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Petiole Anatomical Study in Some Taxa of the Genus Alcea (Malvaceae) from Iran

Maneezheh Pakravan[1] & Azam Javanbakht[1]

Key words: anatomy, cluster analysis, morphometry, Euclidean distance, taxonomy

Ključne besede: anatomija, klastrska analiza, morfometrija, Evklidska razdalja, taksonomija

Abstract

Different species of the genus *Alcea*, due to the presence of mucilaginous compounds, are considered valuable medicinal plants in traditional medicine and pharmaceutical industries. In recent years, numerous biosystematics studies have been conducted to help resolve classification and identification issues of species. In this study, the anatomical characteristics of petioles in 15 taxa of *Alcea* in Iran were investigated. Middle parts of leaf petioles in the lower inflorescence were sectioned, double stained, and photographed under microscope. The most important traits examined in this study included the number of layers of collenchyma, parenchyma and the chlorenchyma cells in the cortex, the thickness and number of layers of sclerenchyma fibres in the phloem and the structure of vascular bundles. Based on the anatomical traits of the petioles, cluster analysis was carried out based on Euclidean distance using the Ward method. The results of the cluster analysis showed that some closely related taxa were grouped together, while the placement of others did not align with taxonomic proximity. The findings of this research indicate that certain anatomical characters of petioles, observed in closely related species, can only be used for species identification, but less so for taxonomic grouping.

Izvleček

Različne vrste rodu *Alcea* zaradi prisotnosti sluznih spojin veljajo za dragocene zdravilne rastline v tradicionalni medicini in farmacevtski industriji. V zadnjih letih so bile izvedene številne biosistematske študije, da bi prispevale k reševanju vprašanj klasifikacije in identifikacije vrst. V tej študiji so bile raziskane anatomske značilnosti pecljev pri 15 taksonih rodu *Alcea* v Iranu. Srednji deli listnih pecljev v spodnjem delu socvetja so bili narezani, dvojno obarvani in fotografirani pod mikroskopom. Najpomembnejše lastnosti, ki so bile preučene v tej študiji, so bile število plasti celic v kolenhimu, parenhimu in klorenhimu v skorji, debelina in število plasti sklerenhimskih vlaken v floemu ter struktura žilnih snopov. Na podlagi anatomskih značilnosti pecljev je bila izvedena klastrska analiza na podlagi evklidske razdalje z uporabo Wardove metode povezovanja. Rezultati klastrske analize so pokazali, da so bili nekateri tesno povezani taksoni združeni, medtem ko se umestitev drugih ni ujemala s taksonomsko bližino. Ugotovitve te raziskave kažejo, da se nekatere anatomske značilnosti pecljev, opažene pri tesno sorodnih vrstah, lahko uporabijo le za identifikacijo vrst, manj pa za taksonomsko združevanje.

Corresponding author:
Maneezheh Pakravan
E-mail: pakravan@alzahra.ac.ir

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1 Faculty of biological sciences, Department of plant sciences, Alzahra University, Tehran, Iran

Introduction

The genus *Alcea* (Malvaceae) comprises approximately 70–75 species worldwide, primarily distributed in Southwest Asia (Escobar et al., 2012), with 34 species recorded in Iran (Pakravan 2008). Linnaeus (1753), following Tournefort (1700), was the first to distinguish between the genera *Alcea* and *Althaea*. However, later botanists such as Willdenow (1800), De'Candolle (1828), and Baker (1890) merged these two genera under the name *Althaea*. Alefeld (1862) moved some *Althaea* species to *Alcea* later. Subsequent taxonomic studies on *Alcea* were conducted by Boissier (1867) and Iljin (1949).

More comprehensive taxonomic investigations were carried out by Zohary (1963a, 1963b) in Southwest Asia, and by Riedl (1976) and Pakravan (2008) within the Iranian flora. Given the high degree of polymorphism in morphological traits (Pakravan, 2001), various biosystematic approaches have been employed to aid in the taxonomy of *Alcea*, including analyses of seed coat characteristics (Ozkan & Uzunhisarciklic, 2007), seed protein electrophoresis (Pakravan, 2025), palynology (Cabi et al., 2009), and anatomical studies (Mohammad et al., 2018). The most significant phylogenetic research on *Alcea* was conducted by Escobar et al. (2012), who confirmed its monophyly and its distinction from *Althaea*.

Anatomical studies are considered valuable tools in plant classification (Metcalfe & Chalk, 1979). Numerous researchers have utilized anatomical traits of stems, leaves, and petioles across various plant families to aid in classification (Ozcan & Demiralay, 2018; Azizian & Cutler, 1982). Notable studies on *Alcea* and *Althaea* include those by Ozkan & Uzunhisarciklic (2007) in Turkey and Shaheen et al. (2010) in Pakistan, both of which provided important insights into species delimitation.

Given that petiole anatomy plays a significant role in defining species and genus boundaries in vascular plants (Thacker et al., 2021), and considering the lack of comprehensive anatomical studies on *Alcea*, this research aims to investigate the anatomical characteristics of several *Alcea* taxa to contribute to their taxonomic classification and species positioning within the genus.

Materials and Methods

In this study, samples from 15 taxa (11 species and 4 varieties) of the genus *Alcea* were examined: *Alcea kurdica* (Schlecht.) Alef var. *kurdica*, *A. kurdica* var. *leiocarpa* Zohary, *A. kurdica* var. *laxiflora* (I. Riedl) Pakravan, *A. rechingeri* (Zohary) I. Riedl, *A. wilhelminae* I. Riedl var. *wilhelminae*, *A. sulphurea* (Boiss. & Hohen.) Aef., *A. tarica* Pakravan, *A. glabrata* var. *glabrata, A. glabrata* var. *microcarpa* (Zohary) Pakravan & Ghahreman, *A. angulata* Freyn & Sint., *A. hyrcana* (Grossh.) Grossh., *A. schirazana* Alef., *A. hohenackeri* (Boiss. & Huet.) Boiss., and *A. wilhelminae* var. *lineariloba* (I. Riedl) Pakravan, *A. sacsachanica* Iljin (Table 1). For anatomical analysis, free hand sectioning from herbarium specimens were carried out. For each tax-

Table 1: Studied taxa with voucher number and collection information.
Tabela 1: Preučevani taksoni s številko vavčerja in podatki o nabirkih.

Taxon	Herbarium sheet ID	Collector	Location
A. angulata	ALUH-26379	Pakravan & Darrehshury	Tehran: Firuzkuh
A. angulata	ALUH-26391	Pakravan & Darrehshury	Robat-karim Tehran:
A. angulata	ALUH-26393	Pakravan & Darrehshury	Tehran: Qom Road
A. glabrata var. *glabrata*	ALUH-26436	Pakravan & H. Moghadam	Tehran: 50 km from Bueen Zahra to Eshtehard
A. glabrata var. *microcarpa*	ALUH-26372	Pakravan & Darrehshury	Tehran: 30 km from Karai to Challus
A. hohenackeri	ALUH-26411	Pakravan & Darrehshury	Azarbaijan: 10 km from Oshnavieh to Urmia
A. sachsachanica	ALUH-26390	Pakravan & H. Moghadam	Azarbaijan: Ghushchi pass.
A. kurdica var. *kurdica*	ALUH-26410	Pakravan & H. Moghadam	Kurdestan: 13 km from Kamyaran to Sanandaj
A. kurdica var. *kurdica*	ALUH-26426	Pakravan & H. Moghadam	Azarbaijan: Tabriz, Bostan-abad to Mianeh
A. kurdica var. *kurdica*	ALUH-26428	Pakravan & Darrehshury	Fars: Estahban to Shiraz, Mian Kotal
A. kurdica var. *kurdica*	ALUH-26395	Pakravan & H. Moghadam	Hamedan: 3 km from Avaj to Razan
A. kurdica var. *laxiflora*	ALUH-26434	Pakravan & H. Moghadam	Tehran: 50 km from Bueen Zahra to Eshtehard
A. kurdica var. *laxiflora*	ALUH-26443	Pakravan & H. Moghadam	Kermanshah: Between Khamseh ane Bisotun
A. kurdica var. *leiocarpa*	ALUH-26400-	Pakravan & H. Moghadam	Hamedan: Lalehjin
A. rechingeri	ALUH-26430	Pakravan & Darrehshury	Fars: Estahban to Shiraz
A. schirazana	ALUH-26394	Pakravan & Darrehshury	Fars: Betweeen Ardakan and Komehr
A. hyrcana	ALUH-12440	Pakravan	Gilan: Talesh to Kallkhal
A. sulphurea	ALUH-26378	Pakravan & Darrehshury	Tehran: 3 km from Darbandsar to Fasham
A. sulphurea	ALUH-14645	Darzi	Tehran: Shemiran
A. tarica	ALUH-26380	Pakravan	Tehran: Damavand, Tar Lake.
A. wilhelminae var. *lineariloba*	ALUH-26437	Pakravan & H. Moghadam	Azarbaijan:15 km from Mianeh to Bostan-abad
A. wilhelminae var. *lineariloba*	ALUH-26419	Pakravan & H. Moghadam	Azarbaijan: Ghushchi pass.
A. wilhelminae var. *wilhelminae*	ALUH-26416	Pakravan & H. Moghadam	Azarbaijan: Ghotur valley
A. wilhelminae var. *wilhelminae*	ALUH-12435	Pakravan	Azarbaijan: Ardebil to Khalkhal

on one to four populations, with at least four individuals in each population and three petioles from each individual were examined. The samples were preserved in the Alzahra University herbarium (ALUH) (Thiers, 2025). In all cases, leaves from the lower inflorescence were selected.

The samples were first boiled in water and then stored in 70% alcohol solution for one week. Free hand sectioning was manually prepared from the middle portion of the petiole using razor blades. The sections were stained using a double-staining method with methyl green and carmine aluminize (Nobarinezhad et al., 2018) and subsequently photographed (at least five microscopic slides) with an Olympus BX51 light microscope equipped with an Olympus DP-12 camera.

Morphometry and clustering analysis

In the analysis, 13 anatomical traits were examined (Table 2), including three qualitative and ten quantitative traits, derived from both the petiole surfaces and their cross-sections. For the quantitative traits, each characteristic was measured, and the mean along with the standard deviation was calculated. Cluster analysis based on Euclidean distance by Ward method was performed in SPSS ver. 25 (2017) software.

Results

Anatomical traits of petiole were examined, including the overall shape of the petiole in cross-section, epidermal structure, cortex, and vascular bundles.

Shape of the petiole: In *Alcea kurdica* var. *kurdica*, *A. kurdica* var. *leiocarpa*, *A. rechingeri*, *A. wilhelminae* var. *wilhelminae*, *A. sulphurea*, *A. tarica*, *A. glabrata* var. *glabrata*, and two populations from Firouzkuh and Robat Karim of *A. angulata*., the petiole shape is circular, whereas in other species, it is triangular (Figures 3 & 4A, B, C).

Epidermis: In most taxa, the epidermis consists of one-layer except in *A. glabrata*. var *glabrata*, *A. schirazana* and *A. angulata* that have two layers of epidermis cells. The epidermis cells are round to cuboidal in shape and covered by a thin cuticle, with stomata interrupting the surface in some areas. Except for *A. schirazana* Alef. and *A. wilhelminae* var. *wilhelminae*, which lack trichomes, all other species exhibit combination of various types of trichomes, including simple, star-shaped, bifurcate, three radiate and glandular (Figure 2).

Cortex: Beneath the epidermis, several layers of chlorenchyma and collenchyma cells form the cortex. Below the epidermis, chlorenchyma layers containing storage material were observed, with 2 to 5 layers of cells. The thickest chlorenchyma layers were found in *A. wilhelminae* var. *wilhelminae* and the thinnest in *A. hohenackeri*. Beneath the chlorenchyma, collenchyma layers consisting of 3 to 10 rows of cells are observed, which were angular in all samples. The minimum number of collenchyma layers were observed in *A. wilhelminae* var. *wilhelminae*, and the most were in *A. sulphurea*. The thickest collenchyma layers were found in *A. sulphurea*, and the thinnest in *A. hyrcana* (Grossh.) Grossh. The thickest parenchyma was found in *A. rechingeri*, and the thinnest in *A. glabrata* var. *microcarpa*. This region also contains secretory cavities (Figures 3 & 4).

Beneath the collenchyma layers, parenchyma cells are found, which consist of 4 to 10 rows of round, oval, or polygonal cells, with intercellular spaces (Figures 4 & 5).

Vascular bundles: Vascular bundles are bicollateral except in *A. angulata*. Arrangement had a great stability and systematic concept. It included sclerenchyma fibre cells, phloem bundles, vascular cambium, xylem, inner phloem, inner xylem and pith. The petiole vascular bundles were divided into two abaxial and adaxial, and unlike in some other members of the Malvaceae family, medullary vascular bundles were not observed in these species. The vascular cylinder consisted of xylem and phloem vascular bundles, which in all samples, had a free and crescent shape, often elliptical, with varying sizes in a ring. A significant variation in the number of vascular bundles was observed in different *Alcea* taxa. Some taxa had large vascular bundles, while others had small vascular bundles, with anywhere from 6 to 8 bundles observed among different species. The minimum number of vascular bundles were found in *A. sulphurea* and *A. hohenackei* (5), and the maximum number of vascular bundles were observed in *A. rechingeri* (16). Additional vascular bundles were observed only in *A. sulphurea* and *A. angulata* (Figures 4 & 5).

Among the studied taxa, most of the them had separate vascular bundles. In some taxa, the bundles were independent and very close to each other, while in the following taxa, continuous vascular bundles were observed:

A. angulata (3 continuous bundles), *A. hyrcana* (3 to 4 continuous bundles), *A. rechingeri* (9 fused bundles), *A. kurdica* var. *kurdica* (4 continuous bundles). U-shaped continuously vascular bundles were observed only in *A. sulphurea* (Figure 3I).

There were one to two rows of cambium cells between the xylem and phloem bundles, but due to the high density of crystals in this area, they are not clearly visible in the images (Figure 4G). Above each large vascular bundle and some small ones, a set of fiber cells with thick walls is located, known as the phloem fiber cap. The number of phloem fiber cap layers varies among the taxa. Based on the height of the cap in the cellular layers, the phloem cap can be classified as large (more than 80 µm), medium (between 50 to 75 µm), or small (between 25 to 45 µm).

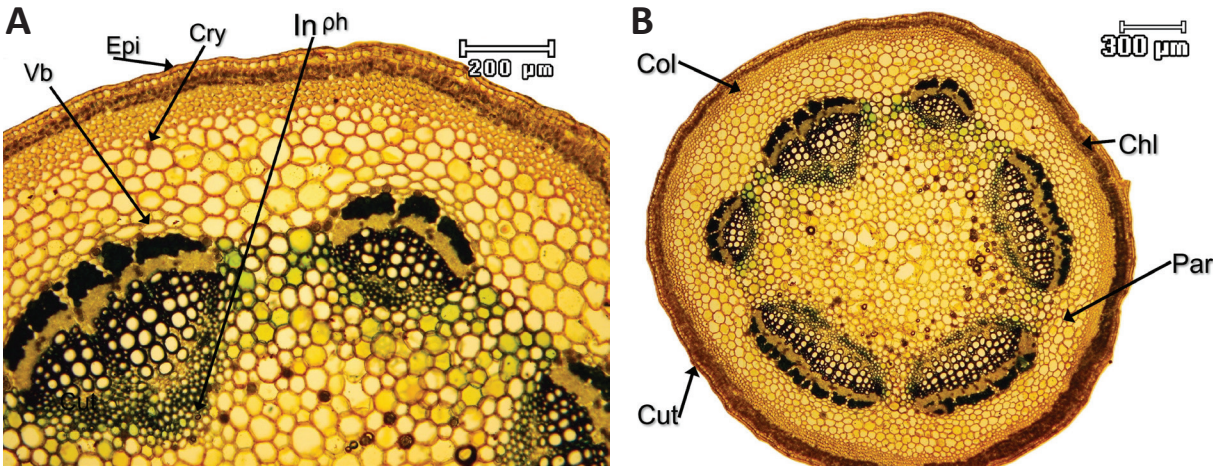

Figure 1. Details of petiole structure in *Alcea wilhelminae* var. *wilhelminae.* Chl: chlorenchyma, Col: collenchyma, Cry: crystal, Cut: cuticle, Epi: epidermis, In ph: internal phloem, Par: parenchyma, V.b: vascular bundle.

Slika 1: Podrobnosti v strukturi listnega peclja pri vrsti *Alcea wilhelminae* var. *wilhelminae*. Chl: klorenhim, Col: kolenhim, Cry: kristal, Cut: kutikula, Epi: povrhnjica, In ph: notranji floem, Par: parenhim, V.b: žilni snop.

Figure 2: Different types of trichomes on the petiole of *Alcea* species. A: simple, B: bifurcate, C & D: glandular, E & F: stellate.

Slika 2: Različne vrste trihomov na listnem peclju vrst v rodu *Alcea*. A: navadni, B: dvokraki, C in D: žlezni, E in F: zvezdasti.

Species such as *A. sulphurea*, *A. sachsachanica, A. tarica*, and *A. angulata* had large fiber caps. The taxa, like *A. hohenackeri*, *A. hyrcana*, *A. glabrata* var. *glabrata*, *A. kurdica* var. *leiocarpa* and *A. wilhelminae* var. *lineariloba* had small fiber caps, while the remaining taxa had medium-sized fiber caps (Figures 4 & 5, Table 2).

Phloem bundles with the widest diameter were found in *A. kurdica* var. *kurdica* (Figure 4C), while the narrowest phloem was found in *A. angulata* (Figure 4E, Table 2).

The xylem bundles with the widest diameter were observed in *A. kurdica* var. *laxifolia* (Figure 5I), while the narrowest were found in *A. hyrcana* (Figure 4I). In all taxa, both xylem and phloem are present internally (except in *A. angulata*). In all taxa, the petiole contains a large area of ground tissue of the pith, which consists of parenchyma cells with thin walls and appears in various shapes such as round, oval, or polygonal, except in *A. hyrcana*, *A. rechingeri* and *A. sacsachanica,* which had hollow pith. Additionally, secondary phloem fibers were observed at the edges of the pith, except in *A. angulata*, where they were sparse and minimal (Figures 4 & 5).

Crystals were also found in various parts of the petiole, including the cortex, phloem vessels, and pith (Figures 4 & 5).

Table 2: Anatomical features of petiole in *Alcea* species.
Tabela 2: Anatomske značilnosti pecljev vrst v rodu *Alcea*.

Taxa \ characters	Parench thick µm	No. Collen	Collen thick (µm)	T. fib scl.	Phlo thick (µm)	Xylem thick (µm)	Chlorench thick (µm)	T. V. B.	Add. V, B	M.V.B	Vas. B. pattern	Epi. Th.	Pet sh
A. angulata	182.3-187.7	5-7	91.1-115.2	74.4-78.3	37.5-46.1	116.8-125.2	51.2-53.7	12	+	**10**	Free bundle Ring	25.8-32.6	C
A. glabrata* var. *glabrata	170.1-175.8	4-6	81.5-93.4	32.-34.2	41.9-45.5	132.2-135.3	36.4-38.2	7	-	4	Free bundle Ring	30.1-32.6	C
A. glabrata* var. *microcarpa	144.3-146.8	4-5	82.7-90.3	54.9-63.7	43.4-45.9	134.2-136.1	46.9-48	7	-	5	Free bundle Ring	21.1-23.6	T
A. hohenackeri	204.2-207.1	2-3	56.1-59.4	43.3-44.51	47.5-52.5	171.9-174.5	30.4-31.9	5	-	4	Free bundle Ring	20.6-23.7	T
A. hyrcana	186.9-187.2	3-7	58.9-110.8	35.6-37.7	50.7-55.4	99.8-103.3	36.9-37.5	12	-	2	Free bundle Ring	26.7-29.8	T
A. kurdica* var. *kurdica	200.3-238.3	4-5	89.5-94.4	52.3-80.65	56.4-**70.7**	121.9-199.6	35.6-49.5	7	-	5	Free bundle Ring	28.5-32	C
A. kurdica* var. *laxiflora	243.9-250	5-6	97.5-100.2	63.1-63.2	63.8-65.7	256.6-**270.5**	42.5-42.6	7	-	4	Free bundle Ring	31.7-34.3	T
A. kurdica* var. *leiocarpa	256.3-270.8	4	87.3-87.7	45.8-48.1	63.7-65.7	129.9-133.7	46.2-50.1	6	-	4	Free bundle Ring	22.6-24.7	C
A. rechingeri	309.8-**330.4**	5-6	99.7-105.4	66.2-67.4	63.5-64.2	186.9-187.6	48.8-51.7	16	-	**10**	Fused bundle Ring	27.8-30.3	C
A. schirazana	257.6-278.4	8-9	103.9-**124.9**	74.7-76.1	60.2-64.2	180.8-182	43.7-47.3	8	-	6	Free bundle Ring	30.7-32.2	T
A. sachsachanica	297.10	6-7	107.3-109.6	76-85.23	60.8-63.1	195.8-198.5	43.8-44.8	6	-	4	Free bundle Ring	28.6-31.5	T
A. sulphurea	229.6-258.8	5-6	100-103.9	60.8-82.4	60.9-63.9	160-167.3	44.5-46.1	5	+	1	U-shape Free bundle	25.8-25.9	C
A. tarica	201.9-215.7	5-6	104.6-108.2	87.9-**93.3**	51.2-54.3	166.6-168.5	31.2-34.5	**8**	-	5	Free bundle Ring	22.8-25.5	C
A. wilhelminae* var. *lineariloba	190.7-192.4	3-4	60.9-81.6	47.2-47.4	39.8-39.9	107.1-118.8	61.6-63.7	6	-	4	Free bundle Ring	31.3-37.0	T
A. wilhelminae* var. *wilhelminae	294.6-297.6	4-5	87.9-102.6	61.2-66.75	55.2-58.2	219.7-227.74	46.2-**87.2**	6	-	4	Free bundle Ring	36.8-**37.2**	C

Parench thick: Parenchyma thickness, No. Collen: Number of collenchyma layers, No. Chloren: Layer number of Chlorenchyma, T. fi. Scl.: Thickness of sclerenchyma fibers, Phlo thick: Phloem thickness, Xylem thick: Xylem thickness, Collen thick: collenchyma thickness, T. V. B.: Total Number of vascular bundles, Add. V, B: Additional vascular bundles, M.V.B: Major vascular bundle, Vas. B. pattern: vascular bundles pattern, Epi. Th.: Epiderm thickness, C: circular, T: triangle, Pet. Sh: Petiole shape, - & +: Presence or absence of additional vascular bundles. The highest value of each trait state is in bold.

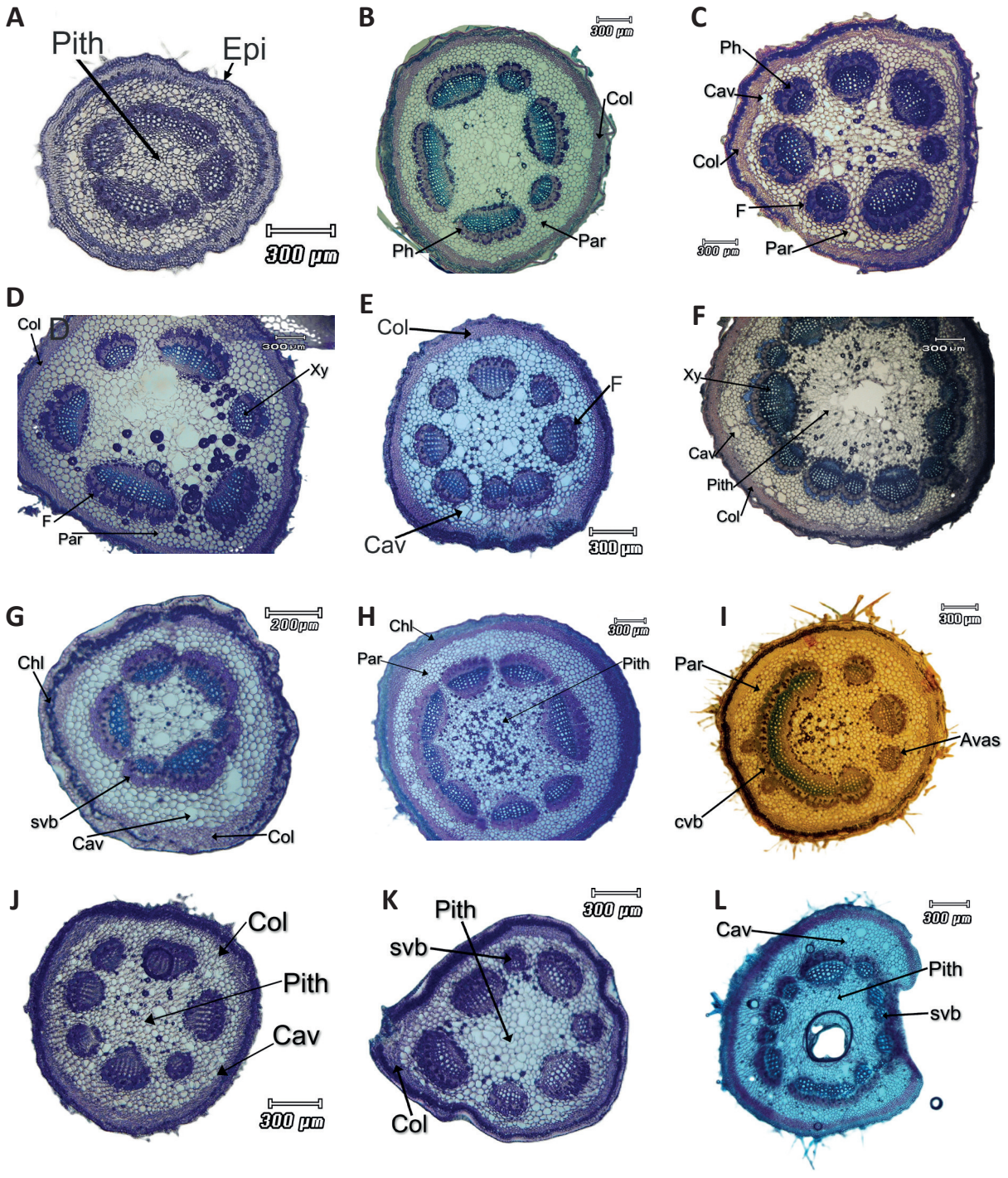

Figure 3: Cross sections of *Alcea* petioles: A: *A. hohenackeri,* B: *A. kurdica* var. *leiocarpa,* C: *A. kurdica* var. *laxiflora,* D: *A. sachsachanica,* E: *A. tarica,* F: *A. rechingeri,* G: *A. wilhelminae* var. *lineariloba,* H: *A. angulata,* I: *A. sulphurea,* J: *A. glabrata* var. *glabrata*, K: *A. glabrata* var. *microcarpa*, L: *A. hyrcana*. Scale bars: 300 µm. Avas: additional vascular bundle, cav: cavity, Chl: chlorenchyma, Col: collenchyma, Cut: cuticle, Cvb: continuous vascular bundles, Epi: epidermis, F: phloem fibre, Par: parenchyma, Ph: phloem, svb: small vascular bundle, Xy: xylem.

Slika 3: Prečni prerezi pecljev vrst v rodu *Alcea*: A: *A. hohenackeri,* B: *A. kurdica* var. *leiocarpa,* C: *A. kurdica* var. *laxiflora,* D: *A. sachsachanica,* E: *A. tarica,* F: *A. rechingeri,* G: *A. wilhelminae* var. *lineariloba,* H: *A. angulata,* I: *A. sulphurea,* J: *A. glabrata* var. *glabrata*, K: *A. glabrata* var. *microcarpa*, L: *A. hyrcana*. Merila: 300 µm. Avas: dodatni žilni snop, cav: votlina, Chl: klorenhim, Col: kolenhim, Cut: kutikula, Cvb: neprekinjeni žilni snopi, Epi: povrhnjica, F: floemsko vlakno, Par: parenhim, Ph: floem, svb: majhen žilni snop, Xy: ksilem.

Clustering analysis

Cluster analysis was conducted based on Euclidean distance by using the Ward method in SPSS software. 25 (2017) based on 11 qualitative petiole anatomical traits. The resulting dendrogram (Figure 6) grouped 15 taxa of the genus *Alcea* into two main clusters (A and B) at an average taxonomic distance of 25 units. The first major cluster (A) is further divided into two sub-cluster, C and D, which separate at a taxonomic distance of 6. Cluster C comprises *A. tarica*, *A. hohenackeri*, *A. sulphurea*, *A. wilhelminae* var. *lineariloba*, which are further split into two smaller sub-clusters, F and G. Cluster D, also separated at a taxonomic distance of 6, includes *A. glabrata* and *A. hyrcana.* The second major cluster (B) consists of *A kurdica* var. *laxiflora*, *A. kurdica* var. *kurdica*, *A. wilhelminae and A. sachsachanica.*

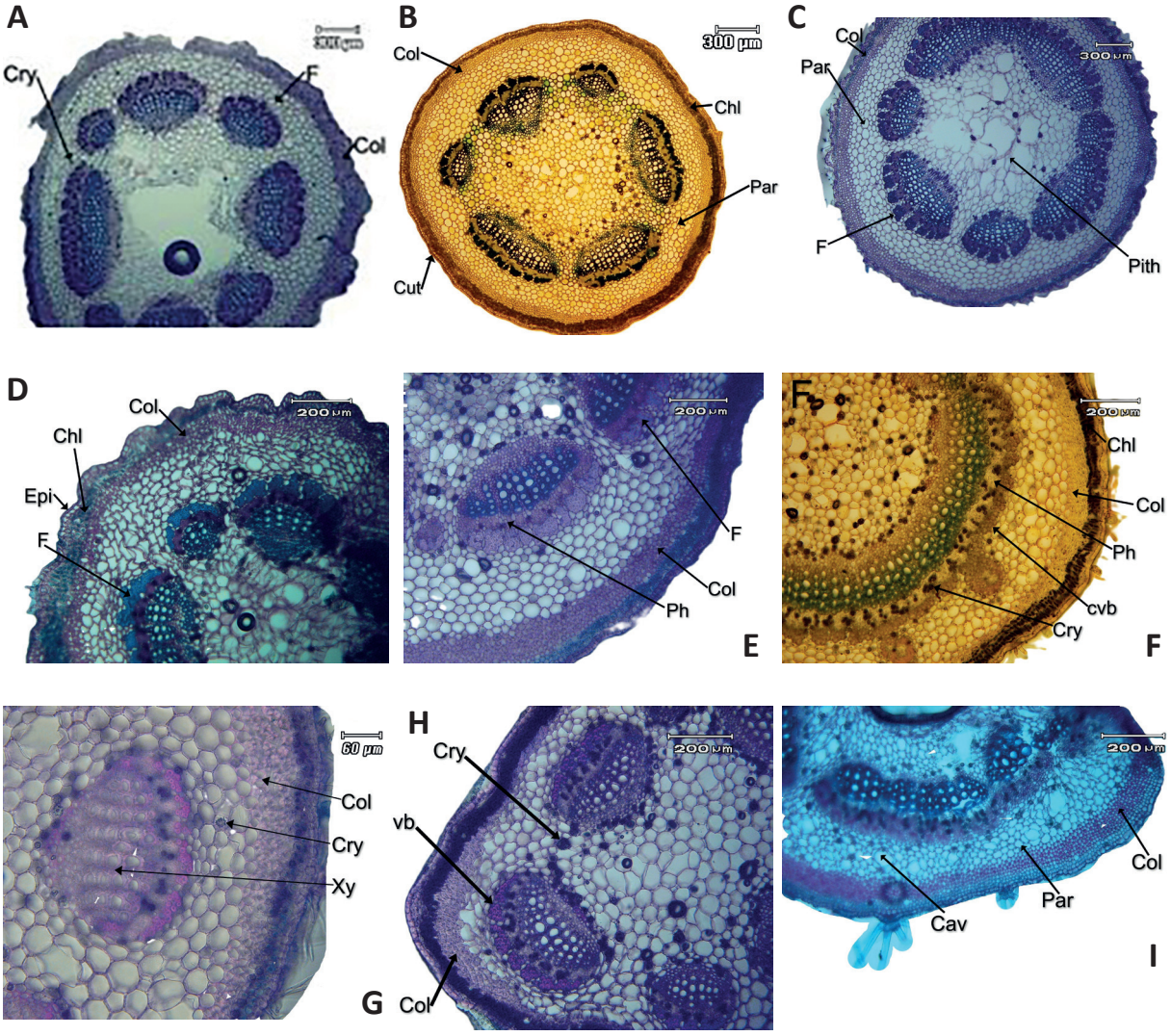

Figure 4: Cross section of *Alcea* petioles: A: *A. schirazana,* B: *A. wilhelminae* var. *wilhelminae,* C: *A. kurdica* var. *kurdica,* D: *A. wilhelminae* var. *lineariloba,* E: *A. angulata,* F: *A. sulphurea*, G: *A. glabrata* var. *glabrata*, H: *A. glabrata* var. *microcarpa*, I: *A. hyrcana*. Scale bars: 300 µm (A, B, C,), 200 µm (D, E, F, H, J), 60 µm (G). cav: cavity, Chl: chlorenchyma, Col: collenchyma, cry: crystal, Cut: cuticle, cvb: continuous vascular bundle, Epi: epidermis, F: phloem fibre, L.vb: lateral vascular bundle, Par: parenchyma, Ph: phloem, svb: small vascular bundle, Xy: xylem.

Slika 4: Prečni prerezi pecljev vrst v rodu *Alcea*: A: *A. schirazana,* B: *A. wilhelminae* var. *wilhelminae,* C: *A. kurdica* var. *kurdica,* D: *A. wilhelminae* var. *lineariloba,* E: *A. angulata,* F: *A. sulphurea*, G: *A. glabrata* var. *glabrata*, H: *A. glabrata* var. *microcarpa*, I: *A. hyrcana*. Merilo: 300 µm (A, B, C,), 200 µm (D, E, F, H, J), 60 µm (G). cav: votlina, Chl: klorenhim, Col: kolenhim, crystal: kristal, Cut: kutikula, cvb: neprekinjen snop. Epi: povrhnjica, F: floemsko vlakno, L.vb: lateralni žilni snop, Par: parenhim, Ph: floem, svb: majhen žilni snop, Xy: ksilem.

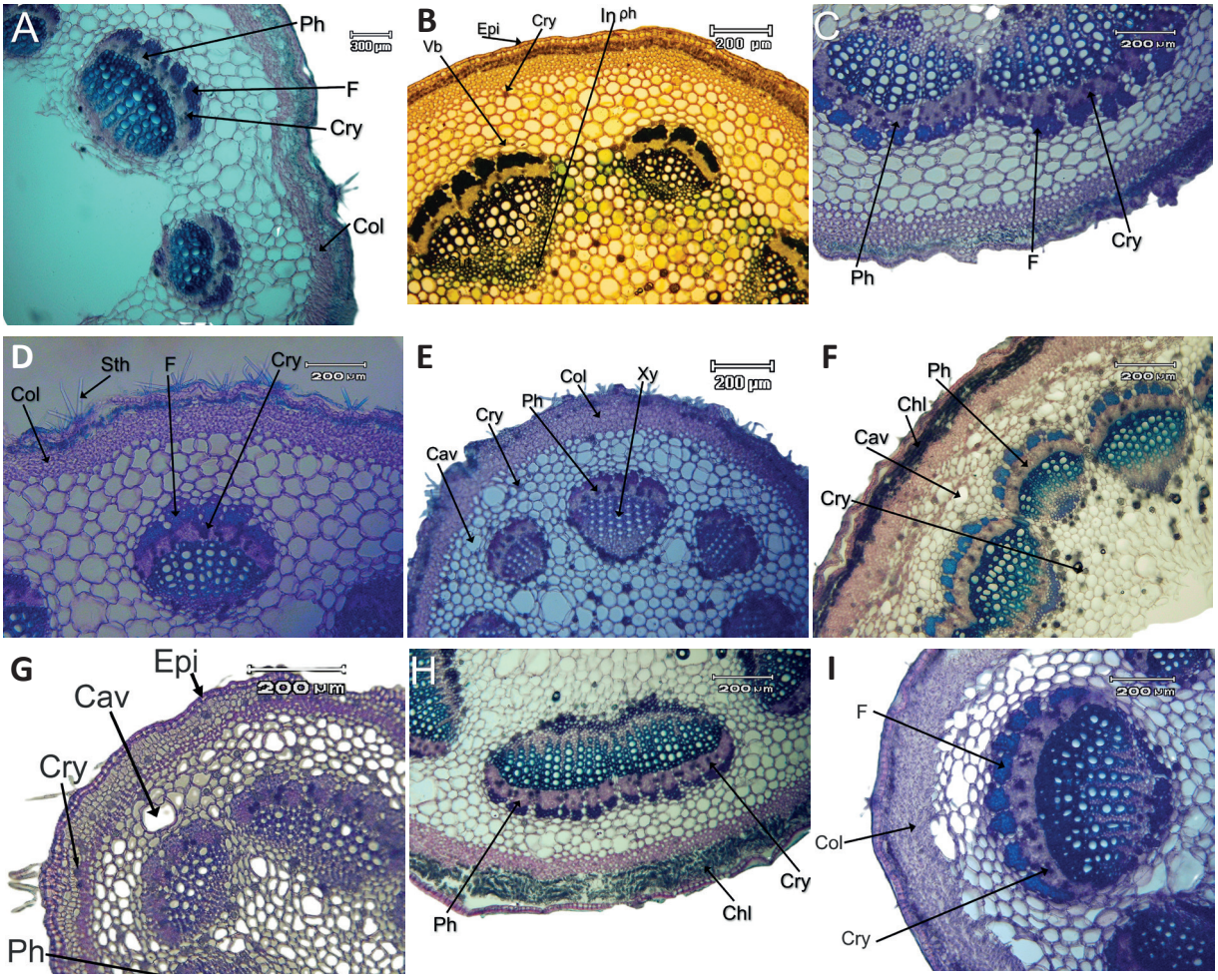

Figure 5: Cross section of *Alcea* petioles: (A): *A. schirazana,* (B): *A. wilhelminae* var. *wilhelminae,* (C): *A. kurdica* var. *kurdica,* (D): *A. sachsachanica,* (E): *A. tarica*, (F): *A. rechingeri,* (G): *A. hohenackeri,* (H): *A. kurdica* var. *leiocarpa,* (I): *A. kurdica* var. *laxiflora.* Scale bars: 200 µm. cav: cavity, Chl: chlorenchyma, Col: collenchyma, cry: crystal, Cut: cuticle, Epi: epidermis, F: phloem fibre, In ph: internal phloem, L.vb: lateral vascular bundle, Par: parenchyma, Ph: phloem, svb: small vascular bundle, Sth: stellate trichome, Xy: xylem.

Slika 5: Prečni prerezi pecljev vrst v rodu *Alcea*: (A): *A. schirazana,* (B): *A. wilhelminae* var. *wilhelminae,* (C): *A. kurdica* var. *kurdica,* (D): *A. sachsachanica,* (E): *A. tarica*, (F): *A. rechingeri,* (G): *A. hohenackeri,* (H): *A. kurdica* var. *leiocarpa,* (I): *A. kurdica* var. *laxiflora.* Lestvice: 200 µm. cav: votlina, Chl: klorenhim, Col: kolenhim, Cut: kutikula, cry: kristal, Epi: povrhnjica, F: floemsko vlakno, In ph: notranji floem, L.vb: lateralni žilni snop, Par: parenhim, Ph: floem, Sth: zvezdasti trihom, svb: majhen žilni snop, Xy: ksilem,

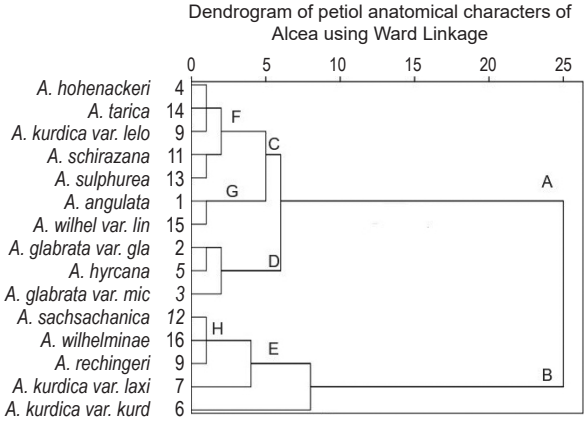

Figure 6: Dendrogram based on quantitative petiole anatomical characters of *Alcea* species.

Slika 6: Dendrogram, ki temelji na kvantitativnih anatomskih značilnostih pecljev vrst v rodu *Alcea*.

Discussion

The findings of this study align with those of Mohammad et al. (2018), who conducted a cladistic analysis based on anatomical traits between different genera of the Malvaceae family. Their research also confirmed that key anatomical features of the petiole, such as sclerenchyma fibres, the structure of vascular elements (including their size, shape, and distribution), the overall vascular pattern, and the presence of crystals, play a crucial role in classification and phylogenetic analysis within the Malvaceae family.

Metcalfe and Chalak (1979) stated that the arrangement of the vascular structures of the petiole changes with the age of the leaf. However, many previous scientists showed that the arrangement of vascular structures in the petiole and midvein of the leaf has a systematic meaning (Moon et al., 2009; Schweingruber et al., 2014; Talip et al., 2017; Thacker et al., 2021; Silva et al., 2018; Marasek-Ciolakowska et al., 2021.

The convergence of *A. schiraziana* to *A. kurdica* var. *leiocarpa* is attributed to their similar characteristics in the thickness of collenchyma, parenchyma, phloem, and chlorenchyma. Similarly, the close positioning of *A. kurdica* var. *laxiflora, A. kurdica* var. *kurdica* and *A. rechingeri* is due to shared traits in collenchyma thickness, phloem structure, petiole shape, and the number of vascular bundles. In contrast, *A. wilhelminae* var. *wilhelminae* and var. *lineariloba* have separated into distinct clusters due to dissimilarity in sclerenchyma and collenchyma width, phloem thickness, and petiole shape, and thus did not group together.

The statistical analysis in this study clearly demonstrates that, while the delimitation of some taxa aligns with previous classifications based on morphological traits, others do not conform to these groupings. In traditional classification systems based on morphology, species such as *A. wilhelminae, A. glabrata,* and *A. sachsachanica* are placed in the same group (Zohary, 1963), while *A. kurdica* and *A. rechingeri* are classified together in another. However, in the dendrogram based on petiole anatomical traits (Figure 6), only closely related accessions appear in adjacent branches. Such as, *A. sulphurea* is positioned next to *A. angulata*, as both share mericarps without wings, leaves with shallow or no indentations, and densely velvety pubescence. Similarly, *A. kurdica* var. *laxiflora* is clustered with *A. kurdica* and *A. rechingeri,* also *A. glabrata* var. *microcarpa* is clustered with *A. glabrata,* and *A. wilhelminae* with *A. sachsachanica*, which confirmed their close taxonomic relationships, while *A. wilhelminae* and *A. lineariloba* are found in a convergent group cluster. These results are completely consistent with the results of phylogenetic analysis based on molecular markers on *Alcea* species in Irano-turanian region (Escobar et. al., 2012).

In contrast, the placement of some other taxa does not correspond to their expected taxonomic relationships (Figure 6). For example, it was beyond our expectation that *A. tarica* was placed beside the *A. kurdica* or *A. wilhelminae* var. *lineariloba* was placed beside the *A. angulata*. It is due to taxonomic relationships being based mostly on morphological characters. They do not always correspond to genetic relationships, just as in molecular systematics, some taxa are not placed in their taxonomic positions based on morphological traits.

Conclusion

Petiole anatomy of 15 taxa of the genus *Alcea* was studied using hand section and double staining, and some for the first time. Some species have identified by their anatomical characters. Clustering analysis based on petiole anatomical traits indicated that while some anatomical traits of the petiole could aid in species identification, they are not particularly useful for grouping closely related species.

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Research data availability

The data supporting this study are available from the corresponding author upon request.

Declaration of competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID iDs
Maneezhes Pakravan https://orcid.org/0000-0002-1683-5569
Azam Javanbakht https://orcid.org/0009-0008-6888-2464

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