Acta Biologica Szegediensis

Volume 69(1):30-37, 2025
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs

DOI:10.14232/abs.2025.1.30-37

ARTICLE

Leaf anatomical study of *Onosma* (Section *Onosma*, Subsection *Haplotricha*) Boraginaceae in Iran

Reyhaneh Jamshidi[1], Maryam Keshavarzi[1],*, Ahmad Reza Mehrabian[2]

[1]Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[2]Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

ABSTRACT The genus *Onosma* (Boraginaceae) comprises a diverse group of species with significant taxonomic complexity. In this study, the leaf anatomical characteristics of 11 species from the *Onosma* section, *Haplotricha* subsection in Iran were examined to identify diagnostic traits that contribute to their classification. The qualitative and quantitative anatomical features were statistically analyzed. Multivariate statistical analyses, such as cluster analysis and principal component analysis, were used to determine species relationships based on the variations in the anatomical traits. The results revealed distinct variations among species, with *O. assadii* and *O. sabalanica* showing the highest divergence. Factor analysis indicated that mesophyll structure, midrib thickness, and trichome characteristics were key differentiating features. These findings support the significance of leaf anatomical traits in the systematic study of *Onosma* and contribute to a more refined taxonomic framework for the genus.
Acta Biol Szeged 69(1):30-37 (2025)

KEY WORDS
Boraginaceae
Iran
leaf anatomy
multivariate analysis
Onosma
taxonomy

ARTICLE INFORMATION
Submitted
12 July 2025
Accepted
19 November 2025
***Corresponding author**
E-mail: m.keshavarzi@alzahra.ac.ir

Introduction

Onosma L. (Lithospermeae, Boraginaceae) is known with 235 species in the world and 74 species in Iran (POWO 2024). The Asian and Mediterranean regions, especially Iran and Turkey, are this genus's main diversity centers (Nasrollahi et al. 2020). *Onosma* comprises biennial or perennial herbs characterised by scabrous, petiolate or sessile leaves with entire margins (Kumar et al. 2013). A key morphological feature of *Onosma* species is the presence of setae and their shapes (Mehrabian et al. 2014).

The *Onosma* species are commonly distributed in dry, sunny, or damp environments, typically in rocky cracks (Kumar et al. 2013). The genus displays significant morphological and karyological diversity, which has led to confusion in the classification. *Onosma* species hold medicinal and economic significance (Binzet and Teke 2014). These plants are used in traditional medicine due to their phytochemical compounds such as alkaloids, naphthoquinones, and flavonoids, which vary based on the species and geographic location. These phytochemicals demonstrate various bioactivities, including cytotoxicity against cancer cells, antioxidant effects, and therapeutic potential for inflammation and skin diseases, making *Onosma* a promising candidate for further medicinal research (Saravanakumar et al. 2021). Some species are used in folk medicine for treating wounds, diabetes, dyspepsia, and other ailments (Binzet and Teke 2014), while others are valued for their attractive flowers and nectar, making them important for beekeeping (Teppner 2011). The flowers of certain species are even consumed as vegetables (Akçin 2009).

The genus *Onosma* comprises a diverse group with a complex taxonomic history. While traditional morphological classifications have relied on features as indumentum, calyx structure, corolla shape and color, number of flowers in cymes, and nutlet morphology and nutlet morphology, these have proven insufficient to fully resolve phylogenetic relationships within the genus (Binzet and Teke 2014). This has led to ongoing debate and revisions in the taxonomic framework, highlighting the need for further investigation using other sources of data. The genus has been divided into sections (*Protonosma*, *Podonosma*, and *Onosma*). *Onosma* section has two subsections as *Asterotricha* and *Haplotricha*, primarily based on indumentum type (Binzet and Teke 2014), but Riedl (1967) highlighted the artificiality of this classification and the need for further investigation (Binzet and Teke 2014).

Despite the taxonomic complexities, the existing literature on the anatomy of *Onosma* is limited. Azizian et al. (2000) focused on the taxonomic significance of leaf anatomical structure in *Onosma* species. Their findings were of diagnostic value in sectional classification. The anatomical features are diagnostic in the systematic study of *Anchusa* (Selvi

Table 1. Geographical information and voucher numbers of sampled species

Species	Voucher details
O. assadii Mehrabian & Mozaff.	Azarbaijan, Horand near Gharesou, 700 m., Assadi 86652
O. bulbotricha Dc.	Azarbaijan, Tabriz, Mehrabian, 2008233 HSBU
	Kermanshah, Paveh, Mehrabian, 2010267 HSBU
O. dichroantha Boiss.	Khorassan, Sabzevar to Neishabur, Mehrabian, 2006552 HSBU
O. gaubae Bornm.	Alburz, Amir Kabir Dam, Mehrabian, 2012654HSBU
O. longiloba Bunge	Tehran, 20 km after Firuzkuh to Semnan, 1870 m., Mehrabian, 2012107 HSBU
O. microcarpa DC	Azarbaijan, Arasbaran, Mehrabian, 2009753 HSBU
	Tehran, Touchal, Mehrabian, 2010243 HSBU
	Mazandaran, Ramsar, Mehrabian, 2009857 HSBU
	Azarbaijan, Sardasht, Mehrabian, 2009583 HSBU
O. pachypoda Boiss.	Tehran, Souhanak, 1900 m., Mehrabian, 20151345 HSBU
O. sabalanica Ponert	Ardebil, Meshkinshahr, Sabalan, 3600 m., Mehrabian, 2010201 HSBU
O. sericea Willd.	Azarbaijan, Silvana, Mehrabian, 2009214 HSBU
	Azarbaijan, Shahindezh, Mehrabian, 2010458 HSBU
O. sheidaii Mehrabian	Kurdestan, 15 km after Paveh to Ravansar, 1780 m., Mehrabian, 20123 HSBU (Holotype)
O. subsericea Freyn.	Azarbaijan, between Kiwi and Khalkhal, Rechinger, W-07869

and Bigazzi 1998, Keshavarzi et al. 2013 a and b). Selvi and Bigazzi (2001) studied leaf anatomy in Boraginaceae s.str. tribe Boragineae. Binzet and Akçin (2012) studied the anatomical properties of *O. frutescens* and *O. inexspectata*. There were differences in the arrangement and size of parenchyma and vascular tissues.

While some studies have explored the anatomical and ecological properties of certain *Onosma* species (Binzet and Teke 2014), a comprehensive anatomical investigation across a wider range of species is lacking. Akçin et al. (2013) and Akçin and Binzet (2019) considered the leaf epidermis of *Onosma* species of Turkey. They found that stomatal size, outer stomatal rims, and peristomatal rims showed some variation in the species studied. Some anatomical studies have been carried out on different parts of the root, stem, and leaves of some *Onosma* species (Güven et al. 2013; Binzet and Teke 2014; Özkan et al. 2016; Teke and Binzet 2017; Selvi et al. 2019; Azizian et al. 2000; Ulcay 2023).

Table 2. Quantitative and qualitative traits used in anatomical studies

Quantitative traits
Length to the width of the adaxial epidermis (basal leaf)
Length to the width of the abaxial epidermis (basal leaf)
Length to the width of the adaxial epidermis (cauline leaf)
Length to the width of the abaxial epidermis (cauline leaf)
Leaf blade thickness of the basal leaf
Leaf blade thickness of the cauline leaf
Midrib thickness of the basal leaf
Midrib thickness of the cauline leaf
Qualitative traits
Hair of basal leaf (glandular/eglandular)
Hair of cauline leaf (glandular/eglandular)
Hair type basal leaf (unicellular (uni)/multicellular (multi))
Hair type cauline leaf (unicellular (uni)/multicellular (multi))
Mesophyll of basal leaf (unilateral (1)/bilateral (2)/both shapes observed (3))
Mesophyll of cauline leaf (unilateral (1)/bilateral (2)/both shapes observed (3))
Midrib shape basal leaf (round/dome-shaped/smooth)
Midrib shape cauline leaf (round/dome-shaped/smooth)

As the *Haplotricha* subsection has the largest number of species in Iran and the diversity of leaf anatomical structure may contain diagnostic features, in the present study, an attempt has been made to evaluate the quantitative and qualitative traits of leaf anatomy of some *Onosma* species, to achieve a set of differential traits efficient in the determination of the boundaries of these species. By examining the leaf anatomy, we aim to identify diagnostic characteristics that can contribute to a more robust taxonomic framework for the genus and enhance our understanding of the species' diversity and relationships.

Materials and Methods

In this study, 16 accessions of 11 selected *Onosma* species (Table 1) collected from various regions of Iran were analyzed to investigate the cauline and basal leaf anatomical structure. The collected specimens were deposited in the Herbarium of Shahid Beheshti University (SHBU). For each species at each location, 3-5 individuals were

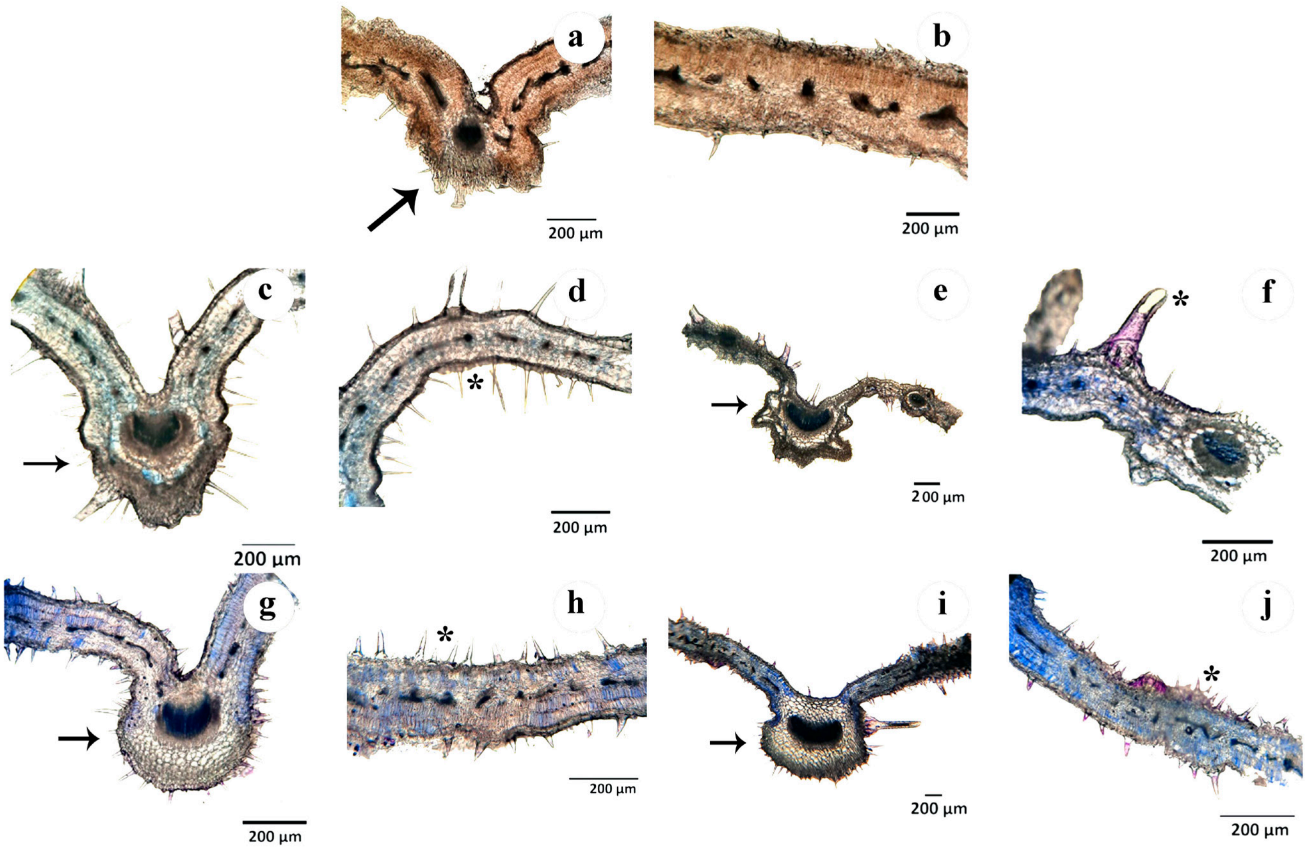

Figure 1. Leaf anatomical structure of midrib and leaf blade. a-b: cauline leaf of *O. assadii*; c-d: basal leaf, e-f: cauline leaf *O. bulbotricha*; g-h: basal leaf, i-j: cauline leaf of *O. dicroantha*. Arrows indicate the midrib, and asterisks indicate trichomes.

sampled. The measurements are based on 10 replicates for each accession per location.

Leaf samples were fixed in a water-alcohol-glycerin solution for anatomical study. Hand-cut cross-sections stained with Toluidine blue. Appropriate sections were photographed with an Olympus light microscope at different magnifications. The quantitative and qualitative traits evaluated are listed in Table 2. Measurements were performed by Digimizer v. 5.3.4 [16] software.

Both quantitative and ordinal anatomical characters were statistically evaluated to determine whether interspecific differences were significant. Prior to analysis, the quantitative variables were tested for normality; however, they did not satisfy the assumptions of normal distribution and homogeneity of variances. Therefore, the non-parametric Kruskal–Wallis test was selected as an appropriate alternative to one-way ANOVA for comparing the median values of the anatomical traits among species. This test is suitable for non-normally distributed quantitative data as well as ordinal variables, as it relies on ranked data and does not require normality or equality of variances. A significance threshold of $p < 0.05$ was applied to identify statistically significant differences. All statistical analyses were conducted using PAST software (version 4.03) (Hammer et al. 2001).

Standardized variables are used for multivariate statistical analysis. Multivariate statistical methods, including cluster analysis, principal component analysis, and factor analysis, were applied to the assessed traits. Average taxonomic distances and squared Euclidean distances are applied as dissimilarity coefficients in the cluster analysis of leaf anatomical data. The mean of measurements was used for quantitative traits, while qualitative features were coded as multistate. To determine the most variable characters among the species studied, factor analysis based on principal components analysis is performed.

Results

Leaf cross sections (Figs. 1-4) at midrib and leaf blades of the cauline and basal leaves of different species studied show some differences in selected features. Although there are great similarities in general leaf anatomy but

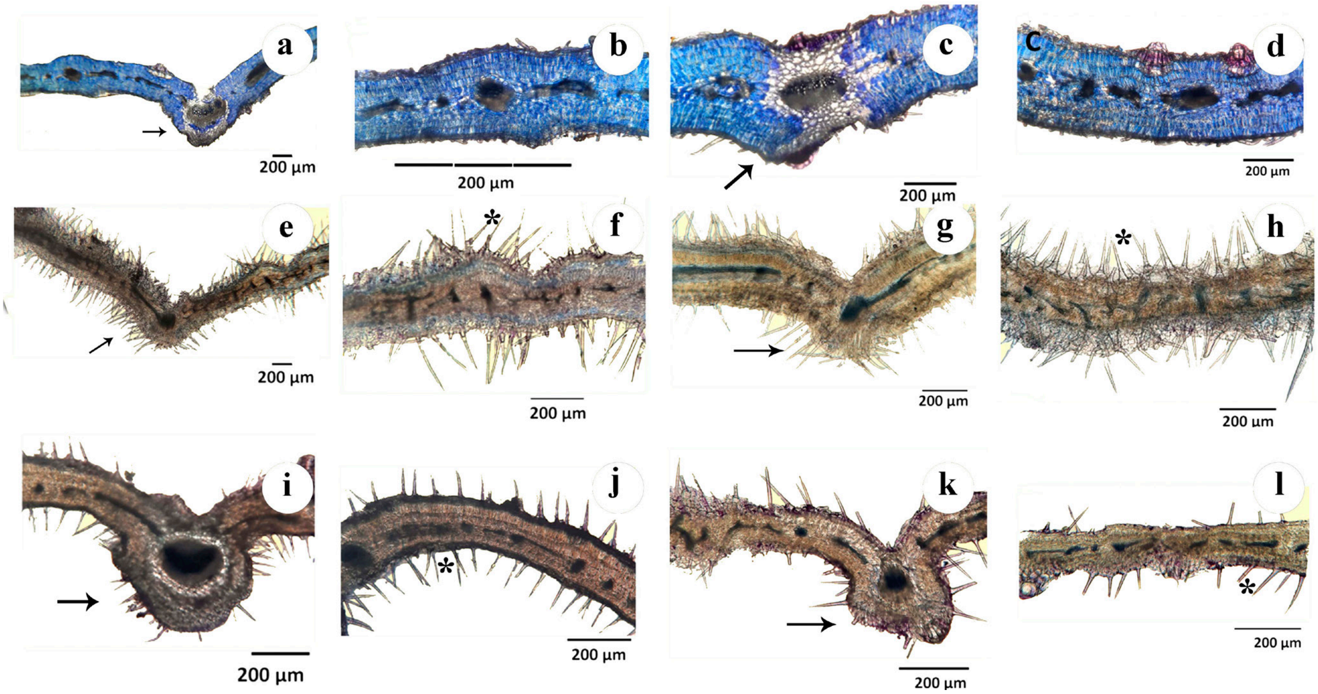

Figure 2. Leaf anatomical structure of midrib and leaf blade. a-b: basal leaf, c-d: cauline leaf of *O. gaubae*; e-f: basal leaf, g-h: cauline leaf of *O. longiloba*; i-j: basal leaf, k-l: cauline leaf *O. microcarpa*. Arrows indicate the midrib, and asterisks indicate trichomes.

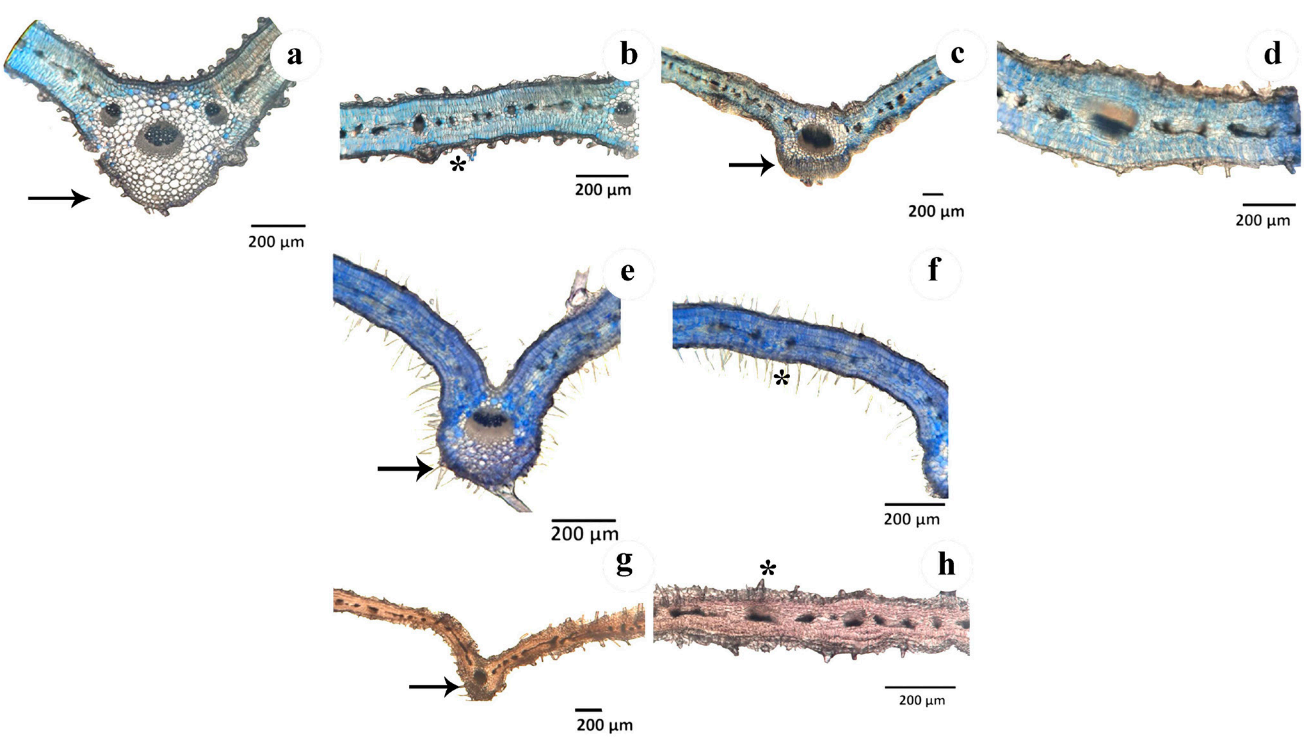

Figure 3. Leaf anatomical structure of midrib and leaf blade. a-b: basal leaf, c-d: cauline leaf of *O. pachypoda*; e-f: basal leaf of *O. sabalanica*; g-h: basal leaf of *O. sericea*. Arrows indicate the midrib, and asterisks indicate trichomes.

Table 3. Qualitative traits of leaf anatomical structure in the studied species.

	Hair on the epidermis		Hair type		Midrib shape		Mesophyll type	
Taxon	Basal leaf	Cauline leaf	Basal leaf	Cauline leaf	Basal leaf	Cauline leaf	Basal leaf	Cauline leaf
O. assadii	NA	Eglandular	NA	uni	NA	Round	NA	1
O. bulbotricha	Eglandular	Eglandular	uni	uni	Dome-shaped	Dome-shaped	3	3
O. dichroantha	Eglandular	Glandular/ Eglandular	uni	uni	Dome-shaped	Dome-shaped	2	2
O. gaubae	Eglandular	Eglandular	uni	uni	Smooth	Smooth	2	2
O. longiloba	Multistate/ Eglandular	Eglandular	uni	multi	Smooth	Round	2	2
O. microcarpa	Eglandular	Eglandular	uni	uni	Dome-shaped	Dome-shaped	3	3
O. pachypoda	Glandular/ Eglandular	Glandular/ Eglandular	uni	uni	Dome-shaped	Round	2	2
O. sabalanica	Glandular/ Eglandular	NA	uni	NA	Dome-shaped	NA	1	NA
O. sericea	Eglandular	NA	uni	NA	Dome-shaped	NA	3	NA
O. sheidaii	Glandular/ Eglandular	Glandular/ Eglandular	uni	uni	Dome-shaped	Dome-shaped	2	2
O. subsericea	Glandular/ Eglandular	Glandular/ Eglandular	uni	uni	Smooth	smooth	1	2

NA: not available.

there are also modifications in the shape of the midrib, the type of hairs and the mesophyll. Anatomical observations of leaf structure confirm variation in quantitative and qualitative traits (Tables 3 and 4).

The study of the divergence and similarity of *Onosma* species based on the evaluated anatomical traits indicates that in the UPGMA diagram (Fig. 5), two main clusters can be distinguished. *O. sabalanica* is grouped in a separate cluster, and the other species in a cluster, which is itself divided into two sub-clusters. *O. assadii* is located under the second sub-cluster, and the other species (*O. subsericea*, *O. longiloba*, *O. gaubae*, *O. sheidei*, *O. pachypoda*, *O. bulbotricha*, *O. microcarpa*, *O. sericea*, and *O. dichroantha*) are located under the first sub-cluster and have the greatest similarity. *O. assadii* and *O. sabalanica* have the greatest divergence from these species.

Factor analysis confirmed that the first three factors together accounted for more than 68% of the variation. In the first factor, with about 34% of the variation, the traits of basal leaf mesophyll thickness, basal and cauline leaf mesophyll structure, midrib thickness in basal leaves, and hair type in basal leaves were identified as the most diverse traits. In the second factor with 19.66% of observed variation, the traits of basal leaf hair type and cauline leaf mesophyll thickness showed the most variation, and in the third factor with 15.2% of observed variation, the length-to-width ratio of lower epidermal cells of cauline leaves accounted for the most variation.

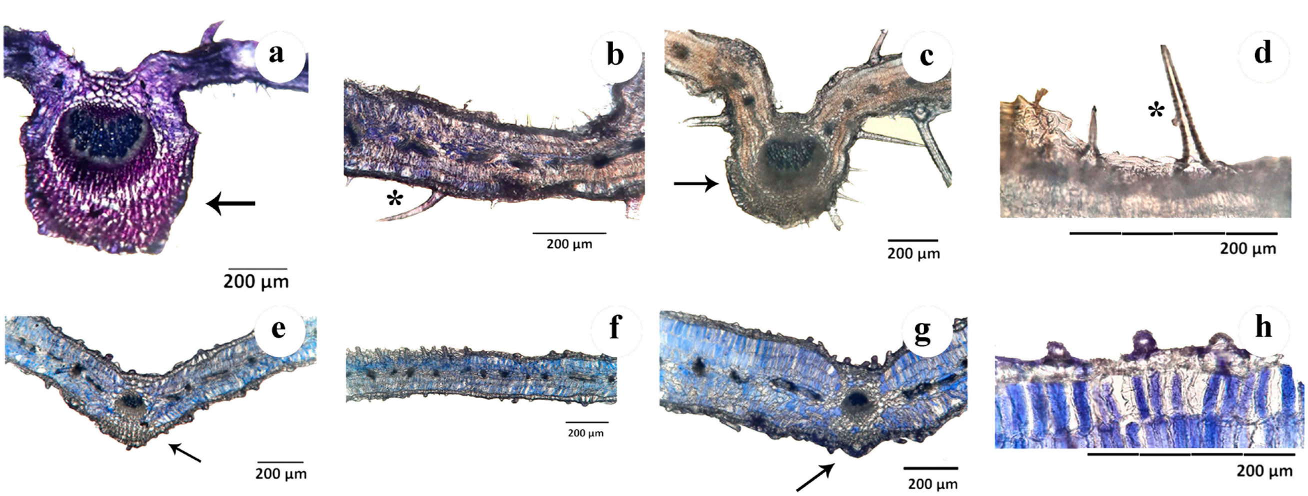

Figure 4. Leaf anatomical structure of midrib and leaf blade. a-b: basal leaf, c-d: cauline leaf of *O. sheidaii*; e-f: basal leaf, g-h: cauline leaf of *O. subsericea*. Arrows indicate the midrib, and asterisks indicate trichomes.

Table 4. Results of measured quantitative traits of leaf anatomical structure in the studied species (μm).

Taxon	Basal leaf blade	Cauline leaf blade	Basal leaf midrib	Cauline leaf midrib	Length/width of adaxial basal leaf	Length/width of adaxial cauline leaf	Length/width of abaxial basal leaf	Length/width of abaxial cauline leaf
O. assadii	NA*	257.78	NA	336.98	NA	0.92	NA	0.8
O. bulbotricha (Tabriz pop.)	220.21	233.49	329.08	413.95	0.86	0.89	0.95	1.64
O. bulbotricha (Paveh pop.)	176.53	216.58	630.64	747.89	1.16	1.15	1.02	1.14
O. dichroantha	217.07	216.07	422.36	643.98	0.98	0.74	1.66	1.51
O. gaubae	343.38	334.73	472.86	408.21	1.14	0.8	1.39	0.98
O. longiloba	244.57	258.01	360.96	333.91	0.96	1.01	0.91	NA
O. microcarpa (Arasbaran pop.)	155.39	128.34	397.03	260.68	1.44	0.72	1.74	1.21
O. microcarpa (Ramsar pop.)	148.58	209.82	289.29	364.81	NA	NA	NA	NA
O. microcarpa (Sardasht pop.)	139.99	173.22	303.18	398.9	1.11	0.96	1.19	1.32
O. microcarpa (Touchal pop.)	219.47	173.92	358.61	429.04	1.55	1.29	1.5	0.93
O. pachypoda	202	303.54	471.73	703.7	1.24	1.16	1.07	1.07
O. sabalanica	137.97	NA	313.92	NA	1.2	NA	0.71	NA
O. sericea (Shahindezh pop.)	228.94	206.7	346.73	518.6	NA	NA	NA	NA
O. sericea (Silvana pop.)	146.75	178.23	307.91	420.24	0.8	1.25	1.26	0.99
O. sheidaii	225.88	215.68	612.96	460.1	0.9	1.02	1.19	1.33
O. subsericea	202.73	241.58	306.53	335.09	1	0.86	1.22	0.77

NA: not available.

O. assadii and *O. sabalanica* have the greatest divergence compared to other species studied, and the other species studied are close together, indicating the similarity of these species, which was also observed in the UPGMA diagram**.**

PCA analysis of leaf anatomical features in different species of *Onosma* studied in Iran revealed that specimens located close together have more similarities in features and are likely to be related species (Fig. 6). Species located apart show strong structural differences. Some species have more common features and are located adjacent to each other. *O. sabalanica*, *O. assadii* are located in different parts of the scatter diagram, indicating structural differences.

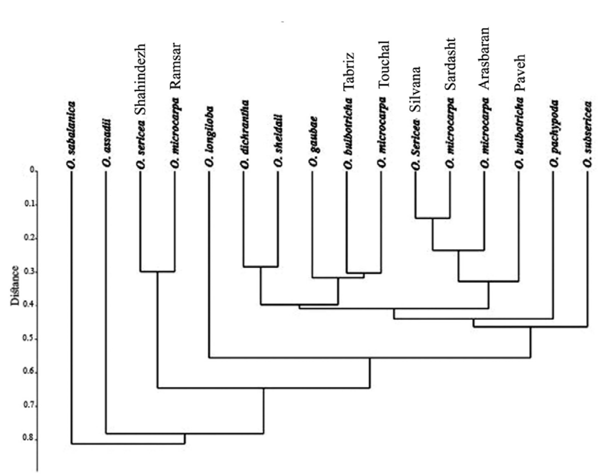

Figure 5. UPGMA diagram of the studied *Onosma* species based on leaf anatomical characters.

Discussion

As stated in the studies of Azizian et al. (2000), the leaf mesophyll structure in the species *O. longiloba*, *O. dichroantha*, and *O. bulbotricha* of the Azerbaijan population is isobilateral, but in the Kermanshah population of *O. bulbotricha*, a unilateral structure was observed. Also, non-glandular hairs are simple. The species *O. dichroantha*, *O. longiloba*, and *O. bulbotricha* have short, unicellular glandular hairs, but in the present study, this type of hair was observed only in the cauline leaves of *O. dichroantha*, and the other species mentioned do not agree with the studies of Azizian et al. (2000) in terms of this trait.

The mesophyll structure of the leaves of *O. sericea* from the Shahindezh population is consistent with the results of Selvi and Bigazzi (2019) and is of bilateral structure. However, the mesophyll structure of *O. sericea* from the Silvana population was observed to be unilateral.

In anatomical studies conducted on the species of the *Haplotricha* suborder, it seems that the shape of the midrib in the same species differs slightly in the cauline and basal leaves, such that the basal leaves are often more curved than the cauline leaves. Also, anatomical studies showed

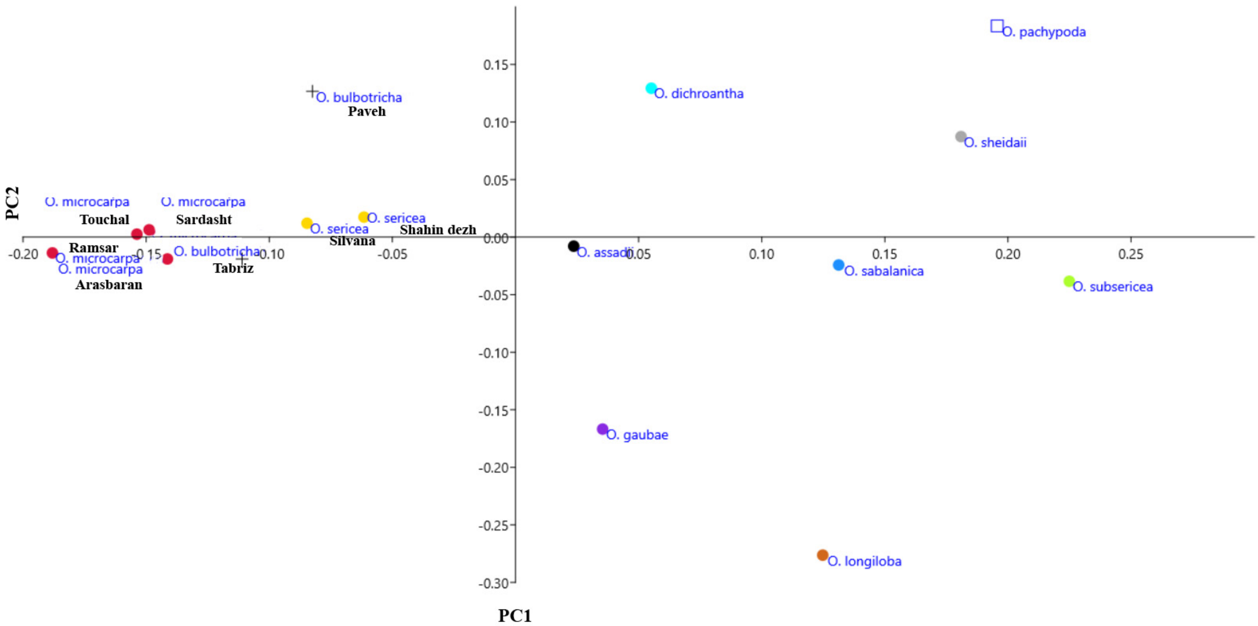

Figure 6. Principal component analysis plot of the studied *Onosma* species based on leaf anatomical traits.

that most of the studied specimens have unicellular and non-glandular hairs, and in a small population, glandular and multicellular hairs were observed.

In the leaf anatomical study of selected *Onosma* species, the significant differences between the cauline and rosette leaves were observed by Belaeva and Butenkova (2020). They pointed out that the palisade mesophyll of the cauline leaves was more developed than the basal leaves. These authors stated that the amount of environmental radiation was different for the cauline and the basal leaves.

Some anatomical features in leaves resemble xeromorphic tendencies, such as cuticle thickness, a well-developed mass of collenchyma on both sides of the midrib, and the number of palisade layers abaxially and adaxially. It should be noted that our findings indicate that leaf anatomical studies in *Onosma* possess diagnostic value.

Acknowledgements

The authors are indebted to the Vice President of Research and Technology of Alzahra University for their partial financial support of this project. We are thankful to the anonymous referees for improving the manuscript. This study followed ethical principles (Code: IR.Alzahra.REC.1400.021).

References

Akçin ÖE (2009) Micromorphological and anatomical studies on petals of 11 Turkish *Onosma* L. (Boraginaceae) taxa. Bangladesh J Plant Taxon 16(2):157-164.

Akçin ÖE, Binzet R (2019) Anatomy of three *Onosma* species from Turkey. Bangladesh J Bot 48(2):329-337.

Akçin ÖE, Şenel G, Akçin Y (2013) Leaf epidermis morphology of some *Onosma* (Boraginaceae) species from Turkey. Turk J Bot 37(1):55-64.

Azizian D, Khatamsaz M, Kasaian J (2000) The taxonomic significance of leaf anatomy in the genus *Onosma* L. (Boraginaceae) in Iran. Iran J Bot 8(2):167-180.

Belaeva TN, Butenkova AN (2020) Leaf blade anatomy of the rare Siberian flora species *Mertensia sibirica* (L.) G. Don fil. (Boraginaceae). Ukr J Ecol 10(5):186-191.

Binzet R, Akçin ÖE (2012) The anatomical properties of two *Onosma* L. (Boraginaceae) species from Turkey. J Med Plants Res 6(17):3288-3329.

Binzet R, Teke HI (2014) The anatomical properties of *Onosma mollis* DC. and *Onosma* halophila Boiss. & Heldr. (Boraginaceae) from Turkey. Pak J Bot 46(5):1663-1668.

Hammer Ø, Harper D, Ryan PD (2001) PAST: Paleontological statistics software package and data analysis. Paleontol Electron 4(1):1-9.

Kumar N, Kumar R, Kishore K (2013) *Onosma* L.: A review of phytochemistry and ethnopharmacology. Pharmacogn

Rev 7(14):14-151.

Mehrabian AR (2015) Distribution patterns and diversity of *Onosma* in Iran: with emphasis on endemism conservation and distribution pattern in SW Asia. Rostaniha 16(1):36-60 [In Persian].

Mehrabian AR, Mozaffarian V (2018) Seven new species of *Onosma* L. (Boraginaceae) with emphasis on their habitats in Iran. Taiwania 63(4):366-388.

Mehrabian AR, Arabameri M, Sadeghi S (2017) Stigma morphology in *Onosma* spp.(Boraginaceae) with emphasis on its systematic implication in Iran. Bangladesh J Bot 46(4):1269-1276.

Mehrabian AR, Sheidai M, Mozaffarian V (2013) Three new species of *Onosma* L. (Boraginaceae) from Iran. Feddes Repert 124(2-3): 69-79.

Mehrabian AR, Sheidai M, Mozaffarian V (2014) Micromorphology of leaf trichomes in *Onosma* (Boraginaceae) and their systematic relevance in Iran. Phytol Balcan 20(1): 41-56.

Mehrabian AR, Sheidai M, Noormohammadi Z, Mozafarian V, Asrei Y (2012) Palynological diversity in the genus *Onosma* L. (Boraginaceae) of Iran. Ann Biol Res 3(8):3885-3893.

Nasrollahi F, Kazempour-Osaloo S, Amini E, Mozaffarian V (2020) Morphological character evolution of *Onosma* (Boraginaceae) in Iran based on nuclear and plastid DNA sequences. Iran J Bot 26(1):35-48.

Özkan M, Kariptaş E, Özdemir C, Kandemir A, Akyol Y (2016) Morphological and anatomical characterization and trace elements composition of *Onosma argentata* Hub. - Mor. (Boraginaceae) endemic to Turkey. Pak J Bot 48(1):187-191.

POWO (2024) (Plants of the World Online) *Onosma* L. Facilitated by the Royal Botanical Gardens, Kew. Plants of the World Online/ Kew Science (Accessed 15 December 2024).

Riedl H (1967) Boraginaceae. In Rechinger KH, Ed. Flora Iranica, Vol 48. Akademische Druk-U. Verlagsanstalt, Graz, Austria.

Saravanakumar K, Sarıkürkçü C, Şahinler SŞ, Sarıkürkçü RB, Wang MH (2021) Phytochemical composition, antioxidant, and enzyme inhibition activities of methanolic extracts of two endemic *Onosma* species. Plants 10(7):1373.

Selvi F, Bigazzi M (2001) Leaf surface and anatomy in Boraginaceae tribe Boragineae with respect to ecology and taxonomy. Flora 196(4):269-285.

Teke HI, Binzet R (2017) Anatomical, morphological and palynological studies of some *Onosma* L. (Boraginaceae) taxa endemic to Anatolia. Pak J Bot 49(2):579-588.

Teppner H (2011) Flowers of Boraginaceae (*Symphytum*, *Onosma*, *Cerinthe*) and. Andrena symphyti (Hymenoptera-Andrenidae)**:** Morphology, pollen portioning, vibratory pollen collection, nectar robbing. Phyton (Horn, Austria) 50(2):145-180.

Ulcay S (2023) A research on the anatomical and ecological characteristics of *Onosma mollis* DC. (Boraginaceae). Sak Univ J Sci 27(4):813-821.