

Anatomical study of some *Erodium* (Geraniaceae) species in Iran

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Keshavarzi M., Najafian E., Nazem Bokaee Z. & Saifali M. (2016):
Anatomical study of some *Erodium* (Geraniaceae) species in Iran. –
Thaiszia – J. Bot. 26 (1): 11-20. – ISSN 1210-0420.

Abstract: The genus *Erodium* (Geraniaceae) composed of 75 species in all continents except Antarctica. Its main center of diversification is Mediterranean region with 62 species. There are 15 annual and perennial *Erodium* species in Iran. In present study stem, leaf and petiole anatomical structures were considered for 16 populations of 7 *Erodium* species. Totally 18 qualitative and 27 quantitative features were evaluated and measured. Anatomical data revealed a significant variation between studied species. The most diagnostic characters were shape of abaxial leaf cross sections and form of gaps, diameter of stem cross section, presence or absence of fiber in petiole structure, number and shape of vascular bundles in stem, stem hair type, shape of adaxial surface of midrib and number of vascular bundles in petiole. Studied taxa were clearly separated by selected anatomical characters.

Keywords: anatomical structure, *Erodium*, Geranicaceae, leaf, stem, petiole.

Introduction

Erodium Aiton (Geraniaceae) with 75 species is distributed in all continents except Antarctica (FIZ et al. 2006). Its main diversification center is Mediterranean region with 62 species (FIZ et al. 2006). Endemics of this genus are limited to 4 in Asia and 1 in Iran (SCHÖNBECK-TEMESY 1970).

KNUTH (1912) subdivided *Erodium* to two sections: *Plumosa* (5 species) and *Barbata* (10 subsections). SCHÖNBECK-TEMESY (1970) followed the Boissier system, but used the *Erodium* name for the *Barbata* section. Guittonneau considered two sections as *Plumosa* and *Barbata* (EL NAGGAR 1991). This classification was based on features of mericarp beak and awn. In *Plumosa* section, beak is caudous and awn is plumose while in *Barbata* section, beak is persistent and awn is not plumose.

In Iran, *Erodium* is grouped in two sections; *Plumosa* Boiss. and *Erodium* Boiss. and three subsections; *Absinthioidea* Brumhard, *Malacoides* Lange and *Cicutaria* Lange (SCHÖNBECK-TEMESY 1970). *Erodium* species are found in different parts of Iran (JANIGHORBAN 2005, SCHÖNBECK-TEMESY 1970). Most of these species are Irano-Touranian and Saharo-Sindian elements. Only one species is endemic in Hyrcanian region (JANIGHORBAN 2005).

Geraniaceae elements are of medicinal and ornamental importance and have secretory hairs in their aerial parts. FODOREA & TĀMAŞ (2005) studied the root, shoot and leaf anatomical structure of *Geranium Palustre* Cent. They found that calcium oxalate crystals are distributed abundantly in all parts of plant and grouped stem vascular bundles as external and internal. They pointed to the differences in presence of bundle sheath. ONSORI et al. (2010) used stem anatomical features as diagnostic characters while recording the presence of *G. linearilobum* in Iran. SALIMPOUR et al. (2009) studied stem and leaf anatomical features of ten *Geranium* species in Iran. They found that stem outline in cross section, number of vascular bundles and presence and shape of crystals and shape of leaf epidermis cells are of diagnostic importance.

Erodium species are not efficiently separated due to morphological similarities. The main aim of present study is to find some anatomical diagnostic features. Species relationship based on anatomical structure in some *Erodium* species is evaluated for the first time in Iran.

Material and methods

Anatomical studies were conducted on 15 accessions of 7 *Erodium* species gathered from different habitats of Iran (Tab. 1). The stem, petiole and leaf cross sections of at least three individuals from each accession were studied. Studied specimens are deposited in Herbarium of Alzahra University (ALUH).

Hand-made sections were soaked in sodium hypochlorite for 45 minutes and double-colored by use of carmine and methylene green. Slides were studied by different magnitude of Olympus light microscope (Bx51). Best sections were photographed by DP12 digital camera. Qualitative and quantitative anatomical features based on AEDO (2007) and our laboratory observations were evaluated and measured (Tab. 2 & 3).

In order to conduct multivariate statistical analysis, qualitative features were coded as binary or multistate characters and mean values for quantitative features were used. Primary statistical analysis was used to clarify the significance of observed quantitative variations. To present the species relationships, cluster analysis, factor analysis and ordination method based on

principal component analysis were used. Factor analysis was done to distinguish the most variable features and to illustrate the contribution of each features in observed variation. Statistical analyses were done using SPSS ver. 19.

Tab. 1. Voucher details of studied accessions.

Section	Species	Accession details
<i>Barbata</i>	<i>E. cicutarium</i> (L.) L'Hér. ex Aiton	Alborz, Karaj, Ardalan, 1427 m., Taghipour, 913. Kermanshah, Goaver, Latechegha village, 1550 m., Najafian, 921. Semnan, Shahroud, 1322 m., Rezaii, 891.
	<i>E. ciconium</i> (Jusl.) L'Hér. ex Aiton	Alborz, Karaj district, 1412 m., Agheli and Tavakoli, 821. Tehran, Jahanekeodak park, 1422 m., Amiri, 681. Lorestan, Khoramabad, 1447 m., Ganjivahed, 651.
	<i>E. gruinum</i> (L.) L'Hér. ex Aiton	Khuzestan, Behbahan district, 319 m., Sanaee, 901. Kermanshah, Gulinagharb, Vignan, 1282 m., Najafian, 922.
	<i>E. malacoides</i> (L.) L'Hér. ex Aiton	Fars, Kazerun, 841 m, Behtash, 902. Kermanshah, Guliangharb, Vignan, 1282 m., Najafian, 924.
	<i>E. moschatum</i> (L.) L'Hér. ex Aiton	Khuzestan, Haft tapeh, 76 m., Keshavarzi, 851. Kermanshah, Gilane gharb, Vignan, 1282 m., Najafian, 924.
	<i>E. neuradifolium</i> Delile ex Godron	Qom, Huze sultan lake, 801 m, Kazemzadeh, 82.
<i>Plumosa</i>	<i>E. oxyrrhynchum</i> M. Bieb.	Alborz, Karaj, Jahanshahr, 1338 m., Taghipour, 892. Tehran, national botanical garden region, 1268 m, Taghipour, 912.

Tab. 2. Studied quantitative features of stem, petiole and leaf sections of *Erodium* in this study.

1. Epidermis thickness in stem sections(μ)	15. Distance between epidermis to pit in stem section (μ)
2. Collenchymas thickness in stem sections (μ)	16. Length of mid vein in leaf (μ)
3. Cortex parenchyma diameter in stem sections (μ)	17. Width of mid vein in leaf (μ)
4. Sclerenchymas fiber diameter in stem sections (μ)	18. Number of Collenchymas layers is leaf ventral surface
5. Collenchymas to epidermis diameter in stem section	19. Depth of fovea in leaf dorsal surface(μ)
6. Collenchymas to parenchyma diameter in stem sections	20. Width of fovea in leaf dorsal surface (μ)
7. Fiber to Collenchymas diameter in stem sections	21. Number of Parenchyma layers in leaf ventral surface
8. Fiber to parenchyma diameter in stem sections	22. Petiole length (μ)
9. Number of vascular bundle in stem section	23. Petiole width (μ)
10. Number of secondary vascular bundle in stem section	24. Epidermis distance to center of petiole(μ)
11. Length of vascular bundle in stem section(μ)	25. Epidermis thickness in leaf(μ)
12. Width of vascular bundle in stem section(μ)	26. Vascular bundle length (μ)
13. Length of stem section (μ)	27. Vascular bundle width(μ)
14. Width of stem section (μ)	

Tab. 3. Qualitative anatomical features evaluated for *Erodium* species of Iran.

No.	Character	State of character
1	Hair type at petiole	Simple(1), simple and glandular(2)
2	Shape of vascular bundle in stem section	Oval(1), oval to elliptic(2)
3	Vascular bundle size in stem section	Same (1), not same(2)
4	Fiber in stem section	Continuous(1), discrete(2)
5	Shape of petiole section	rounded (1), elliptical(2), triangular(3)
6	External surface of petiole	Smooth(1), wavy(2)
7	Fiber in petiole central vascular bundle	Presence(1), absence(2)
8	Shape of leaf mid vein	Rounded(1), elliptic(2)
9	Hair at leaf dorsal mid vein	Presence(1), absence(2)
10	Hair at adaxial mid vein surface of leaf	Presence(1), absence(2)
11	Adaxial surface of mid vein	Smooth(1), wavy (2)
12	Abaxial surface of mid vein	Depressed(1), dished(2)
13	Calcium oxalate crystal shape	Rounded(1), angular(2)
14	Hair type at stem section	Simple(1), simple and glandular(2)
15	Epidermis cells	Regular(1), irregular(2)
16	Stem sections outline	Circular(1), elliptic(2)
17	External stem surface	Smooth(1), wavy(2)
18	Vascular bundle size in petiole	Of Same size(1), not so (2)

Results

Stem sections showed rounded to elliptical outline with smooth or wavy surface. Number, size and shape of vascular bundles, collenchymas, parenchymas and fiber diameter showed variation in studied taxa. Stem anatomical structure is composed of epidermis, collenchymas, cortex parenchyma, fiber, phloem, xylem and pit parenchyma. In *E. cicutarium* accessions, hair frequency and type showed significant modifications. Vascular bundles are 11 and in two sizes. In *E. ciconium* hair frequency show some modifications in different accessions. Vascular bundles are 12 to 15, oval and in two sizes. In *E. gruinum* stem cross section there are 14 to 18 vascular bundles in two or three sizes. 10 ovate vascular bundles in two sizes with elliptic cross section outline were found in *E. malacoides*. In *E. moschatum* there are 14 to 17 vascular bundles, cross section is elliptic shaped, and vascular bundles could be grouped in two or three sizes. In *E. neuradifolium* there are 10 vascular bundles in two sizes. In *E. oxyrhynchum* there are ten elliptic vascular bundles all in a same size.

Petiole cross sections showed different shapes from triangular, rounded to elliptic. Shape and number of vascular bundles is different. In *E. cicutarium* petiole hair frequency and shape showed modifications in different accessions. Number of vascular bundles is 4-5 in two sizes with triangular outline and without fiber in vascular bundle. In *E. ciconium* the number of vascular bundles is 6 to 12. Vascular bundles are in two to three sizes with triangular shape and presence of fiber in bundles. In *E. gruinum* the number of vascular bundles is 6 to 10 with two to three sizes. In *E. malacoides* there are six triangular vascular bundles without fiber. In petiole cross section of *E. moschatum* the number of

vascular bundle is 6 to 10 with two to three sizes. Section outline is triangular, without fiber in vascular bundles. In *E. neuradifolium* there is hair abundance in petiole sections. Vascular bundles are 6 and triangular with fiber. *E. oxyrrhynchum* has 5 vascular bundles in rounded petiole sections in two sizes with fiber presence at bundles.

Leaf sections show circular to elliptical outline at mid rib of studied species. There are different number and size of vascular bundles. In three accessions of *E. ciconium* different hair frequency was observed at mid rib. Vascular bundles were 1 to 2, mid rib was circular and there were two types of hairs (simple and glandular). In the cases of *E. gruinum*, *E. malacoides* and *E. neuradilolium* mid rib shape was round and there was only one vascular bundle at mid rib. In *E. oxyrrhynchum* mid rib was round- elliptic with 1 to 5 vascular bundles.

In stem, leaf and petiole anatomical structure, 21 features of 45 qualitative and quantitative features were of diagnostic importance. In stem and petiole cross sections there were hairs, epidermis, collenchymas, cortex parenchyma, bilateral phloem and pit parenchyma (Figs. 1 & 2). In leaf sections, hairs were observed at both surfaces (Fig. 3).

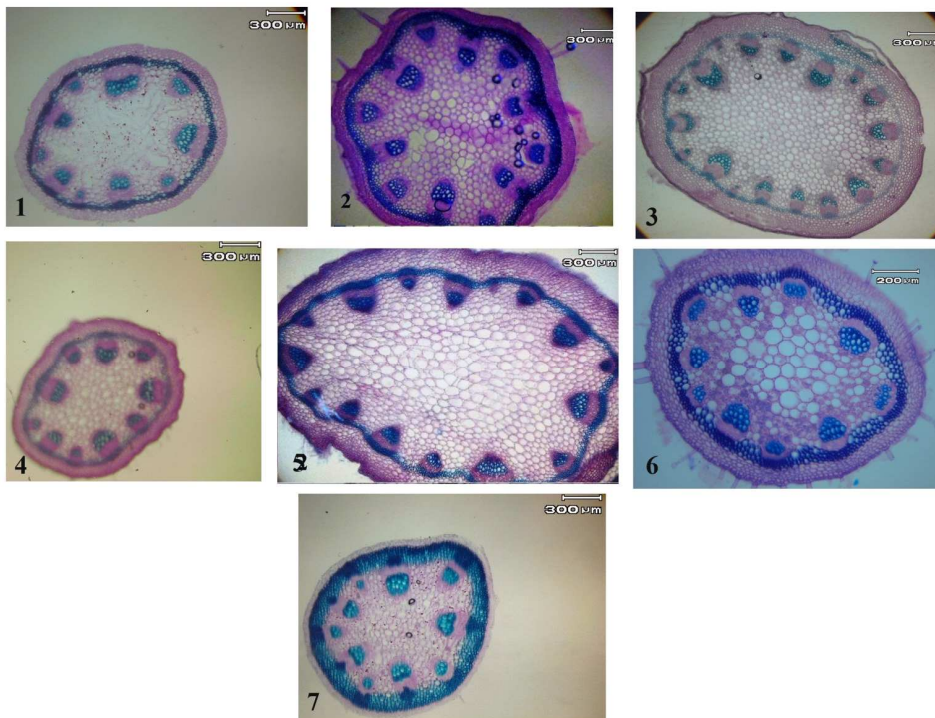


Fig. 1. Stem anatomical structure in 1) *Erodium cicutarium*, 2) *E. ciconium*, 3) *E. gruinum*, 4) *E. malacoides*, 5) *E. moschatum*, 6) *E. neuradifolium*, 7) *E. oxyrrhynchum*.

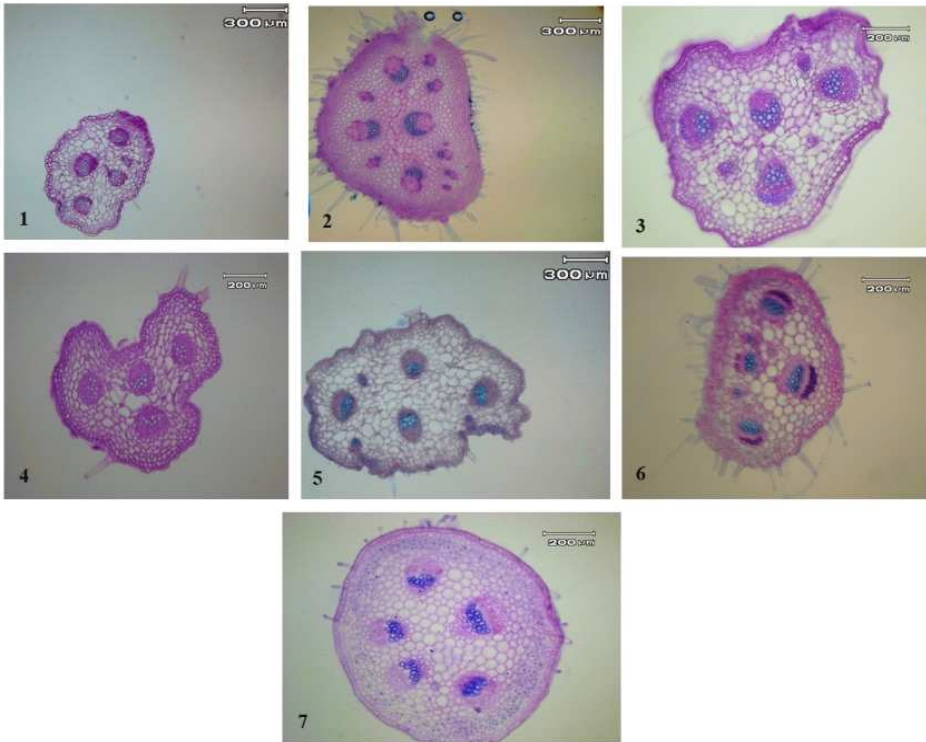


Fig. 2. Petiole anatomical structure in 1) *Erodium cicutarium*, 2) *E. ciconium*, 3) *E. gruinum*, 4) *E. malacoides*, 5) *E. moschatum*, 6) *E. neuradifolium*, 7) *E. oxyrhynchum*.

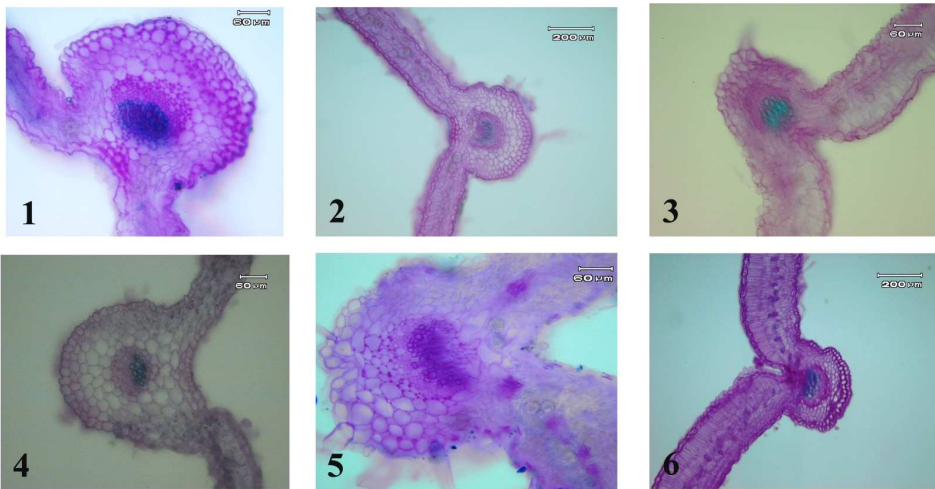


Fig. 3. Leaf anatomical structure in 1) *E. ciconium*, 2) *E. gruinum*, 3) *E. malacoides*, 4) *E. moschatum*, 5) *E. neuradifolium*, 6) *E. oxyrhynchum*.

In order to clarify the species relationships, cluster analysis by WARD method was done based on average of quantitative anatomical features (Fig. 4). The phenogram illustrated that there were two main clusters. Main cluster is divided into two sub-clusters and *E. cicutarium*, *E. malacoides*, *E. moschatum* species are grouped there. *E. cicutarium* is more related to *E. malacoides* than *E. moschatum*. In second cluster *E. gruinum* and *E. ciconium* are grouped. In second main cluster *E. oxyrrhynchum* and *E. neuradifolium* are grouped. Species are clearly separated from each other.

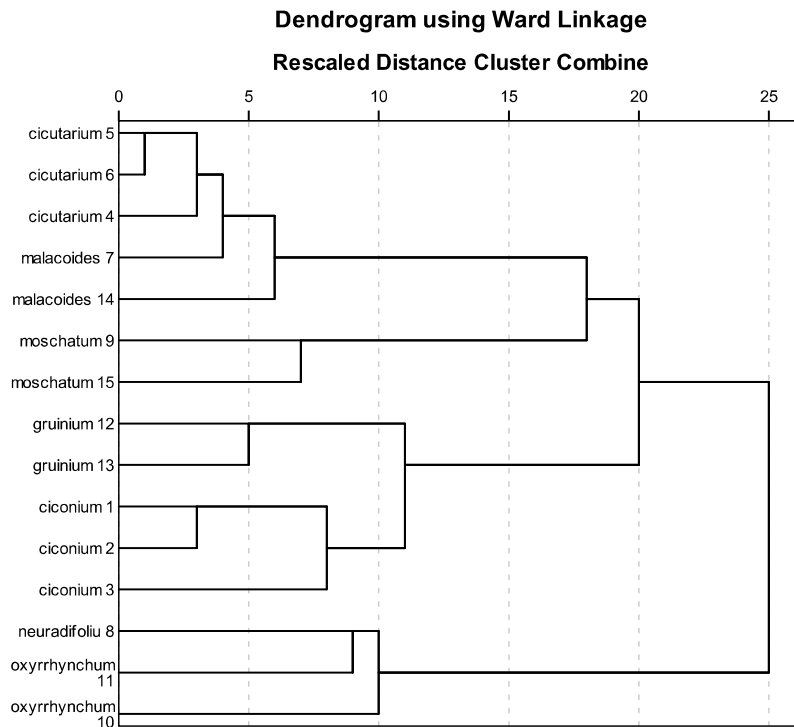


Fig. 4. Phenogram by WARD method based on average of stem, leaf and petiole anatomical features in some *Erodium* species of Iran

Factor analysis revealed that first four factors caused almost 64 % of total observed variation. In first factor by 22 % of total variation, fovea depth in dorsal leaf surface, shape of dorsal mid rib surface, length of stem section, length of vascular bundle, presence of fiber in petiole, number of stem vascular bundles showed most correlation. In second factor with 18 % of total variation, features as length of stem vascular bundles, stem vascular bundle shape, hair type at stem cross section and ventral mid rib shape showed most correlation. In the third factor by 12 % of observed variation, shape of calcium oxalate crystal in leaf showed most correlation and in fourth factor (with 8 % of variation), the effective

character is only the number of vascular bundles in petiole (Tab. 4). PCA scatter diagram based on two main factors revealed the species relationships and distance (Fig. 5).

Tab. 4. Results of factor analysis in studied *Erdoium* species.

Features	1 st factor	2 nd factor	3 rd factor	4 th factor
Fovea depth at leaf dorsal surface	0.82	-	-	-
shape of dorsal surface of mid rib	0.78	-	-	-
Stem section length	0.76	-	-	-
Vascular bundle length	0.75	-	-	-
Presence of fiber in petiole	0.72	-	-	-
Number of stem vascular bundle	0.7	-	-	-
Stem vascular bundle length	-	0.87	-	-
Stem vascular bundles shape	-	0.76	-	-
Hair type at stem section	-	0.74	-	-
Shape of mid rib at adaxial surface	-	0.72	-	-
Shape of calcium oxalate	-	-	0.83	-
Number of subsidiary vascular bundles in petiole	-	-	-	0.82

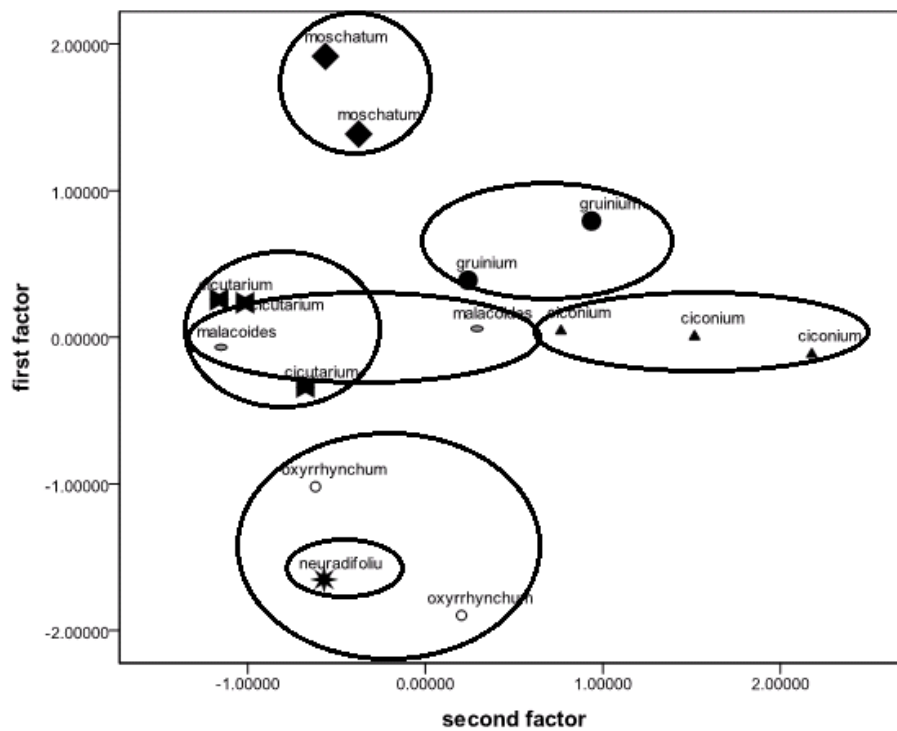


Fig. 5. PCA scatter diagram base on stem, leaf and petiole anatomical features of some *Erdoium* species in Iran.

Discussion

Results of anatomical studies clearly revealed that species are separated at sectional level from each other. Main cluster is composed of *E. cicutarium*, *E. malacoides*, *E. moschatum*, *E. gruinum* and *E. ciconium* all of which are belonged to *Barbata* section. *E. oxyrrhynchum* is nested at second cluster which belonged to *Plumosa* section. FIZ et al. (2006) illustrated such separation while studying the *Erodium* phylogenetic tree.

Neighboring position of *E. neuradifolium* and *E. oxyrrhynchum* can be due to similarities in hair of dorsal and ventral leaf epidermis, dorsal shape of mid rib, outline of stem section and shape of stem vascular bundles. The importance of stem sections outline was mentioned previously by PARK & JIM (1997), who had studied 35 species of *Geranium* and distinguished two subgenera. Results of present study again emphasized on the importance of such features.

SALIMPOUR et al. (2009) and ONSORI et al. (2010) pointed to the diagnostic and taxonomic importance of stem outline, number of parenchyma layers in leaf and presence of calcium oxalate crystals in *Geranium* species of Iran. Our results are in concordance with their findings.

CARLQUIST & BISSING (1976) had studied *Geranium* species of Hawaii Island to evaluate the effects of ecological factors on leaf anatomical features. They believed that although some characters can be affected by environmental condition, hairs, cuticle and vascular bundles features are constant and have taxonomic importance. They mentioned that isolateral leaves with one hypodermal layer beside each leaf surface, two hypodermis like layers beyond epidermis at mid rib and stomata number at lower surface two times of upper one are characteristic of xerophytic nature of species in this genus. In present study only *E. oxyrrhynchum* showed such characters which could be referred as probable xerophytic nature.

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Received: January 07th 2015
Revised: January 23rd 2016
Accepted: January 29th 2016