPS coordinates were recorded with GPS device Garmin GPSmap 60CSx (Garmin Inc. Kansas, U.S.A.).

The data were first collected at the end of April 2011. Location coordinates of the plants, gender, and the number of flowers on a randomly selected stem was determined. Gender was determined for 277 plants in total. Flowers were examined to compare the differences between hermaphrodite and female flowers with those determined by Alonso and Herrera (2001). According to Alonso and Herrera (2001), female flowers have vestigial stamens that do not produce pollen and have shorter corolla tubes ( $4.9 \pm 0.45$  mm, N= 45 flowers) than do hermaphrodites ( $8.1 \pm 0.92$  mm, N= 56). In addition to females (F) and hermaphrodites (H), some undefined plants (N), which did not have flowers, were also noted. Studied plants were at least 3 m apart. The frequency of occurrence of the plants on individual research plots was based on the following scale: individually (1); rare (2); medium often (3); very often (4).

To study sexual dimorphism, a total of 90 plants were analyzed. For morphometric analysis, the five bi-

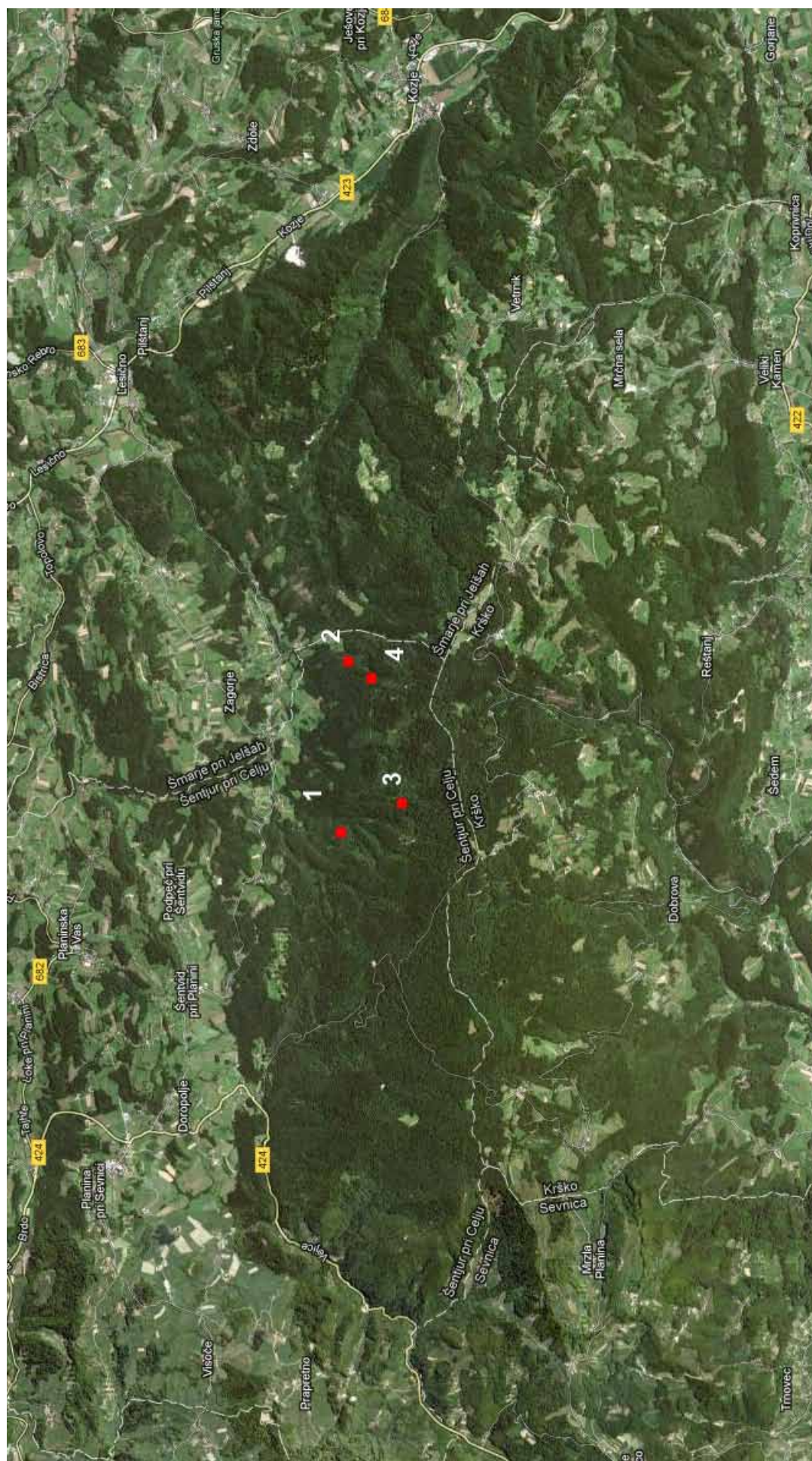
**Table 1:** Main characteristics of the research plots

**Preglednica 1:** Glavne značilnosti raziskovalnih ploskev

Altitudinal belt	450–650 m		650–850 m	
	Plot 1	Plot 2	Plot 3	Plot 4
<b>Altitude</b>	557–641 m	457–498 m	688–781 m	789–843 m
<b>Latitude</b>	46° 04.822' N	46° 04.861' N	46° 04.302' N	46° 04.547' N
<b>Longitude</b>	15° 27.301' E	15° 28.763' E	15° 27.437' E	15° 28.498' E
<b>Inclination</b>	30°	8°	12°	14°
<b>Approximate size</b>	2.4 ha	0.8 ha	20 ha	4.5 ha



**Fig. 2:** Locations of research plots in the Bohor Mountain range (more detailed data for each plot are provided in Table 1)



**Slika 2:** Lokacije raziskovalnih ploskev lovrolistnega volčina na Bohorju (podrobnejše informacije o posameznih ploskvah so podane v tabeli 1)

ggest, fully developed leaves from a randomly selected shoot were collected. All leaves were dried and herbarized and deposited in the herbarium of the Department of Forestry at the Biotechnical Faculty, University of Ljubljana. After herbarization, they were scanned and measured with WinFolia (Régent Instruments Inc. Quebec, Canada) software. On every shrub, the number of stems was counted and the diameter of the thickest stem and the height of the tallest shoot were measured.

Leaves were scanned with an Epson Expression 1680 (Epson America Inc. Long Beach, U.S.A.) scanner and the digital picture was corrected with Adobe Photoshop CS software. Leaves were then analyzed with Winfolia (measured traits are shown in Table 2) and data were statistically processed. Descriptive analysis, a *t*-test, and a hierarchical test were performed.

In the descriptive analysis, only average values of different genders were included. The contribution of each level of treatment to the variability of the leaf morphological traits was determined with a hierarchical test using the following model:

$$Y = B + G + P(B) + S(B,G,P) + \varepsilon.$$

The effects of following factors were included in the model: altitudinal belt (*B*), gender (*G*), nested effect of the plot within the altitudinal belt *P*(*B*), and nested effect individual shrub within the altitudinal belt, gender, and plot *S*(*B,G,P*). Factors *B* and *G* were fixed and factors *P* and *S* were random.

### 3 RESULTS

#### 3 REZULTATI

##### 3.1 Distribution of *Daphne laureola* in the area of Bohor

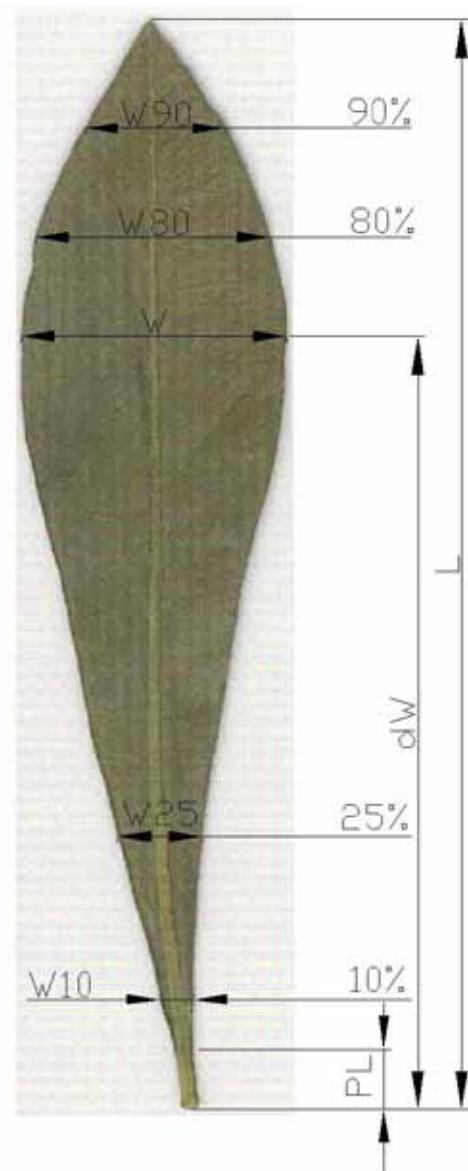
##### 3.1 Razširjenost lovrorolistnega volčina na območju Bohorja

The distribution area of *Daphne laureola* in Bohor is relatively small and isolated. Owing to this isolati-

**Table 2:** List of morphological characters examined

**Preglednica 2:** Seznam preučevanih morfoloških značilnosti

<b>A</b>	Leaf area
<b>L</b>	Leaf length
<b>W</b>	Leaf maximal width
<b>dW</b>	Distance from the leaf base to the point of maximal width
<b>W80</b>	Leaf width at 80% of leaf length from lamina base
<b>W90</b>	Width of leaf on 90% of leaf length from lamina base
<b>W10</b>	Width of leaf on 10% of leaf length from lamina base
<b>W25</b>	Width of leaf on 25% of leaf length from lamina base
<b>PL</b>	Petiole length



**Fig. 3:** Display of measured morphological characters on leaves  
**Slika 3:** Prikaz merjenih morfoloških značilnosti na listih

on and coherent area of distribution, plants have probably evolved independently from other populations. Our results were consistent with the distribution area determined by Jogan *et al.* (2001) (Figure 1). The distribution area lies mostly in quadrant 9958/2, but we also found several plants in quadrant 9959/1, where the species had not yet been recorded.

##### 3.2 Gender structure of populations

##### 3.2 Spolna struktura populacij

The proportion of female plants was lower at higher altitudes (Table 3). The gender ratio in higher altitudes was skewed in favour of hermaphrodites (86.32%), while females represented only 6.84%. In the lower altitudinal belt, the percentage of hermaphrodites was lower (69.83%) due to female plants, whose percenta-



**Table 3:** Partitions and number of plants of different genders of *Daphne laureola* in two altitudinal belts

Altitudinal belt, Plot No.	Gender	Hermaphrodites (Share / No. of plants)	Females (Share / No. of plants)	Undefined (Share / No. of plants)	Total (No. of plants)
	450-650 m	1	43.40/23	47.17/25	9.43/5
	2	82.23/88	12.15/13	5.61/6	107
	Average	69.38	23.75	6.88	
650-850 m	3	90.48/57	3.17/2	6.35/4	63
	4	81.48/44	11.11/6	7.41/4	54
	Average	86.32	6.84	6.84	
Total average (%/n)		74.40/53	18.40/11.5	7.20/4.8	277 69.25

ge rose to 23.75%. The total number of plants in the studied plots was quite different. The highest number of plants was on plot 2 with 107 plants in total (Table 3).

### 3.3 Morphological variability

#### 3.3 Morfološka variabilnost

Populations from different altitudinal belts differ in the average values of the number of flowers (Table 4).

**Table 4:** Average values and standard deviation (sd) of different traits of *Daphne laureola* according to gender

Altitudinal belt, Plot No.	Trait		No. of flowers	No. of stems	Diameter (cm)	Height (cm)
	450-650 m	1	F	27.11	2.68	1.02
		H	26.91	2.18	0.93	54.11
		Average	27.08	2.46	0.98	53.92
		sd	10.22	1.82	0.24	12.40
	2	F	30.11	1.33	1.19	76.93
		H	26.53	1.35	1.01	62.64
		Average	27.77	1.35	1.07	67.59
		sd	12.74	0.63	0.29	22.54
650-850 m	3	F	55.00	1.00	0.80	32.80
		H	35.07	2.33	1.10	69.70
		Average	36.31	2.25	1.08	67.39
		sd	13.79	1.77	0.48	21.19
	4	F	58.17	4.00	1.25	70.22
		H	44.56	1.50	1.22	65.79
		Average	48.27	2.18	1.23	67.00
		sd	26.57	1.79	0.40	18.55
Total female			34.94	<b>2.49</b>	1.11	63.12
Total hermaphrodite			33.66	<b>1.80</b>	1.07	63.70
Total average			34.10	2.03	1.08	63.46
Total sd			18.71	1.59	0.35	19.50

Bold indicates statistically significant differences between female (F) and hermaphrodite (H) plants (t-test, P<0.05)

**Preglednica 3:** Deleži in število osebkov različnih spolov lovrolistnega volčina v dveh višinskih pasovih

Plants from the lower altitude range had fewer flowers on average. Plot No. 1 was characterized by lowest average height values.

Average values of leaf width traits appeared to be lower in the higher altitude range. The only exception was the trait W10, where the average values were slightly higher. On average, the leaves were wider on plot No. 2, again, with the exception of trait W10.

**Preglednica 4:** Povprečne vrednosti in standardni odkloni (sd) različnih znakov na grmih lovrolistnega volčina, ločeno po spolu

### 3.4 Sexual dimorphism

#### 3.4 Spolni dimorfizem

The differences between female and hermaphrodite plants were small with regard to the diameter and plant height. Considerably higher differences appeared in the number of flowers and stems (Table 4). In female plants, average values of the number of flowers were higher on all plots. Plants had on average more stems in the higher altitudinal belt. Differences in the number of stems were most evident on plot No. 4, where female plants had on average two and a half more stems than did hermaphrodites.

When comparing the total average values of traits measured on individuals (*t*-test), it was discovered that statistically significant differences between hermaphrodites and female plants exist only in the number of stems (Table 4). Female plants have on average more stems, while the differences between hermaphrodites and female plants in the number of flowers, diameter, and height were not statistically significant.

Average values measured on the leaves did not differ considerably between genders (Table 5). Leaves of hermaphrodites were on average slightly longer and

had slightly larger leaf areas. The total average values that indicate the width of the leaf did not differ largely and were even identical in some traits. Differences between plants of different genders in the length of the petiole and in the position of dW were also small.

In most of the studied traits, sexual dimorphism was not significant among different plots. Slightly larger differences appeared in the length and the area of the leaf (Table 5). The *t*-test also revealed statistically significant differences in leaf length between plants of different gender (Table 5).

### 3.5 Contribution of specific level to the variability of leaf traits

#### 3.5 Prispevek posameznih nivojev preučevanja k variabilnosti listov

The contribution of the specific level of research to the variability of leaf morphological traits was determined with a hierarchical testing. Differences between shrubs in a single plot accounted for the greatest amount of trait variability (A, L, W, W80, W10, W25). Shares of these traits vary between 50.82% (W10) and 78.76% (L). The variability of the remaining traits

**Table 5:** Average values and standard deviation (sd) of leaf traits of *Daphne laureola* according to gender

**Preglednica 5:** Povprečne vrednosti in standardni odkloni (sd) merjenih znakov na listih lovrorolistnega volčina, ločene po spolu

Altitudinal belt, Plot no.			Trait	A (cm <sup>2</sup> )	L (cm)	W (cm)	dW (%)	W80 (cm)	W90 (cm)	W10 (cm)	W25 (cm)	PL (cm)
450-650 m	1	F		18.02	10.09	3.00	0.68	2.63	1.66	0.48	1.14	0.81
		H		19.17	10.71	3.03	0.68	2.66	1.63	0.48	1.14	0.81
		Average		18.51	10.35	3.01	0.68	2.64	1.65	0.48	1.14	0.81
		sd		2.85	0.97	0.24	0.01	0.22	0.15	0.04	0.09	0.18
	2	F		20.31	10.63	3.16	0.69	2.85	1.90	0.51	1.17	0.92
		H		20.61	11.13	3.11	0.68	2.76	1.78	0.52	1.18	0.83
		Average		20.51	10.96	3.13	0.68	2.80	1.82	0.52	1.18	0.86
		sd		4.42	1.39	0.39	0.02	0.40	0.31	0.06	0.12	0.24
650-850 m	3	F		20.11	11.09	3.00	0.68	2.64	1.66	0.52	1.16	0.89
		H		19.64	11.03	2.97	0.67	2.60	1.63	0.51	1.14	0.89
		Average		20.65	11.59	2.98	0.67	2.58	1.61	0.51	1.13	0.99
		sd		4.53	1.55	0.34	0.02	0.34	0.23	0.03	0.09	0.26
	4	F		17.96	10.82	2.66	0.67	2.30	1.40	0.51	1.09	0.84
		H		18.37	10.78	2.77	0.67	2.40	1.49	0.54	1.12	0.92
		Average		18.26	10.79	2.74	0.67	2.37	1.47	0.53	1.11	0.90
		sd		5.86	2.20	0.40	0.02	0.38	0.23	0.07	0.15	0.30
Total female				18.88	<b>10.48</b>	2.99	0.68	2.64	1.68	0.50	1.15	0.85
Total hermaphrodite				19.68	<b>11.05</b>	2.97	0.67	2.60	1.63	0.52	1.14	0.89
Total average				19.41	10.85	2.97	0.68	2.61	1.65	0.51	1.14	0.88
Total sd				4.53	1.59	0.37	0.02	0.37	0.27	0.06	0.12	0.25

Bold indicates statistically significant differences between female (F) and hermaphrodite (H) plants (*t*-test, *P*<0.05)

**Table 6:** F-values of hierarchical test for analyzed traits measured on the leaves

Source of variance	A	L	W	dW	W80	W90	W10	W25	PL
Gender	0.15n.s.	0.67n.s.	0.02n.s.	0.1n.s.	0.01n.s.	0.32n.s.	0.66n.s.	0.01n.s.	0.03n.s.
Altitudinal belt	0.04n.s.	1.56n.s.	7.71**	5.4*	10.02**	11.96***	2.86n.s.	2.32n.s.	4.03*
Plot (altitudinal belt)	2.23n.s.	1.67n.s.	2.56n.s.	0.8n.s.	2.91n.s.	5.13**	3.45*	0.55n.s.	0.89n.s.
Shrub (plot, alt. belt, gender)	16.54***	20.69***	7.74***	1.9***	6.08***	3.56***	4.37***	5.97***	3.96***

n.s.  $P > 0.05$ ; \*  $0.01 < P < 0.05$ ; \*\*  $0.001 < P < 0.01$ ; \*\*\*  $P < 0.001$

n.s.  $P > 0.05$ ; \*  $0.01 < P < 0.05$ ; \*\*  $0.001 < P < 0.01$ ; \*\*\*  $P < 0.001$

**Preglednica 6:** F-vrednosti hierarhičnega poskusa za analizirane znake na listih

**Table 7:** Partitioning of variation by hierarchical component in all leaf morphological traits

Source of variance	Altitudinal belt	Gender	Plot (alt. belt)	Shrub (plot, alt. belt, gender)	Error
A	4.01	0.13	0.03	76.33	19.50
L	3.10	0.63	1.45	78.76	16.07
W	3.56	0.01	5.35	58.93	32.15
dW	0.54	0.05	1.94	30.67	66.81
W80	3.64	0.00	6.28	53.17	36.92
W90	0.10	0.00	2.24	47.27	50.39
W10	0.04	0.00	0.02	50.82	49.11
W25	0.08	0.00	0.16	58.42	41.35
PL	0.10	0.00	2.24	47.27	50.39

**Preglednica 7:** Prispevek posameznih nivojev preučevanja k skupni variabilnosti morfoloških znakov na listih

(dW, W90, PL) is mostly influenced by the differences within the shrub. The gender has by far the smallest contribution to the leaf variability; in some cases, the contribution is only 0.001% (W80, W90, W25, W10, PL). The F values also show that the individual plot has a rather small impact on leaf variability. Statistically significant differences appeared only in two traits (W90 and W10). The altitudinal belts have a slightly greater influence on the leaf traits, where statistically significant differences appeared in five traits (W, dW, W80, W90, PL). Statistically significant differences with minimum risk appeared in the trait W90.

#### 4 DISCUSSION

#### 4 RAZPRAVA

The distribution area of *Daphne laureola* in the Bohor area is relatively small. It extends over a larger area in lower altitudes, likely due to the better growth conditions there. The presence of the species in a new quadrant (9959/1) was recorded in this part of the distribution range.

There are differences in sexual structure among populations in different altitudinal belts. The sex ratio is highly variable even in the relatively small distribution range of Bohor. In our research, the ratio of female plants varied from 3.17% to 47.17%. This result is simi-

lar to values from past research, where the proportion of female plants reached up to 60% (Mederano *et al.*, 2005). However, in some cases populations were completely hermaphroditic (Alonso and Herrera, 2011).

It can be assumed that the population in which the proportion of female plants was highest is close to dioecy in terms of functional gender. Delph and Wolf (2004) concluded that additional complexities arise when gender allocation of hermaphrodites is plastic, meaning that hermaphrodites can alter their production of seeds or pollen depending on environmental conditions. This was determined for *Daphne laureola* by Alonso and Herrera (2001), where the proportion of female plants was inversely related to altitude. This also appeared to be true in our research, where the proportion of female plants was lower at higher altitudes.

In addition, Alonso (2005) concluded that female quantitative disadvantage in pollination success increased with elevation, suggesting that the higher availability of pollen due to the increased proportion of hermaphrodites could not mitigate the negative effect of other factors associated with elevation.

We found that in populations with a low density of plants there was also a low proportion of female plants. This is similar to the results of Medrano *et al.* (2005). It also indicates that hermaphroditism and self-pollin-



nation occurs as positive selection, where frequencies of individual plants are low, as is typical of newly colonized areas, while ginodicy evolves in more dense populations. We surmise that the higher proportion of hermaphrodites in this area could be a result of their positive selection due to frequent logging.

Contrary to our expectations, there were more flowers per stem on average at higher altitudes. Lower values would be expected because the frequency of the hermaphrodite plants was higher, and it has been discovered that hermaphrodites bear more flowers (Alonso *at al.*, 2007). It is possible that plants at higher altitudes have evolved more flowers in order to improve potential pollination success in response to the lower plant densities resulting from the poorer ecological conditions there.

Most morphological traits, such as the diameter of the thickest stem (10.9 mm) and height (63.97 cm), are consistent with the findings of other studies. Alonso (1996) determined an average stem diameter of 11.2 mm. In our study, the average leaf length was 10.85 cm. This is larger than the values of 3-10 cm described in the literature (e.g. BRUS 2005). Slightly higher average values were expected since we only analyzed the largest leaves from each stem. No data on the average number of stems per individual were found in the literature. The highest number of stems per individual was recorded in plot 1 (2.46). This number would likely be higher if growth conditions were better and population densities higher.

Although some gender differences were discovered, sexual dimorphism of *Daphne laureola* in the area of Bohor is not prominent. Significant differences were discovered between plants of different gender in the number of stems and leaf length. Female plants have probably gained an advantage over hermaphrodites since they produce more stems and flowers (differences were not statistically significant). On the other hand, hermaphrodites have gained some advantages in the development of some vegetative organs such as slightly longer leaves. Alonso *at al.* (2007) also discovered that female plants bore more flowers than hermaphrodites. However, due to their lower pollination success, females did not consistently produce more seeds than hermaphrodites. Because the average number of flowers of female plants was higher, it would be of interest to study the fruit production of female plants in comparison to hermaphrodites. If female plants bore more fruits, it could be concluded that they have gained a reproductive advantage over hermaphrodites. This would explain their persistence in the population. Further studies could also include the

proportion of flowering stems in order to determine if there is any correlation between number of stems and number of flowers.

In contrast to Alonso and Herrera (2001), we found significant differences in leaf length (L) between plants of different gender. The leaves of hermaphrodites were longer compared to those of female plants; however, no uniform distribution pattern of this trait across the populations was found.

The hierarchic test revealed that the variability within the shrub greatly contributes to the common variability of the leaves. Differences that occurred among shrubs nested within the plot can be explained with different ecological conditions for growth that are specific for the individual shrub. For some traits, we can confirm a statistically significant effect of altitudinal belt (W, W80, W90, PL, dW). The nested effect of the plot within the altitudinal belt is statistically significant only for two leaf morphological traits (W90, W10).

## 5 POVZETEK

### 5 SUMMARY

Lovorolistni volčin je ena od šestih samoniklih vrst iz rodu volčinov (*Daphne* L.) v Sloveniji. Je vednozelen grm z usnjatimi temnozelenimi in bleščecimi listi. Manj opazni so rumenozeleni cvetovi. Je sencodržna vrsta, ki najraje raste na apnencu. V Sloveniji uspeva po senčnih in vlažnih gozdovih od nižin do gorskega pasu, največkrat v gorskih bukovih, jelovo-bukovih in drugih svežih mešanih gozdovih. Razmnoževalni sistem, značilen za lovorolistni volčin, kjer v isti populaciji sobivajo obojespolni in ženski osebki, se imenuje ginodiecija. Ta sistem v teoriji velja kot evolucijska pot od hermafrodizma k dieciji. Deleži ženskih oz. obojespolnih osebkov se pri lovorolistnem volčinu med populacijami razlikujejo. Osebki različnih spolov se med seboj razlikujejo v zgradbi cvetov, odkrite pa so bile tudi že nekatere druge razlike med obojespolnimi in ženskimi rastlinami. Lovorolistni volčin je v Sloveniji precej pogosta, a glede spolnega dimorfizma in spolne strukture populacij slabo preučena vrsta. Cilji naloge so bili ugotoviti razširjenost lovorolistnega volčina na Bohorju, ugotoviti, ali poleg razlik v cvetovih obstajajo še druge značilne morfološke razlike med obojespolnimi in ženskimi rastlinami in ugotoviti spolno strukturo populacij in njeno povezavo z rastiščnimi razmerami.

V raziskavo smo vključili štiri ploskve na severnem delu Bohorskega pogorja, in sicer po dve v vsakem višinskem pasu (450 do 650 m in 650 do 850 m). Ploskve smo izbrali, kjer so bile gostote rastlin največje, in jim določili osnovne podatke (GPS koordinate, nadmorsko višino, naklon, okvirno velikost).

Konec aprila 2011 smo v prvi fazi pri vsakem osebku zabeležili koordinate in ugotovili spol ter določili število cvetov na naključno izbranem debelcu. Na vsakem nahajališču smo določili pogostnost pojavljanja. V tej fazi smo rastline, ki smo jih naključno izbrali za podrobnejše preučevanje spolnega dimorfizma, označili z rdečim trakom. Na teh rastlinah smo junija 2011 izbrali po en poganjek in s tega nabrali 5 največjih listov. Liste smo shranili in jih herbarizirali. Vsakemu osebku smo prešteli število debel, izmerili premer najdebelejšega debela pri tleh in višino, ki jo je dosegel najvišji poganjek. Za preučevanje spolne strukture smo skupno popisali 277 osebkov, od katerih smo popisali 90 osebkov za še dodatno preučevanje spolnega dimorfizma. Morfometrijska analiza je obsegala digitalizacijo vsakega lista, analizo s programom Winfolia in statistično obdelavo podatkov. Uporabili smo deskriptivno analizo, *t*-test in hierarhični poizkus.

V spodnjem delu raziskovalnega območja smo lovorolistni volčin odkrili tudi v kvadrantu kartiranja srednjeevropske flore 9959/1, kjer doslej še ni bil zabeležen. Ugotovili smo, da obstajajo razlike v spolni strukturi med populacijami različnih višinskih pasov. Razen razlik v cvetovih, morfološke razlike med obojespolnimi in ženskimi rastlinami po večini niso statistično značilne. Značilne razlike med obojespolnimi in ženskimi osebki smo odkrili le v številu debel in dolžini listov. Treba pa je poudariti, da so te razlike v različnih populacijah različno usmerjene, zato jih je težko interpretirati in bi jih bilo smiselno podrobneje preučiti.

Že na razmeroma majhnem območju Bohorja se je pokazalo, da se lahko razmerje med spoloma precej spreminja. V naši raziskavi se je delež ženskih rastlin gibal med 3,17 % in 47,17 % in je bil obratno sorazmeren z naraščanjem nadmorske višine. Sklepamo lahko, da sta pogostnost pojavljanja in delež ženskih rastlin odvisna od dejavnikov okolja. Od ekoloških dejavnikov pa so odvisne tudi morfološke lastnosti rastlin. Grmi imajo v višjem višinskem pasu več cvetov, listi pa so daljši, z bolj topimi vrhovi listne ploskve in ostrejšim dnom listne ploskve. Razlike med obojespolnimi in ženskimi osebki so bile majhne in po večini niso bile statistično značilne. Značilne razlike so se pokazale le pri dveh znakih, in sicer so imeli ženski osebki značilno več debel, obojespolni osebki pa značilno daljše liste. Sklepamo lahko, da so ženske rastline pridobile prednost pred obojespolnimi rastlinami v razmnoževalnem sistemu s tem, da proizvedejo več debel in več cvetov (razlike sicer niso bile značilne), hermafroditi pa imajo vegetativno prednost, in sicer nekoliko daljše liste.

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