






Morphological variation of reproductive traits in the annual section *Polygonum* taxa and its taxonomic significance

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Abstract: Reproductive traits are important taxonomic characters in section *Polygonum*. However, the taxonomic value of this character remains uncertain because of morphological variability and overlap among taxa. The goal of this research was to evaluate the discriminatory power of a comparative analysis of the quantitative and qualitative morphological characters of the bracts, perianth, and fruit in selected representatives of sect. *Polygonum*. The sample included seven taxa from 54 populations inhabiting the Balkan Peninsula and the Pannonian and Wallachian plains, and 23 quantitative and 9 qualitative morphological characters were analyzed. The results revealed a high degree of similarity between certain taxa, particularly *P. aviculare* and *P. arenastrum*, as well as *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum*. Almost all qualitative characters, with the exception of pericarp ornamentation, were found to be unstable at the taxon level, calling into question their previously emphasized taxonomic significance. Although newly analyzed quantitative characters provided additional information, they did not enable complete separation of the investigated taxa. Overall, the results indicate that morphology alone has limited resolving power within sect. *Polygonum* and highlight the need for an integrative taxonomic approach combining morphological, molecular, and ecological data.

Keywords: decision tree, dichotomous key, knotweed, perianth morphology, pericarp ornamentation

INTRODUCTION

Polygonum L. s. str. (Polygonaceae Juss.) is characterized by a low, predominantly prostrate habit, small axillary flowers, and a silvery or colorless membranous ochrea [1]. The genus comprises 60-75 species and is nearly cosmopolitan, with most species occurring in the temperate Northern Hemisphere [2,3]. Within the genus *Polygonum* s. str., section *Polygonum* is the most numerous and includes approximately 45 species [2]. Section *Polygonum* is distinguished by flowers borne in clusters of 2-4 per node, rarely solitary; flowers are pentamerous, with dendritic tepal vasculature; the outer stamens are occasionally reduced; and the pollen is of the Avicularia type [4]. Within the study area, five species have been reported from Montenegro [5], six from Serbia, Hungary, North Macedonia, and Bosnia and Herzegovina [3,6-8], and 11 from Romania

[9]. Records of multiple infraspecific taxa in these regions suggest a high level of variability in this section throughout the Balkan Peninsula and the Pannonian and Wallachian plains, contributing to taxonomic complexity in the group.

P. aviculare L. is the most investigated and one of the most taxonomically complex members of sect. *Polygonum*. It is a polymorphic aggregate of annual autogamous taxa that inhabit different types of open habitats under intense anthropogenic influence [10]. The high variability of *P. aviculare*, noted by numerous researchers [10-15], has led to the description of numerous taxa and the recognition of the *P. aviculare* aggregate.

The present study follows the taxonomic treatment adopted in Flora Europaea [16] and the monograph of

Polygonaceae [1], in which the *P. aviculare* aggregate consists of four species – *P. arenastrum* Boreau, *P. aviculare*, *P. boreale* (Lange) Small, and *P. rurivagum* Jord. ex Boreau. This treatment is also consistent with previous morphometric studies [12-14].

The second group of annual taxa of sect. *Polygonum* included in this study comprises plants with small bracts, shorter and narrower than the leaves, that usually do not exceed the flowers in length [16]. The Russian and Central Asian steppe and saline habitats host the greatest diversity of species from this group. In the wider area of the Balkan Peninsula and the Pannonian and Wallachian plains, taxa from this group tend to be found in open, often ruderal habitats or as weeds on cultivated land. In the investigated area, *P. albanicum* Jáv, *P. arenarium* Waldst & Kit. subsp. *arenarium*, *P. arenarium* subsp. *pulchellum* (Loisel.) Thell., *P. bellardii* All., and *P. patulum* M. Bieb. have been recorded to date [3,17]. These taxa share several morphological characteristics, occur within the investigated area, and remain insufficiently studied from a taxonomic perspective, and were therefore selected for the present study. All taxa, except *P. albanicum*, which is a transregional obligate serpentinite endemic to the Balkan Peninsula [17-19], have a wide distribution.

Despite the relatively extensive taxonomic treatment of *Polygonum*, species delimitation within sect. *Polygonum* remains problematic, particularly in the *P. aviculare* aggregate [10,11-15,20]. Pronounced morphological variability and overlap of diagnostic characters, together with inconsistent treatments in regional floras and differing taxonomic interpretations [3,5-9], often hinder reliable identification of annual taxa of sect. *Polygonum* in the Balkan Peninsula and adjacent lowland regions.

Previous morphometric studies of the analyzed taxa focused on vegetative organs (mostly the dimensions of leaves, internodes, and ochreas), as well as on the reproductive traits (total perianth length or length of its individual parts, as well as fruit dimensions) [10,12,14,20-22]. These studies have shown that many of the traditionally used morphological characters exhibit considerable variation and similarity among taxa, limiting their diagnostic value. Previously studied qualitative characters mainly included plant habitus and the shape and color of leaves, branches, and fruits.

Previous micromorphological studies of vegetative organs have primarily focused on leaves, examining epidermal, stomatal, and subsidiary cell morphology, as well as stomatal apparatus and trichome types [15,23-25]. Various reproductive traits have also been investigated, including nectary micromorphology [26], the shape, dimensions, and ornamentation of perianth cells [4,24,27-30], and pericarp ornamentation, as well as the shape and surface characteristics of individual pericarp cells [4,29,31-34]. Pollen micromorphological studies included the dimensions, type, shape, and ornamentation of pollen grains [4,24,25,29,35,36].

Although these studies have significantly contributed to the understanding of morphological variation in the group, comprehensive studies integrating morphology with additional sources of evidence remain limited, particularly for taxa occurring in the Balkan Peninsula and the Pannonian and Wallachian plains. Apart from floristic records, detailed taxonomic studies from this region are still relatively scarce. The primary objective of the present study was to reassess morphological variation in selected representatives of sect. *Polygonum* from the Balkan Peninsula and the Pannonian and Wallachian plains using comparative analysis of reproductive characters and multivariate statistical methods. To achieve this objective, the following specific goals were: (i) evaluation of quantitative and qualitative reproductive characters, including several previously understudied traits, and assessment of their taxonomic usefulness in morphologically similar taxa; (ii) critical in-depth comparison of descriptive and multivariate statistics results with previous literature data; and (iii) development of a morphology-based dichotomous key for the investigated taxa.

MATERIALS AND METHODS

Plant material

Plant material was sampled during the July-October period over four years (2018 to 2021) from 54 natural populations (1,129 individuals, seven taxa) growing in an area that includes the western region of the Wallachian Plain, the central and southern parts of the Pannonian Plain, as well as the central and western parts of the Balkan Peninsula (Supplementary Fig. S1, Supplementary Table S1). For each population,

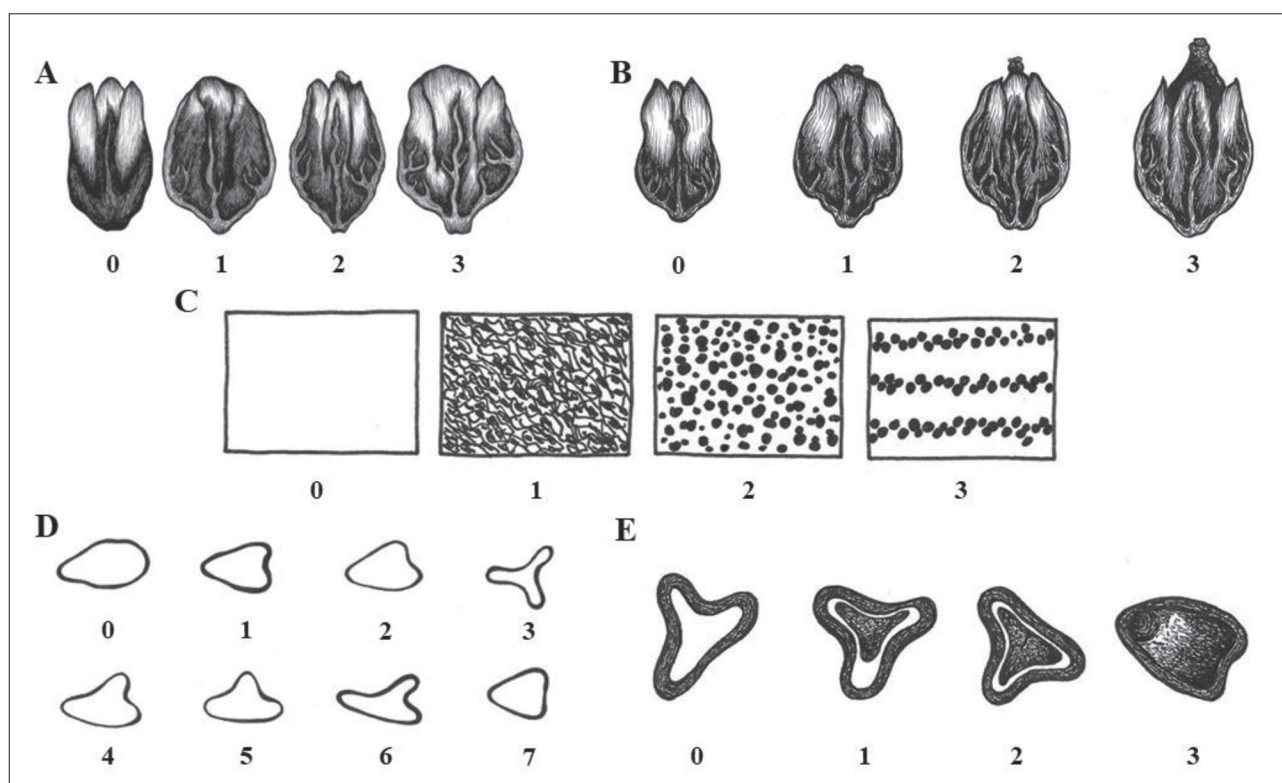


Fig. 1. The states of perianth and fruit qualitative characters pertaining to *Polygonum* representatives. **A** – perianth venation; **B** – fruit position; **C** – pericarp ornamentation; **D** – fruit cross-section shape; **E** – endosperm development. Character states are defined in Table 1.

10-30 individuals were sampled at least 0.5 m apart and subsequently dried using the standard herbarium method [37]. Plant material was determined using appropriate dichotomous keys [1,3,16,38] and deposited in the general collection of the Herbarium of the University of Novi Sad (BUNS). One additional specimen was deposited in the voucher collection of the same herbarium.

Morphological analysis of the plant material

One bract and one mature fruit with accrescent perianth per individual plant were sampled from the upper quarter of the leading stem. The selected perianth and fruit morphological characters were analyzed using a Leica M205C binocular stereomicroscope with a Leica DFC 290 HD camera, while measurements were performed using Digimizer 4.6.10 software. Prior to each measurement, images were calibrated in Digimizer using a 1 mm scale bar included in the photograph. Quantitative measurements were recorded to the nearest 0.001 mm. Qualitative morphological characters

and selected quantitative traits (perianth, segment, and tube length, and perianth width) were first examined on herbarium material. The material was then rehydrated for 24-48 hours in a 4:4:2 solution of water, ethanol, and glycerol. After rehydration, the perianth was dissected to examine individual tepal width and determine the filament dimensions. The selection of characters for analysis was based on previous research [10,12,14,39-41], as well as on personal observations. Among the quantitative characters, 3 pertained to the bracts (2 basic and 1 derived), 14 to the perianth (11 basic and 3 derived), and 6 to the fruit (5 basic and 1 derived) (Supplementary Fig. S2). Nine qualitative characters (5 for the perianth and 4 for the fruit) were also analyzed and subsequently coded. Character codes and corresponding character-state values are presented in Fig. 1 and Table 1.

Because the trigonous nut produces three unequal sides on the accrescent perianth, all perianth-specific characters were measured on its widest side. Filament dimensions were measured on filaments attached to the inner tepals, while fruit width was determined

Table 1. Coded perianth and fruit qualitative characters and their states

Character	Character code	Character state	State code
<i>Perianth</i>			
Perianth margin color	pmc	White	0
		Pink	1
		Red	2
Perianth venation	vep	Not expressed	0
		Weakly expressed	1
		Well expressed	2
		Highly expressed	3
Inner tepal widest part position	wpp I	Near tepal base	0
		Tepal middle	1
		Near tepal apex	2
Outer tepal widest part position	wpp O	Near tepal base	0
		Tepal middle	1
		Near tepal apex	2
Transitional tepal widest part position	wpp T	Near tepal base	0
		Tepal middle	1
		Near tepal apex	2
<i>Fruit</i>			
Fruit position	frp	Enclosed in the perianth	0
		Stigma residues exerted from the perianth	1
		Stigma and style residues exerted from the perianth	2
		Stigma and style residues and part of the fruit exerted from the perianth	3
Pericarp ornamentation	por	Smooth	0
		Slightly convex	1
		Diffused tubercles	2
		Furrowed tubercles	3
Fruit cross-section shape	fcs	Trait states are shown in Fig. 1D	0-7
Endosperm development	end	Undeveloped	0
		< 50% developed	1
		≥ 50% developed	2
		Fully developed	3

from all three sides of the nut. The fruit cross-section classification scheme proposed by Meerts et al. [10] was adapted for the purposes of this research. Pericarp ornamentation was assessed for all analyzed fruits (1,129 individuals) using a stereomicroscope. A JEOL JSM-6460 LV scanning electron microscope was used to document representative patterns of pericarp ornamentation. To facilitate observation, fruit specimens were gold-coated by applying a Ball-Tec SCD 005 Ion Sputter Coater for 180 s at 30 mA. For SEM imaging, two populations of each taxon were selected, and one

fruit from five individuals per population was examined in detail to provide high-resolution visualization of the observed ornamentation types.

Statistical analysis

Mean, minimum, and maximum values, as well as coefficients of variation for the analyzed characters, were calculated using Statistica for Windows ver. 14.1.0.8. Data distributions were assessed using the Shapiro-Wilk test [42]. All non-normally distributed data were subjected to the Box-Cox transformation method [43], and Bonferroni correction was applied. If the data showed normal distribution after transformation, ANOVA and Tukey's HSD post-hoc test were performed; otherwise, the Kruskal-Wallis significance test was conducted, followed by Dunn's test for multiple comparisons as a post-hoc test. The post-hoc test results are included as a superscript next to the mean value for the corresponding character.

To complement significance testing, effect sizes were calculated for all quantitative characters and reported together with P values; η^2 and ϵ^2 were used as effect-size measures for ANOVA and Kruskal-Wallis tests, respectively.

To assess the hierarchical structure of morphological variation, a nested analysis of variance was performed for the quantitative characters, with species treated as a fixed factor and population nested within species.

Principal component analysis (PCA) was conducted to evaluate the contribution of individual characters to overall sample variability and the general structure of variation in the sample. Linear discriminant analysis (LDA) was performed to assess predefined taxon groups, identify the most discriminating characters, and evaluate group separation. Derived indices were not automatically retained; instead, raw measurements and derived ratios were first screened for redundancy. Dimensionality reduction before LDA was carried out by removing highly collinear variables using a two-step screening procedure: pairwise absolute correlation filtering followed by iterative variance inflation factor (VIF) filtering. Variables with $|r| \geq 0.85$ were considered redundant, and variables with $VIF > 5$ were removed

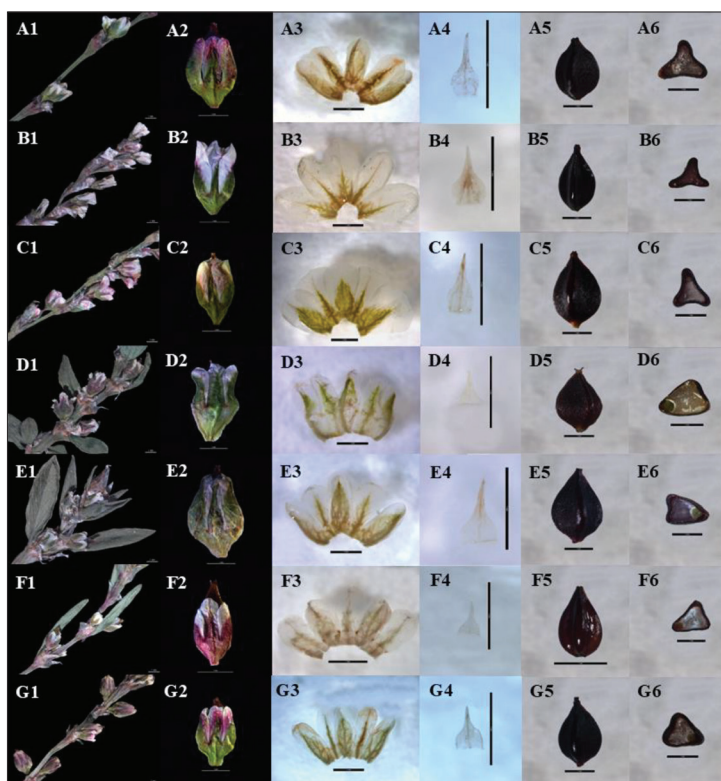


Fig. 2. Reproductive structures (A1-G1), dried perianth with fruit (A2-G2), dissected perianth (A3-G3), inner stamen filament (A4-G4), fruit (A5-G5) and the fruit cross-section shapes that occur most frequently in the analyzed section *Polygonum* taxa (A6-G6). A1-6 – *P. albanicum*, B1-6 – *P. arenarium* subsp. *arenarium*, C1-6 – *P. arenarium* subsp. *pulchellum*, D1-6 – *P. arenastrum*, E1-6 – *P. aviculare*, F1-6 – *P. graminifolium*, G1-6 – *P. patulum*. Scale bar = 1 mm.

sequentially until all retained predictors showed acceptable collinearity.

Classification performance was assessed using multiple validation procedures. For LDA, leave-one-out and repeated stratified cross-validation were performed, and confusion matrices together with class-specific sensitivity and specificity were recorded. The analyzed quantitative characters were also used to construct a decision tree [44]. A determination key for sect. *Polygonum* taxa was developed from the decision tree structure and supplemented with qualitative character data. Decision tree performance was assessed by internal validation, as no independent external dataset was available. The tree was assessed by repeated stratified cross-validation, and both overall accuracy and balanced accuracy were recorded. To assess the robustness of the tree structure, bootstrap resampling was additionally used to quantify the stability

of the primary split. Given the reliance on quantitative thresholds and the absence of external validation, the resulting key should be considered exploratory and interpreted with caution.

Correspondence analysis (CA) was applied to qualitative characters to identify the character states and combinations that best differentiate the analyzed groups. The CA results were presented in tables and graphs showing group centroids and character-state distributions along the first two correspondence axes. CA was selected because it is designed for categorical data and enables direct interpretation of associations between taxa and character states. Unlike distance-based ordination methods, such as principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS), CA operates directly on the contingency structure of categorical variables, allowing more explicit interpretation of character-state contributions to group differentiation. Therefore, CA was considered more appropriate for the aims of this study.

Data distribution testing, significance tests, post-hoc tests, and multivariate statistical analyses were performed in the RStudio ver. 2023.12.1 software package.

RESULTS

Morphology of reproductive traits

The analyzed reproductive structures comprised the upper quarter of the main stem inflorescence, including fruits with accrescent perianths and associated bracts. In most individuals of *P. albanicum*, *P. arenarium* subsp. *arenarium*, *P. arenarium* subsp. *pulchellum*, and *P. patulum*, bracts are absent or scarious and never exceed the flowers in length (Fig. 2A1-C1, 2G1). In contrast, the bracts of *P. arenastrum*, *P. aviculare*, and *P. graminifolium* are leaf-like and always extend beyond the flowers (Fig. 2D1-F1).

The perianth consists of five tepals, each comprising a central region and differently pigmented margins (Fig.

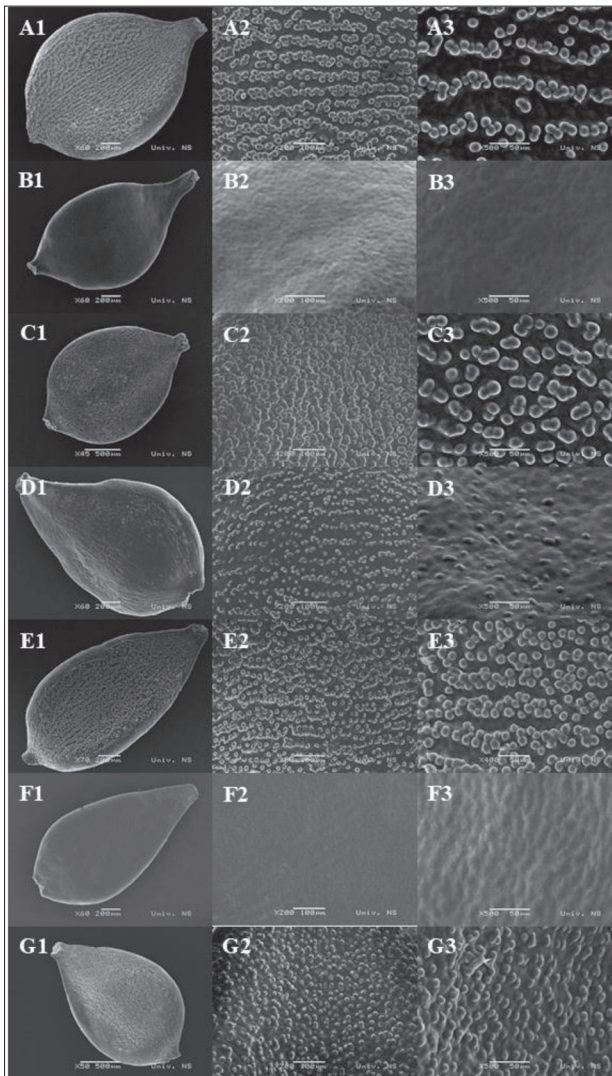


Fig. 3. Pericarp ornamentation of *Polygonum* taxa obtained by scanning electron microscopy. **A1-3** – *P. albanicum*, **B1-3** – *P. arenarium* subsp. *arenarium*, **C1-3** – *P. arenarium* subsp. *pulchellum*, **D1-3** – *P. arenastrum*, **E1-3** – *P. aviculare*, **F1-3** – *P. graminifolium*, **G1-3** – *P. patulum*.

2A2-G2, 2A3-G3). The margins are symmetrical except in the transitional tepal, where the margin overlapped by the outer tepal is wider than that covering the inner tepal. The filaments are basally expanded (Fig. 2A4-G4), with the outer filaments shorter and narrower than the inner ones. The fruit is a trigonous nut (Fig. 2A5-G5) that is either completely enclosed by the perianth (Fig. 2B2) or exerted from it to varying degrees (Fig. 2A2, 2E2-G2). Nut sides vary from concave to flat or convex, reflecting not only genetic differences but also the stage of fruit maturation at sampling, particularly the degree of embryo and endosperm development.

Table 2. Quantitative bract, perianth, and fruit characters of the analyzed sect. *Polygonum* taxa (mean, CV%, and min–max values)

Character/Taxon	P_alb (8/160)	P_arr (10/190)	P_pul (6/141)	P_art (12/239)	P_avi (13/257)	P_gra (3/82)	P_pat (2/60)	Significance test results
Bract								
1 Bract length (mm)	/	/	/	7.27 (51.3) ^C (2.62-23.56)	10.18 (39.1) ^A (4.05-31.04)	8.29 (42.6) ^B (2.35-19.04)	/	96.13 (P<0.001)
2 Bract width (mm)	/	/	/	2.82 (34.9) ^A (1.32-6.92)	2.92 (43.2) ^A (0.91-9.33)	1.09 (22.0) ^B (0.25-1.68)	/	197.61 (P<0.001)
3 Bract index ¹	/	/	/	2.52 (24.5) ^C (1.45-5.41)	3.67 (26.6) ^B (2.04-7.35)	7.57 (34.2) ^A (3.36-13.03)	/	331.17 (P<0.001)
Perianth								
4 Perianth length (mm)	2.12 (8.8) ^C (1.76-2.60)	2.30 (9.4) ^B (1.72-2.85)	2.23 (9.4) ^B (1.59-2.71)	2.46 (10.6) ^A (1.78-3.27)	2.51 (11.4) ^A (1.91-3.18)	1.84 (8.0) ^D (1.56-2.38)	2.21 (6.5) ^{BC} (1.88-2.51)	446.99 (P<0.001)
5 Perianth segment length (mm)	1.41 (11.7) ^B (1.08-2.00)	1.57 (11.7) ^A (0.90-2.04)	1.53 (10.5) ^A (1.12-1.96)	1.31 (13.0) ^C (0.82-1.80)	1.52 (12.9) ^A (1.06-2.62)	1.08 (11.1) ^D (0.87-1.43)	1.34 (11.3) ^{BC} (0.63-1.68)	429.57 (P<0.001)
6 Tube length (mm)	0.65 (10.5) ^D (0.49-0.85)	0.64 (13.1) ^{DE} (0.47-0.86)	0.62 (13.7) ^{DE} (0.42-0.85)	1.05 (12.4) ^A (0.71-1.59)	0.91 (14.9) ^B (0.52-1.27)	0.59 (13.3) ^E (0.38-0.83)	0.77 (11.4) ^C (0.63-1.30)	833.05 (P<0.001)
7 Perianth index ²	2.19 (13.3) ^B (1.46-3.15)	2.49 (13.4) ^A (1.65-3.45)	2.50 (14.5) ^A (1.62-3.49)	1.26 (14.2) ^E (0.77-2.07)	1.69 (13.7) ^D (1.26-2.65)	1.88 (17.5) ^C (1.37-3.04)	1.79 (9.25) ^{CD} (1.49-2.19)	873.40 (P<0.001)
8 Tube % ³	30.72 (8.8) ^C (26.00-38.93)	27.80 (8.5) ^D (21.29-33.73)	27.76 (10.1) ^D (20.67-37.41)	42.83 (7.6) ^A (32.78-55.31)	36.20 (8.1) ^B (25.56-43.80)	31.86 (12.0) ^C (22.65-41.09)	34.38 (6.26) ^B (30.42-39.10)	881.53 (P<0.001)

Table 2. continued

9 Perianth width 1 (mm)	1.52 (10.6) ^B (0.76-1.98)	1.25 (10.6) ^D (0.96-1.67)	1.37 (12.6) ^C (0.69-1.74)	1.59 (12.4) ^B (0.90-2.11)	1.72 (12.7) ^A (0.80-2.27)	1.23 (12.6) ^D (0.92-1.61)	1.38 (8.5) ^C (1.13-1.68)	593.14 (P<0.001)
10 Perianth width 2 (mm)	1.35 (12.6) ^B (0.76-1.79)	1.07 (10.8) ^E (0.75-1.49)	1.16 (12.7) ^{CD} (0.52-1.52)	1.57 (12.1) ^A (0.81-2.11)	1.63 (14.0) ^A (0.78-2.16)	1.12 (11.8) ^{DE} (0.86-1.43)	1.26 (9.4) ^{BC} (1.07-1.57)	746.45 (P<0.001)
11 Perianth width 3 (mm)	0.47 (17.4) ^{CD} (0.21-0.61)	0.36 (17.4) ^F (0.20-0.69)	0.38 (16.6) ^{EF} (0.19-0.55)	0.69 (16.2) ^A (0.36-1.07)	0.60 (19.7) ^B (0.25-1.01)	0.48 (18.5) ^C (0.29-0.70)	0.41 (13.0) ^{DE} (0.32-0.56)	754.34 (P<0.001)
12 I width (mm)	0.90 (13.1) ^{BC} (0.66-1.38)	1.24 (12.7) ^A (0.83-1.72)	1.25 (11.9) ^A (0.96-1.79)	0.88 (14.1) ^C (0.54-1.34)	0.93 (13.3) ^B (0.64-1.34)	0.75 (10.7) ^D (0.47-0.95)	0.72 (7.0) ^D (0.63-0.87)	732.16 (P<0.001)
13 O width (mm)	0.97 (11.3) ^C (0.70-1.36)	1.22 (12.1) ^A (0.69-1.64)	1.23 (11.1) ^A (0.92-1.71)	0.94 (13.6) ^{CD} (0.67-1.41)	1.07 (12.5) ^B (0.78-1.53)	0.87 (11.0) ^E (0.51-1.09)	0.89 (8.3) ^{DE} (0.68-1.10)	577.44 (P<0.001)
14 T width (mm)	1.05 (12.1) ^B (0.78-1.37)	1.41 (12.7) ^A (0.79-1.95)	1.41 (11.2) ^A (1.09-1.96)	0.95 (12.5) ^C (0.67-1.33)	1.08 (12.6) ^B (0.72-1.54)	0.96 (10.8) ^C (0.58-1.12)	0.91 (8.1) ^C (0.65-1.04)	710.45 (P<0.001)
15 Filament length (mm)	0.64 (12.8) ^B (0.45-0.87)	0.73 (12.5) ^A (0.47-0.98)	0.74 (13.7) ^A (0.48-1.02)	0.58 (17.3) ^C (0.32-0.95)	0.64 (14.5) ^B (0.40-0.89)	0.48 (15.0) ^D (0.32-0.75)	0.59 (8.8) ^C (0.49-0.76)	432.50 (P<0.001)
16 Filament base width (mm)	0.35 (13.9) ^{DE} (0.22-0.52)	0.36 (14.9) ^D (0.26-0.53)	0.39 (15.7) ^C (0.22-0.58)	0.51 (17.3) ^A (0.27-0.84)	0.45 (15.3) ^B (0.27-0.66)	0.33 (14.2) ^{EF} (0.24-0.49)	0.31 (10.9) ^F (0.23-0.42)	591.58 (P<0.001)
17 Filament index ⁴	1.84 (14.7) ^B (1.36-3.29)	2.05 (18.6) ^A (1.28-3.34)	1.92 (19.3) ^{AB} (1.17-3.10)	1.17 (19.4) ^D (0.59-2.06)	1.44 (13.9) ^C (0.89-2.26)	1.46 (16.2) ^C (1.03-2.21)	1.89 (12.0) ^{AB} (1.45-2.73)	714.67 (P<0.001)
Fruit								
18 Fruit length (mm)	2.10 (7.4) ^C (1.23-2.41)	2.03 (9.8) ^D (1.20-2.63)	2.04 (8.7) ^{CD} (1.58-2.49)	2.30 (9.9) ^B (1.75-2.99)	2.45 (9.4) ^A (1.78-2.98)	2.07 (10.2) ^{CD} (1.03-2.40)	2.07 (5.4) ^{CD} (1.82-2.28)	458.74 (P<0.001)
19 Height of the widest fruit part (mm)	0.82 (11.7) ^{BC} (0.54-1.14)	0.79 (12.4) ^C (0.58-1.01)	0.81 (12.3) ^{BC} (0.53-1.10)	0.84 (16.6) ^B (0.45-1.35)	0.93 (12.9) ^A (0.59-1.23)	0.66 (16.3) ^E (0.32-0.85)	0.75 (9.0) ^{CD} (0.61-0.91)	75.12 (P<0.001)
20 Fruit width 1 (mm)	1.23 (10.1) ^A (0.96-1.51)	1.04 (9.7) ^B (0.86-1.44)	1.18 (10.0) ^A (0.88-1.47)	0.85 (18.0) ^D (0.41-1.33)	1.07 (14.4) ^B (0.61-1.52)	0.93 (17.0) ^C (0.56-1.28)	1.07 (10.4) ^B (0.83-1.27)	534.08 (P<0.001)
21 Fruit width 2 (mm)	1.35 (8.5) ^B (1.09-1.68)	1.13 (10.7) ^D (0.91-1.91)	1.28 (11.5) ^C (0.96-2.24)	1.31 (11.8) ^C (0.86-1.72)	1.44 (11.8) ^A (0.83-1.87)	1.11 (12.9) ^D (0.65-1.38)	1.17 (9.5) ^D (0.94-1.40)	472.59 (P<0.001)
22 Fruit width 3 (mm)	1.46 (8.8) ^B (1.12-1.86)	1.22 (9.6) ^D (0.96-1.61)	1.37 (9.8) ^C (1.01-1.71)	1.46 (10.7) ^B (0.99-2.20)	1.60 (11.3) ^A (1.04-2.01)	1.22 (12.4) ^D (0.75-1.49)	1.30 (8.3) ^{DC} (1.05-1.49)	544.35 (P<0.001)
23 Fruit index ⁵	1.44 (7.2) ^E (0.77-1.73)	1.67 (9.4) ^{AB} (1.06-2.17)	1.49 (8.5) ^D (1.21-1.87)	1.58 (9.9) ^C (1.00-2.38)	1.54 (7.4) ^D (1.28-2.08)	1.71 (8.3) ^A (1.38-2.03)	1.60 (7.8) ^{BC} (1.35-1.94)	322.03 (P<0.001)

Number of population/Number of individuals; Character measurements performed on dried material; Character measurements performed on rehydrated material; Abbreviations: I – inner tepal, O – outer tepal, T – transitional tepal, Bract length/Bract width; ²Perianth segment length/Tube length; ³Tube length as a percentage of the total perianth length; ⁴Filament length/Filament base width; ⁵Fruit length/Fruit width 3. The values marked with different letters in the superscript are statistically significantly different at the P≤0.05 level, according to the *post-hoc* test results. Perianth width 1 was measured at the widest part of the perianth, Perianth width 2 at the point where the tube transitions into the perianth segments, while Perianth width 3 represents the distance between the tepals at the transition of the perianth tube into the perianth segments. The bolded values of significance test result indicate the ANOVA test results, while the others indicate the results of the Kruskal-Wallis test. Abbreviations: P_alb – *P. albaticum*, P_arr – *P. arenarium* subsp. *arenarium*, P_art – *P. arenastrum*, P_avi – *P. aviculare*, P_gra – *P. graminifolium*, P_pat – *P. patulum*, P_pul – *P. arenarium* subsp. *pulchellum*.

Table 3. Loadings (%) of perianth and fruit morphological characters on the first two principal components and canonical structure coefficients of quantitative characters on the first two discriminant functions in sect. *Polygonum*

Characters	PCA 1	PCA 2	LD 1	LD 2
Perianth length	9.6840	0.9728	-	-
Perianth segment length	2.8773	11.7956	0.3452	-0.2657
Tube length	7.4690	3.9107	-0.8087	-0.3875
Perianth width 1	11.6478	0.3843	-	-
Perianth width 2	11.4245	2.1576	-	-
Perianth width 3	7.7186	6.0490	-0.8139	-0.1384
Inner tepal width	0.0985	17.3323	0.6654	-0.5759
Outer tepal width	0.6646	17.0005	0.5469	-0.4403
Transitional tepal width	0.0028	19.5780	-	-
Filament length	0.6060	10.7555	0.4933	-0.3014
Filament base width	7.1208	0.5944	-0.5483	-0.4957
Fruit length	10.1109	0.2074	-0.5172	-0.0571
Fruit width 1	0.7204	8.0388	0.4745	0.4350
Fruit width 2	9.9302	0.1393	-0.3177	0.1364
Fruit width 3	11.5977	0.0011	-	-
Height of the widest fruit part	8.3270	1.0825	-0.1883	-0.1233
Axis contribution	43.9%	27.3%	72.9%	15.6%

Note: Values in bold in the PCA columns represent characters contributing most strongly to total sample variability. Values in bold in the LD columns represent the strongest associations with a discriminant axis. Dashes indicate characters excluded before the final LDA after correlation and VIF screening.

Eight fruit cross-sectional shapes were distinguished based on observations at the point of greatest width (Fig. 1D). Although nearly all shapes were recorded in each analyzed taxon, one or two predominated (Fig. 2A6-G6). The pericarp surface is smooth in *P. arenarium* subsp. *arenarium* and *P. graminifolium*, slightly convex in *P. arenastrum*, or tuberculate, with tubercles either diffusely distributed or arranged in furrows in *P. albanicum*, *P. arenarium* subsp. *pulchellum*, *P. arenastrum*, *P. aviculare*, and *P. patulum* (Fig. 3).

Morphometry of reproductive traits

Bract traits were measured only in *P. arenastrum*, *P. aviculare*, and *P. graminifolium*, the only taxa with bracts extending beyond the flowers. Among these traits, bract length showed the greatest interspecific differences and high variability, with coefficients of variation ranging from 39.1% to 51.3% (Table 2). The average perianth length of the examined taxa ranged from 1.84 to 2.51 mm (Table 2). The lowest values for this character were recorded in *P. graminifolium* and the highest in *P. aviculare*, while the coefficients of variation in most taxa were below 10%. Perianth segment

and tube lengths showed low variability, with coefficients of variation of 10-15%, slightly higher than that of overall perianth length. The perianth index, expressing the ratio of segment to tube length, was lowest in *P. arenastrum* (slightly above 1), whereas values for the remaining taxa ranged from 1.69 to 2.50. The proportion of tube length relative to total perianth length was also calculated. In *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum*, the tube comprises about one-quarter of the total perianth length; in *P. albanicum*, *P. aviculare*, *P. patulum*, and *P. graminifolium*, about one-third; and in *P. arenastrum*, about one-half. *P. arenarium* subsp. *arenarium* and *P. graminifolium* have the narrowest perianths, whereas *P. aviculare* has the widest. The smallest distance between tepals at the point where the tube transitions into the perianth segment was recorded in *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum*, and the largest in *P. arenastrum*. Characters related to perianth width showed moderate variation (8.5-19.7%).

The widths of individual perianth tepals were also measured (Table 2). The transitional tepals are generally the widest, followed by the outer ones, while the inner tepals are the narrowest, with the exception of *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum*, where the inner tepals are equal in width to the outer ones. Tepal widths showed coefficients of variation ranging from 7.0% to 14.1%. The inner filament dimensions differ among taxa. The shortest filaments were recorded in *P. graminifolium*, while *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum* are characterized by the longest filaments. The narrowest filaments were measured in *P. patulum*, and the widest in *P. arenastrum*.

In most taxa, fruit length ranged within the 2-2.10 mm range (Table 2), while *P. arenastrum* (2.3 mm) and *P. aviculare* (2.45 mm) have somewhat longer fruits. This character exhibits a low coefficient of variation (<10%). Unlike fruit length, fruit width varied more among taxa, and differences among the widths of individual fruit sides were also observed, typically not exceeding 0.1 mm. *P. arenastrum*, *P. aviculare*, and *P. graminifolium* deviate from this pattern, as one side of their fruits is significantly narrower than the other

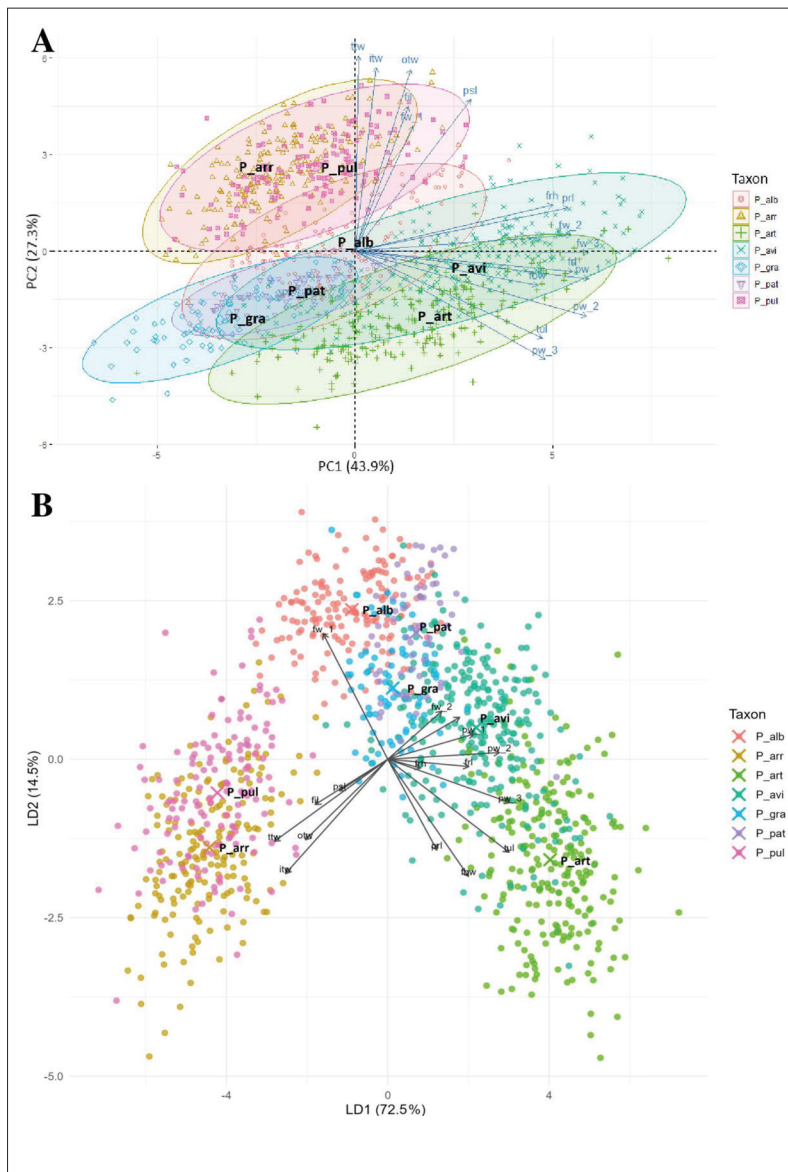


Fig 4. Position of specimens of *Polygonum* taxa in the space bound by the first two principal component (A) and discriminant axes (B). Abbreviations: P_alb – *P. albanicum*, P_arr – *P. arenarium* subsp. *arenarium*, P_art – *P. arenastrum*, P_avi – *P. aviculare*, P_gra – *P. graminifolium*, P_pat – *P. patulum*, P_pul – *P. arenarium* subsp. *pulchellum*.

two. In *P. graminifolium*, the widest part of the fruit is located nearest the carpopodial zone, whereas in *P. arenarium* subsp. *pulchellum* it is nearest the stylopodial zone. The lowest length-to-width ratio (fruit index) was recorded in *P. albanicum*, while *P. graminifolium* had the most elongated fruits.

Nested analysis of variance

Across all quantitative characters, 51.51% of the total variance was attributable to differences among species, 15.49% to differences among populations within species, and 33.00% to within-population variation. Species-level differentiation thus accounted for the largest proportion of morphological variation, although substantial variation was also observed among populations and within populations.

Principal component analysis

The first principal component is primarily associated with perianth widths, perianth and tube length, filament base width, fruit length and widths, and the position of the fruit's widest part. The second principal component is mainly associated with tepal widths, perianth segment length, filament length, and fruit width 1 (Table 3). Together, the first two principal components explain 71.2% of the total variance.

Along the first two principal component axes, *P. graminifolium* occupies the negative region of the second axis, whereas *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum* cluster in the positive region (Fig. 4A).

Linear discriminant analysis

The taxa were primarily distinguished by perianth tube length, perianth width 3, inner and outer tepal widths, filament base width, and fruit length (Table 3). The analysis was further supported by three complementary approaches. First, effect-size estimates showed that several quantitative traits had not only highly significant P values but also strong discriminatory power, particularly tube length, transitional and inner tepal widths, and perianth widths 2 and 3 (Supplementary Table S2). Reporting effect sizes alongside P values showed that several quantitative characters were not

only statistically significant but also strongly discriminatory. For example, tube length showed $\eta^2 = 0.7356$ and $\epsilon^2 = 0.7371$, while perianth width 3 showed $\eta^2 = 0.6375$ and $\epsilon^2 = 0.6670$ (Supplementary Table S2). Thus, the revised presentation distinguishes traits with large biological separation from those that are only statistically significant because of sample size. Second, multicollinearity screening reduced the initial set of 16 quantitative predictors to 11 non-redundant variables suitable for LDA. Highly redundant variables, including perianth width 2, fruit width 3, and transitional tepal width, were excluded during correlation screening, whereas perianth length and perianth width 1 were removed during VIF filtering (Supplementary Table S3). The final predictor set comprised perianth segment length, tube length, perianth width 3, inner and outer tepal widths, filament base width, filament length, fruit length, the position of the fruit's widest part, and fruit widths 1 and 2 (Supplementary Table S4). The first two discriminant functions explained 88.5% of the discriminatory variation. All discriminant functions were statistically significant according to Wilks' lambda test ($P < 0.001$) (Supplementary Table S5). Classifier performance remained consistent after validation. LDA yielded an apparent accuracy of 0.8733, a leave-one-out cross-validated accuracy of 0.8663, and a repeated stratified cross-validation accuracy of 0.8642 ± 0.0174 , with a mean balanced accuracy of 0.8693 ± 0.0190 (Supplementary Table S6). The confusion matrix shows overall strong discrimination among taxa, but also identifies the principal areas of overlap, especially between *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum* and *P. arenastrum* and *P. aviculare* (Supplementary Fig. S3).

The distribution of sect. *Polygonum* individuals along the first two discriminant axes shows *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum* clustered in the negative region of the first axis (Fig. 4B). These taxa are characterized by wider transitional and inner tepals and longer filaments than the remaining taxa. In contrast, *P. arenastrum* and *P. aviculare* occupy the positive region of the same axis and are distinguished by a longer perianth tube and wider perianths and filaments. The remaining taxa are grouped mainly in the positive region of the second axis, reflecting their narrower inner tepals and filaments.

Decision tree analysis

The decision tree showed moderate but consistent classification performance under repeated stratified cross-validation, with a mean accuracy of 0.7388 ± 0.0268 and a mean balanced accuracy of 0.7276 ± 0.0329 (Supplementary Fig. S4). Bootstrap analysis demonstrated high stability of the primary split, with tube length retained as the root split in all 200 bootstrap resamples. However, the tree performed less well than LDA, and substantial overlap among taxa remained, indicating limited discriminatory power despite a stable primary pattern in the data. Cross-validation showed that LDA retained high predictive performance, whereas the decision tree was less accurate despite its structural stability, indicating that some characters contribute more reliably to classification than others.

Qualitative character analysis

Analysis of qualitative character states revealed that almost all characters occurred in all recorded states, albeit at different frequencies, indicating their instability (Supplementary Table S7). Nonetheless, some characters are stable in certain taxa, such as margin color in *P. arenastrum* and *P. graminifolium* (white margins) and pericarp ornamentation in *P. arenarium* subsp. *arenarium* and *P. graminifolium* (smooth pericarp), as well as in *P. arenarium* subsp. *pulchellum* and *P. patulum* (diffusely distributed tubercles on pericarp surface).

Correspondence analysis

The centroid positions allow for a low separation of the examined taxa. The taxa are distributed in each of the quadrants of the area bounded by the first two correspondence axes, and the characters with which the grouped taxa correspond well are organized accordingly (Fig. 5). *P. arenastrum* stands out in the positive part of the first correspondence axis, and the characters that distinguish it and separate it from other taxa are the position of the widest part of the inner tepal (widest in the first third of the tepal), perianth margin color (which is typically white), weakly expressed perianth venation, and nut with one narrower concave and two wider convex sides. The second group comprises *P. albanicum* and *P. aviculare*, characterized by an

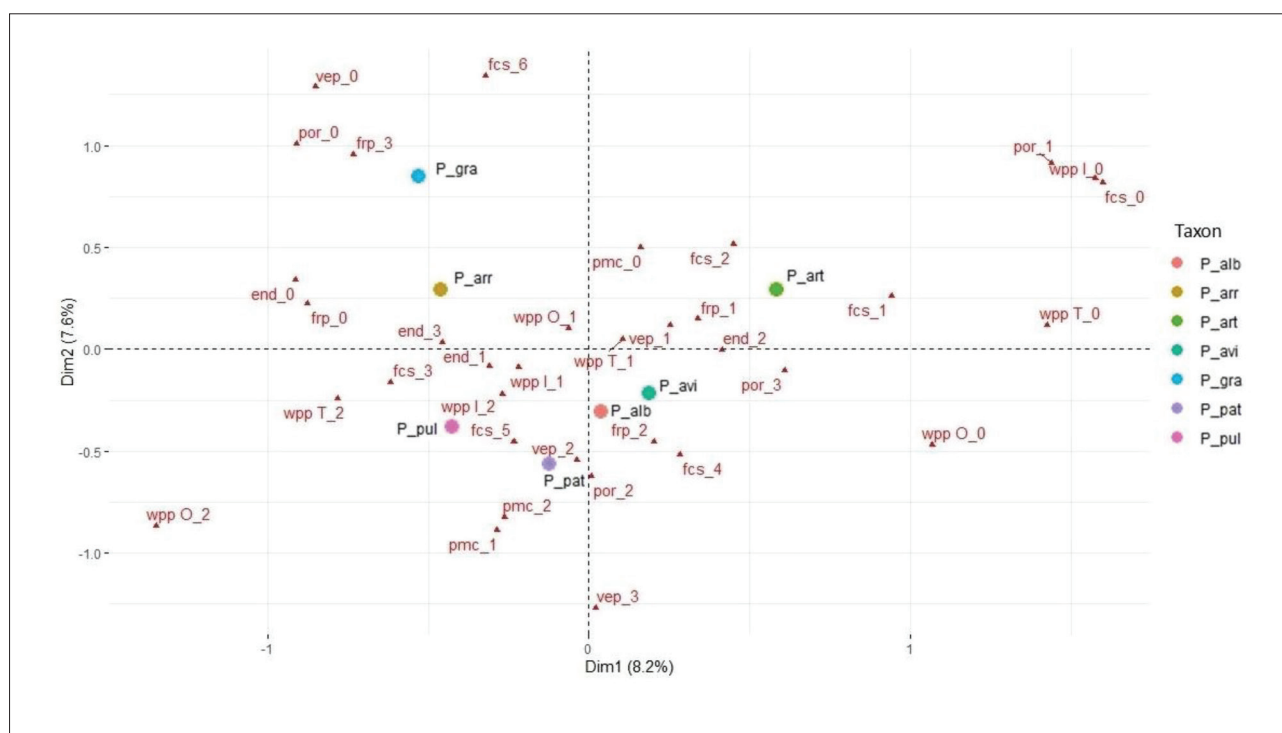


Fig. 5. State of perianth and fruit morphological and micromorphological characters with the centroid position of the analyzed section *Polygonum* taxa in the space bounded by the first two correspondence axes. Abbreviations: pmc – perianth margin color, vep – perianth venation, por – pericarp ornamentation, fcs – fruit cross-section shape, wpp – tepal widest part position, frp – fruit position, end – endosperm development, I – inner tepal, O – outer tepal, T – transitional tepal. *P.*_{alb} – *P. albanicum*, *P.*_{arr} – *P. arenarium* subsp. *arenarium*, *P.*_{art} – *P. arenastrum*, *P.*_{avi} – *P. aviculare*, *P.*_{gra} – *P. graminifolium*, *P.*_{pat} – *P. patulum*, *P.*_{pul} – *P. arenarium* subsp. *pulchellum*. The states of individual characters are shown in Table 1.

ornamented pericarp and fruits with exerted style and stigma remnants. *P. arenarium* subsp. *arenarium* and *P. graminifolium* occupy the positive region of the second correspondence axis and share a smooth pericarp and usually weakly expressed perianth venation. *P. arenarium* subsp. *pulchellum* and *P. patulum* are located in the negative region of both axes and are characterized by predominantly pink perianth margins, inner tepals widest in the middle or upper third, and diffusely distributed pericarp tubercles. Overall, discrimination among taxa based on the first two correspondence axes was weak, as they explained only 15.8% of the total variation (8.2% and 7.6%, respectively; Supplementary Table S8).

Determination key for annual taxa of sect. *Polygonum* taxa (*Polygonum* s. str.) from the Balkan peninsula and the Pannonian and the Wallachian plains based on the bract, perianth, and fruit morphological characters

A dichotomous key based on bract, perianth, and fruit morphology was developed from the decision tree results (Supplementary Fig. S4) and supplemented with the qualitative characters analyzed in this study. Although most qualitative characters showed substantial variability among taxa, certain character states proved relatively stable and were selectively incorporated into the key when supported by consistent frequency patterns.

1a Perianth tube length < 0.8 mm	2
1b Perianth tube length ≥ 0.8 mm	11
2a Inner tepal width < 1 mm	3
2b Inner tepal width ≥ 1 mm	8
3a Perianth segment length < 1.1 mm	<i>P. graminifolium</i>
3b Perianth segment length ≥ 1.1 mm	4
4a Inner tepal width < 0.8 mm	5
4b Inner tepal width ≥ 0.8 mm	6
5a Tepal margins pink, pericarp with diffusely distributed tubercules, bracts in the upper quarter of the leading stem do not exceed the flower length or absent	<i>P. patulum</i>
5b Tepal margins white, pericarp smooth, bracts in the upper quarter of the leading stem significantly exceed the flower length	<i>P. graminifolium</i>
6a Width of the narrowest fruit side < 1.1 mm	7
6b Width of the narrowest fruit side ≥ 1.1 mm	<i>P. albanicum</i>
7a Pericarp smooth	<i>P. graminifolium</i>
7b Pericarp with diffusely distributed tubercules, or with furrowed tubercules	<i>P. aviculare</i>
8a Width of the narrowest fruit side < 1 mm	<i>P. arenarium</i> subsp. <i>arenarium</i>
8b Width of the narrowest fruit side ≥ 1 mm	9
9a Perianth width at the point where the perianth tube transitions into segments < 1.4 mm, pericarp with diffusely distributed tubercules or smooth	10
9b Perianth width at the point where the perianth tube transitions into segments ≥ 1.4 mm, pericarp with furrowed tubercules	<i>P. albanicum</i>

10a Pericarp smooth	<i>P. arenarium</i> subsp. <i>arenarium</i>
10b Pericarp with diffusely distributed tubercules	<i>P. arenarium</i> subsp. <i>pulchellum</i>
11a Width of the narrowest fruit side < 1 mm	12
11b Width of the narrowest fruit side ≥ 1 mm	<i>P. aviculare</i>
12a Bracts 2-3 times longer than wide	<i>P. arenastrum</i>
12b Bract more than 3 times as long as wide	<i>P. aviculare</i>

DISCUSSION

This study examined morphological variations in the reproductive traits of selected representatives of sect. *Polygonum* from the Balkan Peninsula and the Pannonian and Wallachian plains using comparative analysis and multivariate statistical methods.

The examined taxa varied in morphological distinctness, with some readily distinguishable and others showing substantial overlap in diagnostic characters. Accordingly, the discussion focuses on the taxa that proved most difficult to discriminate morphologically.

Due to their lower plasticity, reproductive traits are generally considered more informative than vegetative characters in flowering plant taxonomy [45]. Because vegetative characters in sect. *Polygonum* have been extensively studied [10,12,14,21,40,41] and are known to exhibit high phenotypic plasticity [12,13,20,46-48], this study focused on reproductive structures (perianth, fruit, and bracts) with previously demonstrated taxonomic value within the section [10,12,14,20,39,41].

Perianth length, together with perianth segment and tube length and fruit dimensions, are among the most frequently studied quantitative characters in this group, particularly in *P. aviculare* and *P. arenastrum* [10-14,20,21,32,39-41]. In contrast, perianth and tepal widths and filament dimensions have received little attention and were therefore included here to assess their additional discriminatory value.

In most taxa examined in this study, except *P. aviculare*, perianth length was broadly consistent with previous reports [1,3,16]. In *P. aviculare*, the values recorded here were lower than those reported from more northerly regions, including former Czechoslovakia [11], Great Britain [12], and Germany [14], partly supporting the latitudinal trend proposed by Scholz [49]. However, measurements from Russia [21,34,40,41], despite its substantially higher latitude, closely matched our results, suggesting that this pattern is not consistent across all regions.

More information is available on the relative proportions of the perianth segments and tube than on their absolute dimensions [1,10,12-14,16,17]. Our results generally agree with previous findings [3,7,16], although some discrepancies were observed, particularly in *P. arenastrum*. In this taxon, the perianth tube accounted for a larger proportion of total perianth length than in the other taxa examined, averaging about 43% rather than 50%. The observed difference likely reflects less precise or inconsistent estimation of tube and segment lengths in previous studies of *P. arenastrum*, or differences in the definition of measurement boundaries between perianth parts. This interpretation is supported by studies in which tube and segment lengths were measured separately [39,41], yielding a ratio of approximately 45:55. It should also be noted that the relationship between perianth parts was analyzed using two approaches: the perianth index, which is more commonly reported in the literature, and percentage contribution of the tube to the total perianth length, which provides a more intuitive and visually interpretable assessment of perianth proportions.

Most of the measured fruit characters agreed with previous literature reports. However, some differences were observed in fruit width in *P. arenarium* subsp. *arenarium*, where the values obtained in the present study (1.2 mm) were lower than those reported in earlier studies (up to 1.6 mm) [3], as well as in *P. aviculare*, where higher values have also been reported in some investigations [11,12,14]. Newly analyzed traits (perianth width, tepal width, filament dimensions, and the height of the widest part of the fruit) have not been widely studied, but they did not substantially improve discrimination among taxa.

Qualitative perianth and fruit characters have often been regarded as taxonomically informative [50-54]

and have been widely studied within Polygonaceae and *Polygonum* [10-12,14,27,32-34]. These studies consistently emphasized their taxonomic importance. In the present study, all analyzed qualitative perianth and fruit characters, except pericarp ornamentation and margin color in certain taxa, showed limited diagnostic value due to their high variability and the occurrence of multiple character states within most taxa.

With the exception of *P. graminifolium*, which is characterized by tepals with white margins, tepal margin color varied both among and within taxa. As noted by Schmid [14], margin color varies among flowers on the same individual and depends on insolation, indicating an environmental influence on this trait. Changes during fruit development further reduce the taxonomic reliability of this character, which can vary relative to the perianth even within the same individual [12,14].

Although pericarp ornamentation appeared relatively stable in our study, Yurtseva [34] reported that it varies with fruit ripening stage and environmental conditions. Moreover, similar ornamentation types may occur in different taxa, further limiting its diagnostic value.

Fruit cross-section shape may also be influenced by environmental factors such as trampling intensity and habitat type, although this aspect has received little attention in the literature. The diversity of reported shapes may partly reflect the subjective nature of their assessment [10]. The fruit ripening stage should also be considered; in the present study, this factor was addressed by evaluating endosperm development. Our results suggest that the degree of endosperm development can be related to the fruit cross-section shape in *P. graminifolium* only, while in the remaining taxa, species-specific characteristics with a genetic basis may represent the main contributing factor. Taken together with previous studies, fruit cross-section shape appears to be a highly variable character at the taxon level and is therefore of limited taxonomic value.

The nested analysis of variance indicated that the largest proportion of total morphological variation was attributable to differences among species. Smaller proportions were associated with differences among populations within species and among individuals within populations, likely reflecting environmental heterogeneity, including variation in trampling and grazing intensity.

Because this study was based on field-collected material, the relative contributions of environmental and genetic factors to the observed morphological variation could not be assessed directly. Controlled cultivation experiments and molecular data are therefore needed to distinguish environmentally induced plasticity from genetically based differentiation.

LDA of quantitative perianth and fruit characters achieved a relatively high classification success but did not completely separate the examined taxa. Two groups of morphologically similar taxa were consistently identified: *P. arenarium* subsp. *arenarium* with *P. arenarium* subsp. *pulchellum*, and *P. arenastrum* with *P. aviculare*, whereas the remaining taxa occupied intermediate positions. Principal component analysis revealed a similar pattern with even greater overlap among individuals, while correspondence analysis of qualitative characters produced inconsistent groupings.

Another way to reduce redundancy is to combine correlated measurements into biologically interpretable composite indices. In this study, however, we prioritized transparent screening of raw and derived characters before classification, allowing direct validation of the retained predictor set. Nevertheless, composite indices may serve as useful complementary descriptors, particularly when summarizing coherent aspects of organ shape or proportion. Potential candidates include indices describing perianth proportions, tepal-width balance, or fruit shape relationships. However, such indices should be retained only when they remain morphologically interpretable and improve parsimony without obscuring diagnostic variation.

P. arenastrum and *P. aviculare* belong to a broader aggregate that also includes *P. rurivagum* and *P. boreale* [1,16]. The taxonomic complexity of this aggregate is reflected in the large number of synonyms reported for its taxa [55]. Consistent with this, our results revealed substantial overlap among the analyzed taxa, indicating that morphology alone is insufficient to resolve relationships within the group and that additional data types and broader sampling are needed.

The observed morphological overlap among some taxa may reflect several non-exclusive processes, including phenotypic plasticity, retention of ancestral character states, or gene flow among closely related taxa. However, because this study was based solely

on morphological data, no conclusions regarding hybridization can be drawn; molecular and reproductive studies are needed to evaluate this possibility.

An even greater overlap in the LDA results was observed between *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum*. Although several characters differed significantly, the differences were small in magnitude, and substantial overlap in qualitative characters further limited reliable distinction between the two taxa.

The two subspecies also differ in habitat and geographic distribution. *P. arenarium* subsp. *arenarium* occurs in sandy habitats of northern Serbia, central Hungary, and along the Danube in Serbia and Romania, whereas *P. arenarium* subsp. *pulchellum* occupies geographically isolated populations on saline and loamy soils in southeastern Serbia and North Macedonia. The two subspecies also differ in general habit, with *P. arenarium* subsp. *arenarium* typically being more robust, more branched, and larger than *P. arenarium* subsp. *pulchellum*. However, these differences are likely environmentally influenced and therefore do not provide clear taxonomic boundaries.

Dichotomous keys for the genus *Polygonum* based on morphological characters are a standard component of numerous publications [1,3,16,38]. The key presented here incorporates both commonly used and newly analyzed characters, such as perianth and tepal width, and the width of the narrowest fruit side. On the other hand, qualitative characters such as tepal overlap, fruit cross-section shape, and fruit position, which were frequently used in the past, were not considered when constructing the key presented in this paper due to their apparent instability. Many determination keys include vegetative characters, which may provide additional discriminatory information. However, numerous studies have shown that many vegetative traits are highly variable [12,13,47-48]. In addition, herbarium specimens often lack leaves and ochreas because they are damaged or were not collected, whereas flowers and fruits are more commonly preserved. For these reasons, the proposed key focuses primarily on floral and fruit characters. Given the observed variability and overlap among taxa, it should be regarded as an exploratory rather than definitive identification tool. Nevertheless, the study identifies several reproductive characters with potential taxonomic value and provides

a morphometric framework for future integrative research within this complex group.

CONCLUSIONS

Based on quantitative and qualitative analyses of reproductive characters in seven annual taxa of sect. *Polygonum* sampled from 54 populations across the Balkan Peninsula, the Pannonian Plain, and the Wallachian Plain, complete morphological separation of the investigated taxa was not achieved. The greatest overlap was observed between *P. aviculare* and *P. arenastrum*, and between *P. arenarium* subsp. *arenarium* and *P. arenarium* subsp. *pulchellum*. Several traditionally used characters, as well as newly analyzed traits such as tepal width and filament dimensions, provided only limited taxonomic resolution. These findings indicate that reproductive morphology alone is insufficient for robust taxon delimitation within this group and suggest an important role for phenotypic plasticity and environmental variation in shaping morphological diversity. Future studies integrating molecular phylogenetic data, population-level morphometrics, and controlled cultivation experiments are needed to distinguish genetically based differentiation from environmentally induced variation and to achieve a more robust taxonomic framework for sect. *Polygonum*.

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Data availability: The data supporting this article are available in the online dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Obradov%20et%20al_Dataset.xlsx

REFERENCES

1. Akeroyd JR. Docks and knotweeds of Britain and Ireland. 2nd ed. London: Botanical society of Britain and Ireland; 2014. 258 p.
2. Galasso G, Banfi E, De Mattia F, Grassi F, Sgorbati S, Labra M. Molecular phylogeny of *Polygonum* L. s.l. (Polygonaceae), focusing on European taxa: preliminary results and systematic considerations based on rbcL plastidial sequence data. *Atti Soc Ital Sci Nat Mus Civ Stor Nat Milano*. 2009;150(1):113-48.
3. Anačkov G, Boža P, Jovanović S. 4. *Polygonum* L. In: Stevanović V, Niketić M, editors. *Flora Srbije 3*. Beograd: Srpska akademija nauka i umetnosti; 2022. p. 176-87.
4. Ronse De Craene LP, Hong S-P, Smets EF. What is the taxonomic status of *Polygonella*? Evidence of floral morphology. *Ann Mo Bot Gard*. 2004;91:320-45.
5. Stešević D, Caković D. *Katalog Vaskularne Flore Crne Gore 1*. Podgorica: Crnogorska akademija nauka i umjetnosti; 2013. 363 p.
6. Király G. II. *Polygonum* L. s.str. – Porcsinkeserűfű. In: Király G, editor. *Új Magyar fűvészkönyv. Magyarország hajtásos növényei. Határozókulcsok. Jósvárfő: Aggteleki Nezetmeti Park Igazgatóság; 2009. p. 111.*
7. Micevski K. 1. *Polygonum* L. In: Lozanovski R, editor. *Flora na Republika Makedonija 3*. Skopje: Makedonska akademija na naukite i umetnostite; 1995. p. 438-49.
8. Stupar V, Milanović Đ, Brujić J. *Vaskularna Flora Republike Srpske*. Banja Luka: Šumarski fakultet Univerziteta u Banja Luci; 2021. 499 p.
9. Oprea A. *Lista Critică a Plantelor Vasculare din România. Iași: Editura Universității “Alexandru Ioan Cuza”; 2005. 668 P.*
10. Meerts P, Briane J-P, Lefévre C. A numerical taxonomic study of the *Polygonum aviculare* complex (Polygonaceae) in Belgium. *Plant Syst Evol*. 1990;173:71-89. <https://doi.org/10.1007/BF00937764>
11. Chrtek J. Proměnlivost druhu *Polygonum aviculare* L. v ČSR. *Preslia*. 1956;28:362-8.
12. Styles BT. The taxonomy of *Polygonum aviculare* and its allies in Britain. *Watsonia*. 1962;5(4):177-214.
13. Raffaelli M. Contributi alla conoscenza del genere *Polygonum* L. 4. Le specie italiane della sect. *Polygonum*. *Webbia*. 1982;35(2):361-406. <https://doi.org/10.1080/00837792.1982.10670232>
14. Schmid K. Untersuchungen an *Polygonum aviculare* s. l. in Bayern. *Mitteilungen der Botanischen Staatssammlung München*. 1983;19:29-149.
15. Mosaferi S, Sheidai M, Keshavarzi M, Noormohammadi Z. Genetic diversity and morphological variability in *Polygonum aviculare* s.l. (Polygonaceae) of Iran. *Phytotaxa*. 2015;233(2):166-78. <https://doi.org/10.11646/phytotaxa.233.2.4>
16. Webb DA, Chater AO, Akeroyd JR. *Polygonum* L. In: Tutin TG, Burges NA, Chater AO, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SM, Webb DA, editors. *Flora Europaea Vol 1*. 2nd ed. Gateshead: Cambridge University Press; 1993. p. 91-7.

17. Akeroyd JR. Two overlooked species of *Polygonum* from SE Europe. *Bot J Linn Soc.* 1987;95:251-57. <https://doi.org/10.1111/j.1095-8339.1987.tb01858.x>
18. Stevanović V, Tan K, Iatrou G. Distribution of the endemic Balkan flora on serpentine I. – obligate serpentine endemics. *Plant Syst Evol.* 2003;242:149-70. <https://doi.org/10.1007/s00606-003-0044-8>
19. Maslo S, Šarić Š. Remarks on recent distribution of *Polygonum albanicum* Jáv. in Bosnia and Herzegovina. *Glasilo Future.* 2021;4(5-6):1-9. <https://doi.org/10.32779/gf.4.5-6.1>
20. Scholz H. Die Systematik des europäischen *Polygonum aviculare* L., II. Die Arten und Sippen aus der Verwandtschaft des *Polygonum heterophyllum* Lindman. *Ber Deutsch Bot Ges.* 1959;71:63-72. <https://doi.org/10.1111/j.1438-8677.1959.tb01413.x>
21. Yurtseva OV, Voylokova VN, Troitsky AV, Bobrova VK. Morphological and molecular evidences of *Polygonum patulum* and *Polygonum arenastrum* (Polygonaceae) hybridization. *Bot J.* 2007;92(9):1320-31.
22. Mosaferi S, Sheidai M, Keshavarzi M, Noormohammadi Z. Species delimitation and population structure analysis in *Polygonum* species (section *Polygonum*). *Wulfenia.* 2016;23:98-112.
23. Haraldson K. Anatomy and taxonomy in Polygonaceae subfam. Polygonoideae Meissn. emend. Jaretzky. *Acta Univ Ups Symb Bot Ups.* 1978;22:1-95.
24. Yasmin G, Khan MA. Taxonomic studies of *Polygonum* L. (complex) and *Rumex* L. Polygonaceae. Saarbrücken: Lambert Academic Publishing; 2010. 337 p.
25. Hameed A, Zafar M, Ullah R, Shahat AA, Ahmad M, Cheema SI, Lubna, Haq I, Sultana S, Usma A, Majeed S. Systematic significance of pollen morphology and foliar epidermal anatomy of medicinal plants using SEM and LM techniques. *Microsc Res Tech.* 2020;83:1007-22.
26. Ronse De Craene LP, Smets E. The Floral Nectaries of *Polygonum* s.l. and related genera (Persicarieae and Polygoneae): Position, Morphological Nature and Semophylisis. *Flora.* 1991;185:165-85. [https://doi.org/10.1016/S0367-2530\(17\)30468-1](https://doi.org/10.1016/S0367-2530(17)30468-1)
27. Ronse De Craene LP, Akeroyd JR. Generic limits in *Polygonum* and related genera (Polygonaceae) on the basis of floral characters. *Bot J Linn Soc.* 1988;98:321-71. <https://doi.org/10.1111/j.1095-8339.1988.tb01706.x>
28. Hong S-P, Ronse De Craene LP, Smets E. Systematic significance of tepal surface morphology in tribes Persicarieae and Polygoneae (Polygonaceae). *Bot J Linn Soc.* 1998;127(2):91-116. <https://doi.org/10.1111/j.1095-8339.1998.tb02091.x>
29. Mosaferi S, Keshavarzi M. Micro-morphological study of Polygonaceae tribes in Iran. *Phytologia Balcanica.* 2011;17(1):89-100.
30. Shiha MA. Tepal surface micromorphology and its taxonomic implications in some species of Polygonaceae in Egypt. *Egypt J Exp Biol.* 2019;15(2):261-68. <https://doi.org/10.5455/egyjebb.20190712101824>
31. Moore DN, Mertens TR, Highwood JE. Cytotaxonomic notes on genus *Polygonum*, section *Polygonum*. *Proc Indiana Acad Sci.* 1969;79:396-400.
32. Wolf SJ, McNeill J. Synopsis and achene morphology of *Polygonum* section *Polygonum*. *Rhodora.* 1986;88(856):457-79.
33. Ronse De Craene LP, Hong S-P, Smets E. Systematic significance of fruit morphology and anatomy in tribes Persicarieae and Polygoneae (Polygonaceae). *Bot J Linn Soc.* 2000;134:301-37. <https://doi.org/10.1111/j.1095-8339.2000.tb02356.x>
34. Yurtseva OV. Ultrasculpture of achene surface in *Polygonum* section *Polygonum* (Polygonaceae) in Russia. *Nord J Bot.* 2001;21:513-28. <https://doi.org/10.1111/j.1756-1051.2001.tb00805.x>
35. Hedberg O. Pollen morphology in the genus *Polygonum* L. s. lat. and its taxonomical significance. *Svensk Botanisk Tidsskrift.* 1946;40(4):371-404.
36. Hong S-P, Oh IC, Ronse De Craene LP. Pollen morphology of the genera *Polygonum* s. str. and *Polygonella* (Polygoneae: Polygonaceae). *Plant Syst Evol.* 2005;254:13-30. <https://doi.org/10.1007/s00606-005-0334-4>
37. Anačkov G. Botanički praktikum. Online book: ECOBIAS Erasmus [Internet]. University of Novi Sad. [cited 2026 Feb 11]. Available from: https://www.ecobiaserasmus.com/wp-content/uploads/2023/07/Task-2_5_Final_Practicum-in-ecology_botany.pdf
38. Grulich V, Májeková J, Eliáš P jun., Dítě D. 7. *Polygonum* L. In: Goliašová K, Michalková E, editors. *Flóra Slovenska VI/4.* Bratislava: VEDA – vydavateľstvo Slovenskej akadémie vied; 2016. p. 502-15.
39. Yurtseva OV. Self-pollination in species of *Polygonum* subsection *Polygonum*. *Bulletin of Moscow Society of Naturalists, Biological Series.* 1998;103(5):61-7.
40. Yurtseva OV, Kramina TE. Variability of species of the subsection *Polygonum* of the genus *Polygonum* (Polygonaceae) in connection with their possible hybridization. *Bot J.* 2003;88(1):9-25.
41. Yurtseva OV, Bobrova VK, Voylokova VN, Troitsky AV, Kramina TE. Morphological variability and genetic polymorphism of *Polygonum aviculare* group (Polygonaceae). *Botanical Journal.* 2006;91(5):697-716.
42. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika.* 1965;52(3-4):591-611. <https://doi.org/10.1093/biomet/52.3-4.591>
43. Box GEP, Cox DR. An analysis of transformations. *J R Stat Soc Series B Stat Methodol.* 1964;26(2):211-52. <https://doi.org/10.1111/j.2517-6161.1964.tb00553.x>
44. Mienye ID, Jere N. A Survey of Decision Trees: Concepts, Algorithms, and Applications. *IEEE Access.* 2024;12:86716-27. <https://doi.org/10.1109/ACCESS.2024.3416838>
45. Stuessy TF. *Plant taxonomy, the systematic evaluation of comparative data.* 2nd ed. New York: Colombia University Press; 2009. 539 p.
46. Lindman CAM. Wie ist die kollektivart *Polygonum aviculare* zu spalten? *Sven Bot Tidskr.* 1912;6(3):673-96.
47. Meerts P, Vekemans X. Phenotypic plasticity as related to trampling within a natural population of *Polygonum aviculare* subsp. *aequale*. *Acta Oecol.* 1991;12(2):203-12.
48. Meerts P. Phenotypic plasticity in the annual weed *Polygonum aviculare*. *Bot Acta.* 1995;108:414-24. <https://doi.org/10.1111/j.1438-8677.1995.tb00515.x>
49. Scholz H. Bemerkungen zur Merkmalsgeographie des *Polygonum aviculare* insbesondere des *P. arenastrum*. *Verh Bot Ver Prov Brandenburg.* 1977;113:13-22.

50. Budantsev AL, Lobova TA. Fruit morphology, anatomy and taxonomy of tribe Nepeteae (Labiatae). *Edinb J Bot.* 1997;54(2):183-216.
<https://doi.org/10.1017/S096042860004029>
51. Abdel Khalik K, Abd El-Ghani M, El Kordy A. Fruit and seed morphology in *Galium* L. (Rubiaceae) and its importance for taxonomic identification. *Acta Bot Croat.* 2008;67(1):1-20.
52. Shahla S, Asghar Maassoumi A, Mohammad S, Hamdi M, Mehregan I, Nejadstattari T. Fruit morphology of the genus *Rumex* L. (Polygonaceae) in Iran. *J Biol Env Sci.* 2014;5(1):655-63.
53. Çilden E. Significance of fruit and seed morphology in current taxonomy of the genus *Iberis* L. (Brassicaceae) in Turkey. *Nord J Bot.* 2022;2022(8):e03671.
<https://doi.org/10.1111/njb.03671>
54. Jang JE, Baasanmunkh S, Nyamgerel N, Oh S-Y, Song J-H, Yusupov Z, Tojibaev K, Choi HJ. Flower morphology of *Allium* (Amaryllidaceae) and its systematic significance. *Plant Divers.* 2024;46:3-27.
<https://doi.org/10.1016/j.pld.2023.06.009>
55. Royal Botanic Gardens, Kew. Plants of the World Online [Internet]. Richmond (UK): Royal Botanic Gardens, Kew; 2017. *Polygonum* L.; [cited 2026 Feb 11]. Available from: <https://powo.science.kew.org/>
56. Shorthouse DP. SimpleMappr: an online tool to produce publication-quality point maps [Internet]. Ottawa (ON): David P. Shorthouse; 2010 [cited 2026 Feb 11]. Available from: <https://www.simplemappr.net>

SUPPLEMENTARY MATERIALS

The Supplementary Materials are available in the supplemental online dataset:

https://www.serbiosoc.org.rs/NewUploads/Uploads/Obradov%20et%20al_Supplementary%20Materials.pdf