

**A short glance on leaf anatomy and taxonomy of subfamily *Caryophylloideae* in Iran**

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*Caryophyllaceae* is the fifth largest Iranian plant family in terms of number of species where most of its species are Irano-Turanian. About 30% of these species are endemic of Iran. There are continuously uncertainties regarding the circumscription of genera, and especially because of new molecular and phylogenetic studies that in many cases do not confirm the traditional generic circumscription, the need for tangible traits to distinguish between the genera is felt. Accordingly, in this study, leaves' anatomical characteristics of some species of *Caryophylloideae* from Iran have been described. Forty-nine characters (including 16 qualitative and 33 quantitative characters) of leaves' transverse sections and epidermis have been considered. No anatomical characteristics are constant in all species of the genera, but some of them such as stomatal patterns, limb symmetry, collenchymatous tissue and layers of fiber around the central vascular bundles may be useful to separate some genera. Contemplating morphological and anatomical features, two new combinations and a key for the genera of this subfamily in Iran have been provided. *Psammophiliella* has been recorded for the first time from Iran.

**Keywords:** *Caryophyllaceae*, new combination, *Petrohragia*, *Psammophiliella*, qualitative and quantitative characters**نگاهی به تشریح برگ و آرایه‌شناسی زیر تیره کاربوفیلوئیده در ایران**

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**خلاصه**

تیره میخکیان پنجمین تیره بزرگ ایران از نظر تعداد گونه است که غالب گونه‌های آن پراکنش ایران-تورانی دارند. حدود ۳۰٪ گونه‌های این تیره انحصاری کشور ایران هستند. پیوسته ابهاماتی در تعیین حدود جنس‌های میخکیان وجود داشته و به خصوص براساس مطالعات مولکولی و تبارشناختی جدید که در بسیاری موارد حدود سنتی جنس‌ها را تایید نمی‌کنند، لزوم یافتن صفات ملموسی برای تشخیص جنس‌ها از یکدیگر احساس می‌شود. بر این اساس در این پژوهش، خصوصیات تشریحی برگ برخی گونه‌های ایرانی زیر تیره کاربوفیلوئیده شرح داده شده است. چهل و نه صفت (شامل ۱۶ صفت کیفی و ۳۳ صفت کمی) برش عرضی و اپیدرم برگ‌ها مورد مطالعه قرار گرفته است. همه صفات تشریحی در بین گونه‌های بررسی شده یک جنس ثابت نیستند، ولی بعضی از آن‌ها مانند الگوی روزنه‌ها، تقارن پهنک، وجود بافت کلانشیم و وجود لایه‌های فیبر در اطراف دسته آوندی مرکزی، می‌توانند در جدایی برخی جنس‌ها مفید باشند. با توجه به صفات ریخت‌شناختی و تشریحی، دو ترکیب جدید آرایه‌شناختی و کلید شناسایی برای جنس‌های این زیر تیره در ایران تهیه شده و جنس *Psammophiliella* برای نخستین بار از ایران گزارش شده است.

**واژه‌های کلیدی:** پتروژایا، پساموفیلیلا، ترکیب جدید، صفات کیفی و کمی، میخکیان

## Introduction

*Caryophyllaceae* Juss. is a large and cosmopolitan flowering plant family; however, the systematic of many of its taxa has been ambiguous, due to a lack of definite morphology and complete matching of morphological and molecular studies' results (Greenberg & Donoghue 2011, Madhani *et al.* 2018). As a matter of fact, floral features such as calyx characters and the number of styles are very important in circumscription of taxa in *Caryophyllaceae*. (Boissier 1867, Schischkin 1936, Rechinger 1988) However, in delimitation of some genera like *Saponaria* L., and *Silene* L. some of these characters sometimes have been neglected. For instance, in spite of their difference in the number of styles and capsule teeth, *Lychnis* L. has been mentioned as a synonym for *Silene* in Flora Iranica (Melzheimer 1988) and many researchers have put various sets of taxa in *Saponaria* (Barkodah 1962, Rechinger 1988).

The subfamily *Caryophylloideae* Rabeler & Bittrich with nearly 240 species comprises about 3% of angiosperms' flora of Iran (Ghahremaninejad *et al.* 2017). *Caryophylloideae* includes *Acanthophyllum* C.A.Mey., *Agrostemma* L., *Allochrusa* Bunge ex Boiss., *Ankyropetalum* Fenzl, *Dianthus* L., *Diaphanoptera* Rech.f., *Gypsophila* L., *Petrorhagia* Link, *Psammophiliella* Ikonn., *Saponaria*, *Silene*, *Vaccaria* Wolf, and *Velezia* L. in Iran. *Silene* with around 117 species (Gholipour & Sheidai 2009, Nejadi Edalatiyan *et al.* 2010, 2011, Gholipour & Parsa Khanghah 2015, Gholipour *et al.* 2016, Hoseini & Assadi 2016, Gholipour 2017, Gholipour & Aminirad 2017, Nejadi Edalatiyan *et al.* 2017, Gholipour 2018), *Dianthus* with nearby 38 species (Assadi 1985, Rechinger 1988, Vaezi *et al.* 2014), *Gypsophila* with 38 species (Nejad Falatoury *et al.* 2015a, b, 2016a, b, c, Nejad Falatoury & Assadi 2018), and *Acanthophyllum* (Basiri Esfahani *et al.* 2011, Mahmoudi Shamsabad *et al.* 2012) with nearly 24 species are respectively large genera in this subfamily in Iran.

The main objective of this study, was to achieve the morphological and anatomical differences through

leaf anatomy and stomata morphology among 11 genera of *Caryophylloideae* (*Acanthophyllum*, *Agrostemma*, *Allochrusa*, *Dianthus*, *Gypsophila*, *Petrorhagia*, *Psammophiliella*, *Saponaria*, *Silene*, *Vaccaria*, and *Velezia*), and also to contribute some useful information for the systematic of these genera.

## Materials and Methods

### - Anatomical study

In the present study, leaf anatomy of 23 species of eight genera of the subfamily *Caryophylloideae* out of 13 genera in the Flora Iranica area were investigated (Table 1). The sampling strategy was to include the representatives of problematic genera from the perspective of phylogenetic studies (Greenberg & Donoghue 2011, Pirani *et al.* 2014, Madhani *et al.* 2018). Where available, more than two species were considered for one genus to ensure certainty about the stability of anatomical characters among different species of a certain genus. The study is based on herbarium specimens of the following herbaria: FAR, T, and TARI. A list of voucher specimens including the source of plant materials as well as collection data is given in table 1. Herbarium abbreviations follow "Index Herbariorum" (Thiers 2016, continuously updated; <http://sweetgum.nybg.org/science/ih/>).

Leaf samples were rehydrated with boiling and fixed in FAA solution (formalin-acetic acid-alcohol: 90:5:5) for 24–36 hours, then preserved in 70% ethanol. Cross sections were made by hand using commercial razor blades. The sections were transferred into 30% sodium hypochlorite solution for about 20 min washed in several changes of water, then transferred into acetic acid 5% for 30–60 seconds and rinsed in water. The sections were double stained with methyl green and brown bismarck colors (Culter *et al.* 2007).

For stomata studies, leaves were excised from the mid-lamina regions of adaxial and abaxial surface. Paradermal sections were transferred into 30% sodium hypochlorite solution for about one minute and rinsed in water then stained with Fuchsin (Culter *et al.* 2007).

- Data analysis analyzed by PAUP 4.0b10 software with UPGMA and Neighbor-joining methods. The FigTree Ver. 1.4.2 has been used for drawing the phenograms (Figs 8–9).

Forty-nine characters (Metcalf & Chalk 1979) (including 16 qualitative and 33 quantitative characters) (Table 2) have been considered. To determine the similarity of examined taxa, the results have been

Table 1. List of taxa used in anatomical study along with their voucher specimens

No.	Taxon	Locality (Iran)
1	<i>Acanthophyllum mucronatum</i> C.A.Mey.	Fars province: Marvdasht, Sad-e Doroodzan, 1755 m, 6 Mar. 2011, Khosravi 12512 (T)
2	<i>A. sordidum</i> Bunge ex Boiss.	North Khorasan province: Esfarayen, Saluk National Park, Istgah-e Mohitbani, 1162 m, 26 Mar. 2007, Ezazi 4401(T)
3	<i>Agrostemma githago</i> L.	Tehran province: southern slopes of central Alborz around the Vardij and Varish villages, 1848 m, 22 May 2009, Gilani 8199 (T)
4	<i>Allochrusa lutea</i> Falat. & Mahmoodi	Zanjan province: 20 km on the Zanjan-Tabriz road, beginning of Esfejin to Mirjan road, 36°44'22.38"N, 48°14'35.88"E, 1570 m, 19 Jun. 2014, Mahmoodi 100635 (TARI!)
5	<i>Dianthus armeria</i> L.	Guilan province: Rudbar, Siahrud, Atayi 3887 (T)
6	<i>D. crinitus</i> Sm.	Fars province: Marvdasht, sad-e Doroodzan, 1782 m, 5 May 2011, 12364 (T)
7	<i>D. szowitzianus</i> Boiss.	Tehran province: a part of southern slopes of central Alborze, around Vardij and Varish villages, 2291 m, 5 Jun. 2009, Gilani 8281 (T)
8	<i>Gypsophila acantholimoides</i> Bornm.	Esfahan province: Natanz, 2358 m, 17 Jun. 2015, Zeraatkar 15913 (T)
9	<i>G. bicolor</i> (Freyn. & Sint.) Grossh.	Mazandaran province: Ghar-e Berke, 27 Jun. 1991, Azadbakht 007134 (T)
10	<i>G. caricifolia</i> Boiss.	Zanjan province: Tharum, south western of Khanchay village, 2600 m, 29 Jul. 1996, Mousavi 002666 (T)
11	<i>G. linearifolia</i> Boiss.	Khuzistan province: 5 km from Ramhormoz to Izeh, 50 m, 19 Apr. 1982, Assadi and Abohamzeh 38754 (TARI)
12	<i>G. pallida</i> Stapf	Fars province: Marvdasht, sad-e Doroodzan, 1748 m, 6 Jan. 2011, Zera'atkar 12481 (T)
13	<i>G. pilosa</i> Huds.	Tehran province: southern slopes of central Alborz around Vardij and Varish villages, 1699 m, 10 Sept. 2008, Gilani 7966 (T)
14	<i>Petrorhagia saxifraga</i> (L.) Link	Mazandaran province: Neka, Hezarjarib protected area, 1576 m, 24 Oct. 2009, Azadbakht 7533 (T)
15	<i>Psammodiella bellidifolia</i> (Boiss.) Ikonn.	Sistan-va-Baluchestan province: 6 km on road from Rask to Iranshahr, 450 m, 9 Mar. 1977, Runemark, Assadi & Sardabi 22540 (TARI)
16	<i>P. esfandiarii</i> (Assadi) Falat., Assadi & F.Ghahrem.	Hormozgan province: Bandar Abbas, Ghotbabad, Baghestan; Damtang village, Baz mt, 500–2000 m, 28 Apr. 1985, Mozaffarian 49988 (TARI)
17	<i>Saponaria viscosa</i> C.A.Mey.	Tehran province: southern slopes of central Alborz, around the Vardij and Varish villages, 1853 m, 22 May 2009, Gilani 8188 (T)
18	<i>Silene bupleurioides</i> L.	East Azarbayegan province: Marakan protected area, 1239 m, 5 Jul. 2009, Nejati Edalatiyan 10745 (T)
19	<i>S. eriocalycina</i> Boiss.	Fars province: Marvdasht, Sad-e Doroodzan, 1825 m, 5 Oct. 2011, 12406 (T)
20	<i>S. latifolia</i> Poir.	Mazandaran province: Neka, Hezarjarib protected area, 2078 m, 26 Oct. 2009, Azadbakht 7557 (T)
21	<i>S. vulgaris</i> (Moench) Garcke	?: 29 May 1998, 023087 (T)
22	<i>Vaccaria hispanica</i> (Mill.) Rauschert	Kurdistan province: Badr and Parishan, Maroofi 15529 (T)
23	<i>Velezia rigida</i> L.	Guilan province: Rudbar, Siahrud, Atayi 3701 (T)

## Results and Discussion

Transverse sections of leaves are usually u- or v-shape and sometimes plain. The epidermis in both surfaces is composed of one layer of polyhedral, circular or stellate cells, with straight or undulate

anticlinal walls, which are covered by cuticle. Leaves of all examined genera are amphistomatic and stomatal index of adaxial and abaxial sides do not show meaningful differences. The stomata are typically diasytic (Figs 4–7), but there are some exceptions in the

genera *Acanthophyllum*, *Allochrusa*, *Saponaria*, and *Psammophiliella* Ikonn. which their stomatal patterns are more similar to anemocytic pattern (Fig. 5). Mesophyll is centric or dorsiventral, but in the genera *Agrostemma* (Fig. 2 A–B), *Acanthophyllum* (Fig. 1 B–C), *Allochrusa* (Fig. 1 A), *Gypsophila* (Fig. 3 A–H), and *Vaccaria* (Fig. 1 L) merely centric feature has been recognized. In all genera, there are 1–4 layers of palisade parenchyma (at least on one side), and then 1–7 layers of spongy parenchyma depending on the species. Midrib in transverse sections is circular, semicircular or pyriform. Sometimes there are 1–3 (scarcely eight) layers of collenchymatous tissue below the phloem or above the xylem tissue. Most species have parenchymatous bundle sheath (Table 4, Figs 1–3).

According to the phenogram resulting from UPGMA (Fig. 8 A) and Neighbor-joining (Fig. 8 B), all anatomical characteristics are not constant in species of the genera, but some of them such as stomatal patterns, limb symmetry, collenchymatous tissue and layers of fiber around the central vascular bundles may be useful

to separate some genera. It is probable that, a wider sampling would lead to finding a set of features that could more match to the classification based on morphology and phylogeny.

However, none of the phenograms (Fig. 8) show precisely categories corresponding traditional genera, but the resulting phenogram obtained with NJ has categories that are more acceptable. All examined specimens of *Dianthus*, *Velezia*, and *Petrorhagia* are located in one branch, of course it should be mentioned that, *Saponaria viscosa* is in the same branch. Except for *Silene eriocalycina*, the other three species of *Silene* are arranged in one branch. However, *Gypsophila* and *Acanthophyllum* species are scattered in different branches. In fact, the anatomical features of *Gypsophila* species, as well as their morphological characteristics, are diverse. An important feature that seems to have led to the separation of some species from the expected branch is variation in stomatal type found in some species of different genera.

Table 2. Character states and coding

Character (code)	State (code)			
(1) Number of upper collenchyma layers	0 (0)	1 (1)		
(2) Number of lower collenchyma layers	0 (0)	1–3 (1)	8 (2)	
(3) Upper collenchyma type	Absent (0)	Tangential (1)		
(4) Lower collenchyma type	Absent (0)	Tangential (1)	Angular (2)	
(5) Shape of transverse section of midrib	Circular (0)	Semicircular (1)	Pear-shaped (2)	Circular to pear-shaped (3)
(6) Number of upper parenchyma layers	1 (0)	2 (1)	3 (2)	
(7) Number of lower parenchyma layers	<3 (0)	3–7 (1)	>7 (2)	
(8) Thickness of midrib (µm)	<120 (0)	120–320 (1)	>320 (2)	
(9) Thickness of xylem of midrib (µm)	<50 (0)	50–100 (1)	>100 (2)	
(10) Thickness of phloem of midrib (µm)	<100 (0)	100–200 (1)	200–300 (2)	>300 (3)
(11) Thickness of upper paranchyma (µm)	<150 (0)	150–250 (1)	250–350 (2)	>350 (3)
(12) Thickness of lower paranchyma (µm)	<150 (0)	150–300 (1)	300–450 (2)	>450 (3)
(13) Number of xylem's row of midrib	<3 (0)	3–7 (1)	>7 (2)	
(14) Vertical diameter of midrib (µm)	<100 (0)	100–200 (1)	>200 (2)	
(15) Horizontal diameter of midrib (µm)	<150 (0)	150–300 (1)	300–450 (2)	>450 (3)
(16) Vertical to horizontal diameter ratio of midrib	<0.5 (0)	0.5–1 (1)	>1 (2)	
(17) Number of trichoms above midrib	0 (0)	1 (1)	2 (2)	

**Table 2. (contd)**

(18) Number of trichoms below midrib	0 (0)	1–3 (1)	>3 (2)		
(19) Fibers	Present (0)	Absent (1)			
(20) Midrib's thickness to limb's thickness	<1 (0)	1–2 (1)	>2 (2)		
(21) Midrib's phloem thickness to midrib's xylem thickness	<2 (0)	2–4 (1)	>4 (2)		
(22) Sclerenchymatous sheath	uniseriate with various cell size (0)	Multiseriate (1)			
(23) Shape of transverse section	Linear (0)	U-shaped (1)	V-shaped (2)		
(24) Number of spongy parenchyma layers	1–2 (0)	3–5 (1)	6–7 (2)		
(25) Number of upper palisade parenchyma layers	0–2 (0)	3–4 (1)			
(26) Number of lower palisade parenchyma layers	0–2 (0)	3–4 (1)			
(27) Length of upper palisade parenchymatous cells (µm)	Absent (0)	<100 (1)	100–300 (2)	>300 (3)	
(28) Length of lower palisade parenchymatous cells (µm)	Absent (0)	100–300 (1)	>300 (2)		
(29) Cell wall of palisade parenchymatous cells	Entire (0)	Undulate (1)	Entire to repand (2)		
(30) limb symmetry	Isolateral (0)	Dorsiventral (1)			
(31) Limb thickness (µm)	<100 (0)	100–200 (1)	>200 (2)		
(32) Anticlinal wall of upper epidermis	Entire (0)	Undulate (1)			
(33) Anticlinal wall of lower epidermis	Entire (0)	Undulate (1)			
(34) Shape of upper epidermis cell	Polygonal (0)	Elongate polygonal (1)	Circular (2)	Circular to angulate (3)	Irregular stellate (4)
(35) Shape of lower epidermis cell	Polygonal (0)	Elongate polygonal (1)	Circular (2)	Circular to angulate (3)	Irregular stellate (4)
(36) Stomatal type of upper epidermis	Diacytic (0)	Anemocytic (1)			
(37) Stomatal type of lower epidermis	Diacytic (0)	Anemocytic (1)			
(38) Length of guard cell in upper epidermis (µm)	<45 (0)	45–65 (1)	>65 (2)		
(39) Length of guard cell in lower epidermis (µm)	<45 (0)	45–65 (1)	>65 (2)		
(40) Width of guard cell in upper epidermis (µm)	<40 (0)	>40 (1)			
(41) Width of guard cell in lower epidermis (µm)	<40 (0)	>40 (1)			
(42) Length of stomata in upper epidermis (µm)	<20 (0)	20–30 (1)	>30 (2)		
(43) Length of stomata in lower epidermis (µm)	<20 (0)	20–30 (1)	>30 (2)		
(44) Length to width of guard cell in upper epidermis	<1.3 (0)	>1.3 (1)			
(45) Length to width of guard cell in lower epidermis	<1.3 (0)	>1.3 (1)			
(46) Stomatal index in upper epidermis	<15 (0)	15–40 (1)	>40 (2)		
(47) Stomatal index in lower epidermis	<20 (0)	20–40 (1)	>40 (2)		
(48) Stomatal position in upper epidermis	Irregular & scattered (0)	Regular & parallel (1)			
(49) Stomatal position in lower epidermis	Irregular & scattered (0)	Regular & parallel (1)			

***Acanthophyllum* and *Allochrusa***

Recent molecular phylogenetic analyses (Greenberg & Donoghue 2011, Pirani *et al.* 2014) have shown that, *Allochrusa* belongs to a clade together with *Acanthophyllum* and maybe it must be considered as a section of *Acanthophyllum* (Pirani & Rabeler 2017, Madhani *et al.* 2018), but because of significant

morphological differences, such as not being spiny and not having exerted stamens in *Allochrusa*, this genus has been treated as a separate one (Mahmoodi & Nejad Falatoury 2016). Lacking of multiple layers of fiber around the central vascular bundles in *Allochrusa* supports separation of these two genera (Fig. 1 A–C).

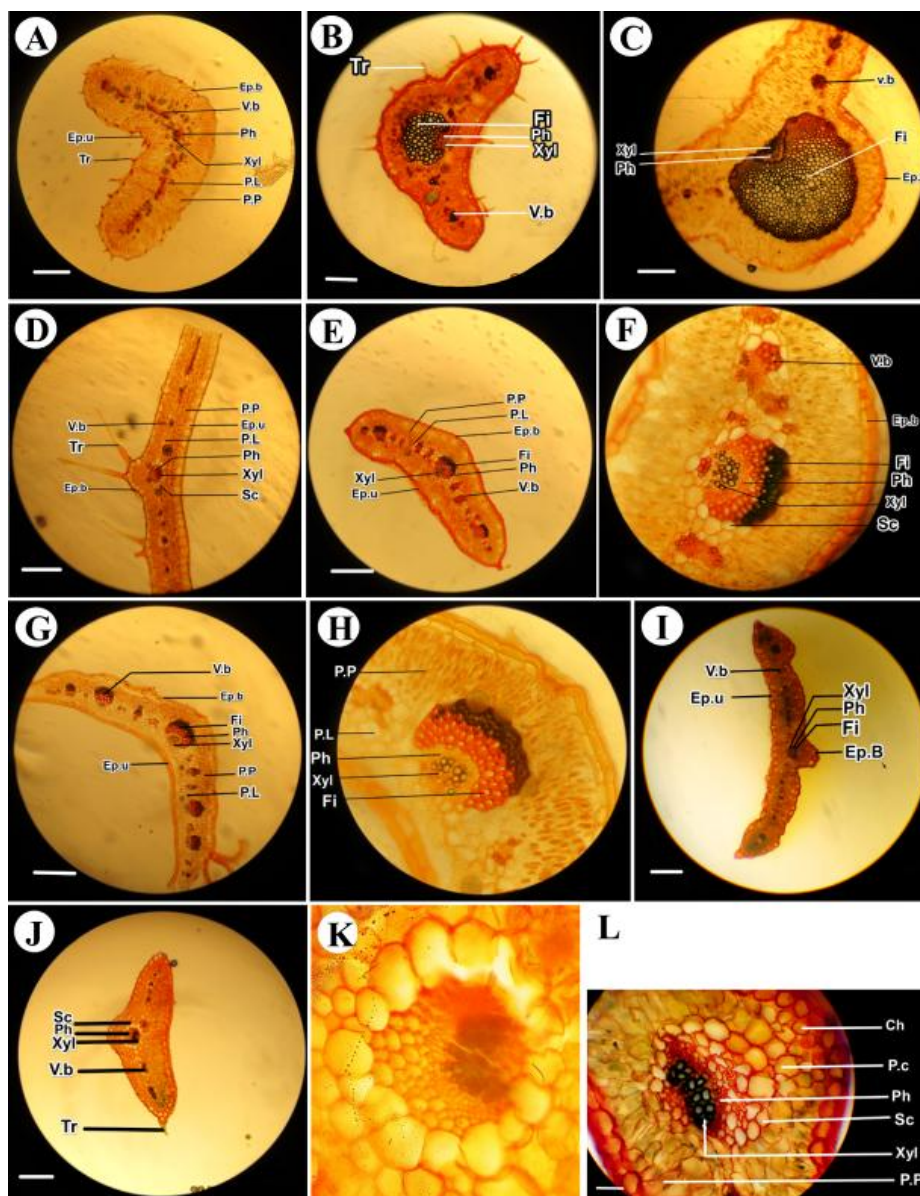


Fig. 1. Transverse sections of leaves: A. *Allochrusa lutea*, B. *Acanthophyllum mucronatum*, C. *A. sordidum* D. *Dianthus armeria*, E, F. *D. crinitus*; G, H. *D. szowitzianus*; I. *Velezia rigida*; J, K. *Petrorhagia saxifraga*; L. *Vaccaria hispanica*. Ch: collenchyma, Ep.b: lower epidermis; Ep.u: upper epidermis; Fi: fibers; Mv: midrib; P.c: paranchyma; Ph: phloem; P.L: spongy paranchyma; P.P: palisade paranchyma; Sc: sclerenchyma; Tr: trichome; V.b: vascular bundle; Xyl: xylem (Bars = 50  $\mu$ m).

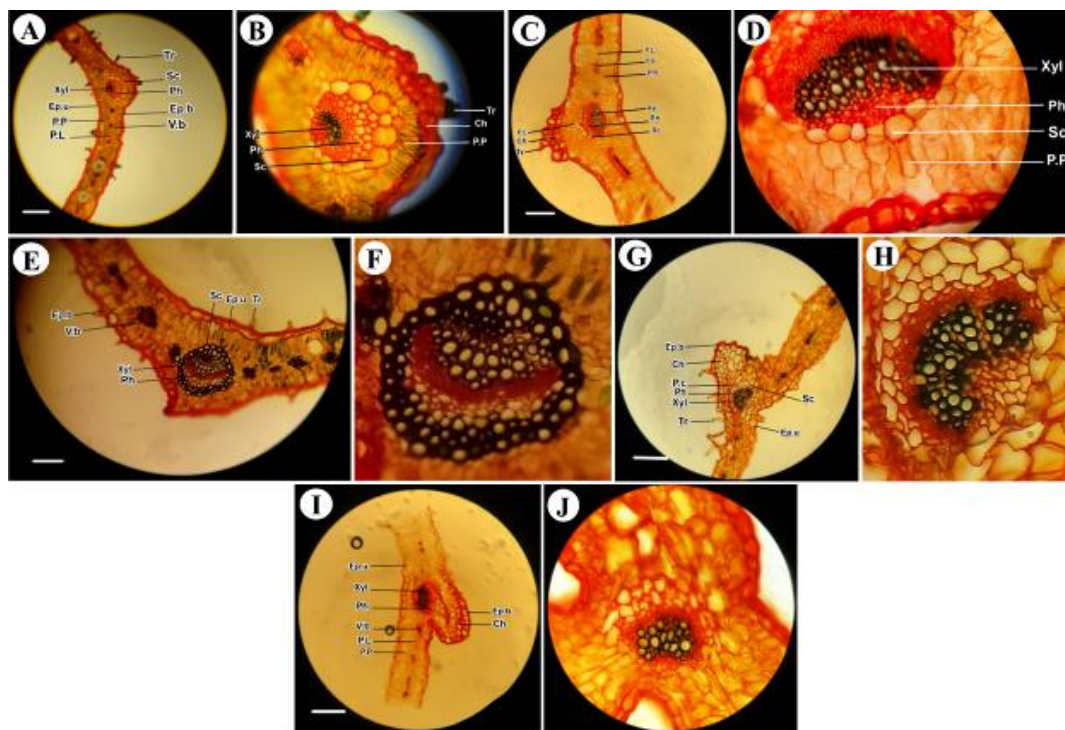


Fig. 2. Transverse sections of leaves: A, B. *Agrostemma githago*, C, D. *Silene bupleurioides*, E, F. *S. eriocalycina*, G, H. *S. latifolia*, I, J. *S. vulgaris*. Ch: collenchyma, Ep.b: lower epidermis, Ep.u: upper epidermis, Fi: fibers, Mv: midrib, P.c: paranchyma, Ph: phloem, P.L: spongy paranchyma, P.P: palisade paranchyma, Sc: sclerenchyma, Tr: trichome, V.b: vascular bundle, Xyl: xylem (Bars = 50  $\mu$ m).

### *Gypsophila*, *Saponaria*, and *Psammophiliella*

In recent molecular analyses *Gypsophila muralis* L. resolves as sister to the *Dianthus-Petrorhagia* clade (Greenberg & Donoghue 2011, Pirani *et al.* 2014, Madhani *et al.* 2018), and the remaining species of *Gypsophila* are found in two additional clades. This result as well as morphological diagnostic characters of *G. muralis*, support splitting of this species and its allied taxa including *Gypsophila bellidifolia* Boiss. = *Saponaria barbata* Barkoudah (1962) and *Saponaria esfandiarrii* Assadi (1989) as a separate genus sensu Ikonnikov (1976) named as *Psammophiliella* Ikonnikov. Diagnostic morphological characters which split *Psammophiliella* of *Gypsophila* are the narrow hyaline intervals in calyx and elongate stigmatic surface of styles vs. capitate stigma in *Gypsophila* species (Ikonnikov 1976) (Table 3). According to the phenogram resulting from NJ (Fig. 8 B), two examined species of *Psammophiliella* are located in one branch, therefore,

they are common in many anatomical features that support the closeness of these two taxa (Fig. 3 I, K).

***Psammophiliella esfandiarrii*** (Assadi) Falat., Assadi & F.Ghahrem. **comb. nov.**

Basionym: *Saponaria esfandiarrii* Assadi, Iran. J. Bot. 4(2): 198 (1989).

Type: Iran: Hormozgan province, ca. 100 km SE of Lar, above the village Sartang, Kuh-e Shab, 500–900 m, 19 April 1983, Assadi & Sardabi 42042 (holotype TARI!).

Specimens examined: *Psammophiliella esfandiarrii*: Iran: Hormozgan province, Bandar Abbas, Ghotbabad, Baghestan, Damtang village, Baz mt, 500–2000 m, 28 April 1985, Mozaffarian 49988 (TARI); *Psammophiliella bellidifolia*: Bandar-abbas, Bashagard, 42 km from Senderk to Anguhran, between Darpahn and Davari, 750 m, 3 May 1982, Mozaffarian, Banihashemi and Shahinzadeh 39296 (TARI); Bashagard, 62 km from Senderk to Anguhran, close to Jakdan, 700 m, 4 May 1982, Mozaffarian, Banihashemi and Shahinzadeh 39334

(TARI); Bashagard, 105 km from Senderk to Anguhran, between Jakdan and Sardasht, 680–1000 m, 8 May 1983, Mozaffarian 44416 (TARI); Sistan-va-Baluchestan province: 6 km on road from Rask to Iranshahr, 450 m, 9 March 1977, Runemark, Assadi and Sardabi 22540

(TARI), 6 km from Sarbaz to Iranshahr, 1000 m, 17 April 1991, Mozaffarian 70134 (TARI); Chahbahar, Gardanehe Hodar, Rask road, 350 m, 7 March 1974, Foroughi 10734 (TARI).

Table 3. Diagnostic morphological characters of *Psammophiliella* compared with *Saponaria* and *Gypsophila*

Diagnostic character	<i>Saponaria</i>	<i>Psammophiliella</i>	<i>Gypsophila</i>
Stigma	Often elongate	Elongate	Capitate
Calyx shape	Oblong-cylindric, terete	Narrow campanulate to short cylindric	Obconic to campanulate, terete to 5-angled
Calyx hyaline intervals	Absent	Narrow	Distinctive
Petal shape	With distinctive claw	With indistinctive claw	Without claw
Coronal appendages of petals	Often present	Absent	Absent
Flowers	Often integrated at end of stem	Often spreading along the stem	Various

#### *Petrorhagia* (Ser.) Link

A new species of *Gypsophila* has been described from Iran as *Gypsophila elymaitica* Mozaff. (Mozaffarian 2008). By studying the type specimen of *G. elymaitica*, we found some characters which did not approve belonging of this specimen to the genus *Gypsophila*. Considering having bracteoles near the calyces, peltate seeds with a facial hilum and straight embryo, this species is not a *Gypsophila* but it belongs to the genus *Petrorhagia*. Thus, a new combination is proposed:

***Petrorhagia elymaitica* (Mozaff.) Falat., Assadi & F.Ghahrem. comb. nov.**

Basionym: *Gypsophila elymaitica* Mozaff., Iran. J. Bot. 14(2): 89 (2008)

Type: Iran: Ilam province: Ilam to Darreshahr, Kulm, N. slope of rocky deep gorge of Kabirkuh, S. of Kulm, 23 VIII 2004, V. Mozaffarian 88385 (holotype TARI!).

Other examined specimens: Ilam province, Ilam to Darreshahr, Badre, Tange Kafari, 700 m, V. Mozaffarian 88396 (TARI!); Khuzistan: Dehdez, kuh-e Gharoon, from Absefid valley, 1000–1500 m, V. Mozaffarian 74483 (TARI!).

It is worth to mention that, despite the phylogenetic results (Greenberg & Donoghue 2011, Pirani 2014, Madhani *et al.* 2018), due to the obvious morphological evidences, here, the merging of the genera *Allochrusa* with *Acanthophyllum*; *Velezia* with *Dianthus*; *Ankyropetalum*, *Vaccaria* with *Gypsophila*, and *Agrostemma* with *Silene* were avoided.



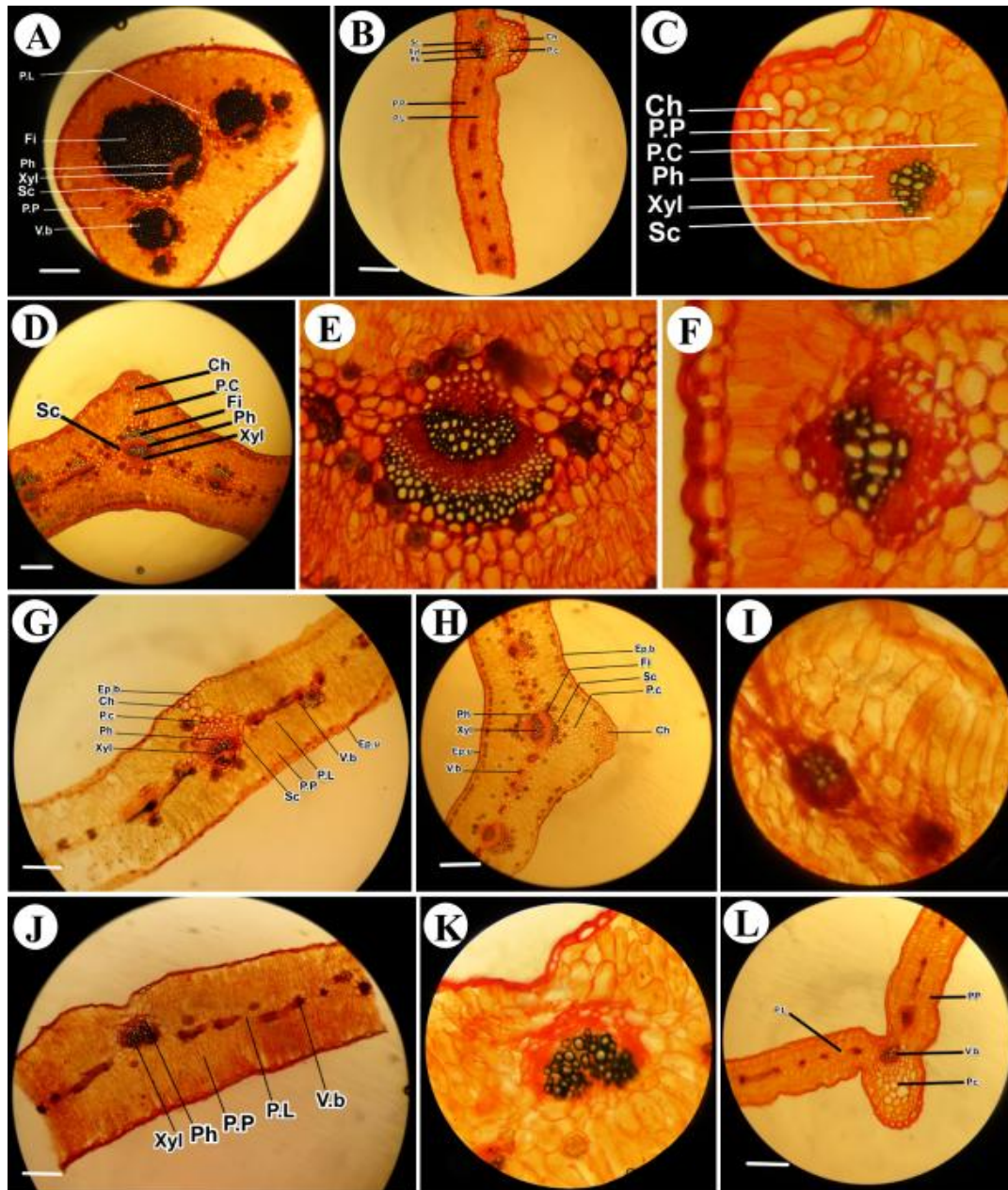


Fig. 3. Transverse sections of leaves: A. *Gypsophila acantholimoides*, B, C. *G. bicolor*, D, E. *G. caricifolia*, F. *G. linearifolia*, G. *G. pallida*, H. *G. pilos*, I. *Psammophiliella bellidifolia*, J, K. *P. esfandiarii*, L. *Saponaria viscosa*. Ch: collenchyma; Ep.b: lower epidermis; Ep.u: upper epidermis; Fi: fibers; Mv: midrib; P.c: paranchyma; Ph: phloem; P.L: spongy paranchyma; P.P: palisade paranchyma; Sc: sclerenchyma; Tr: trichome; V.b: vascular bundle; Xyl: xylem (Bars = 50  $\mu$ m).

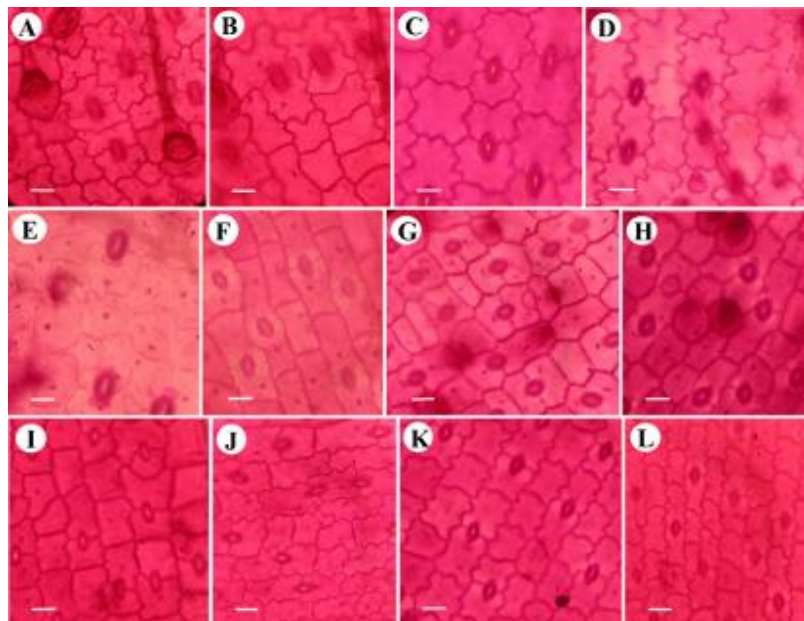


Fig. 4. Epidermis and stomata: A, C, E, G, I & K. Upper epidermis, B, D, F, H, J & L. Lower epidermis, A, B. *Agrostemma githago*, C, D. *Dianthus armeria*, E, F. *D. crinitus*, G, H. *D. szowitzianus*, I, J. *Petrorhagia saxifraga*, K, L. *Velezia rigida* (Bars = 50  $\mu$ m).

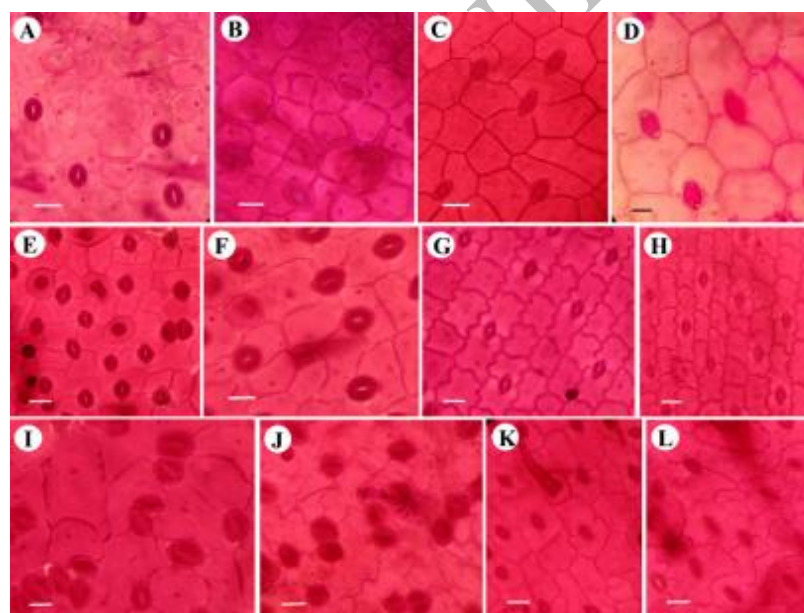


Fig. 5. Epidermis and stomata: A, C, E, G, I & K. Upper epidermis, B, D, F, H, J & L. Lower epidermis, A, B. *Acanthophyllum mucronatum*, C, D. *A. sordidum*, E, F. *Allochrysa lutea*, G, H. *Psammophiliella bellidifolia*, I, J. *P. esfandiarii*, K, L. *Saponaria viscosa* (Bars = 50  $\mu$ m).

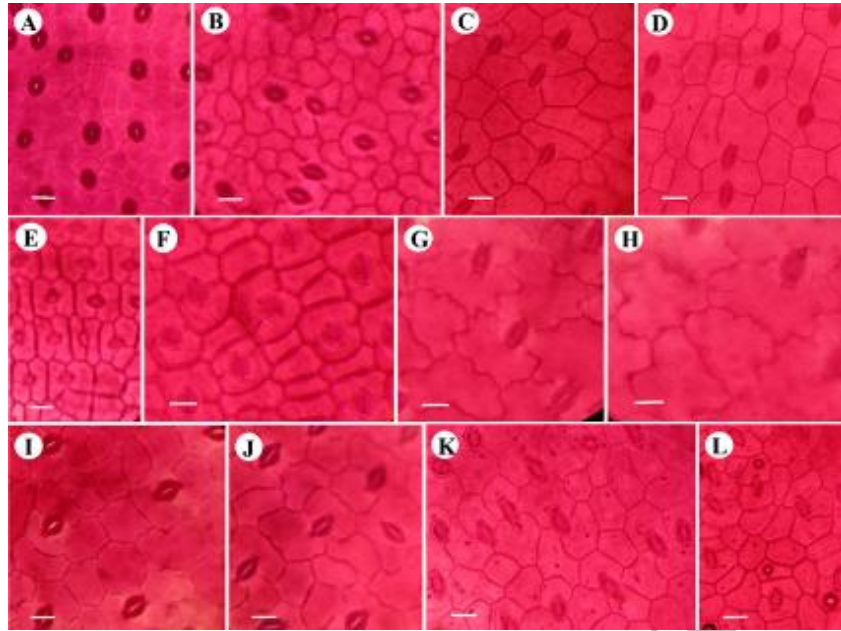


Fig. 6. Epidermis and stomata: A, C, E, G, I & K. Upper epidermis, B, D, F, H, J & L. Lower epidermis, A, B. *Gypsophila acantholimoides*, C, D. *G. bicolor*, E, F. *G. caricifolia*, G, H. *G. linearifolia*, I, J. *G. pallida*, K, L. *G. pilosa* (Bars = 50  $\mu$ m).

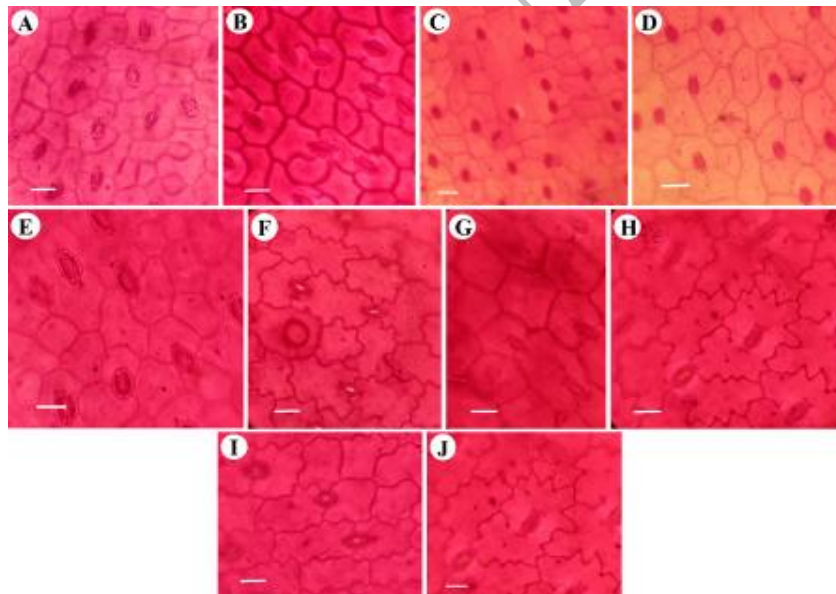


Fig. 7. Epidermis and stomata: A, C, E, G, I & K. Upper epidermis; B, D, F, H, J & L. Lower epidermis. A, B: *Silene bupleurioides*, C, D. *S. eriocalycina*, E, F. *S. latifolia*, G, H. *S. vulgaris*, I, J. *Vaccaria hispanica* (Bars = 50  $\mu$ m).

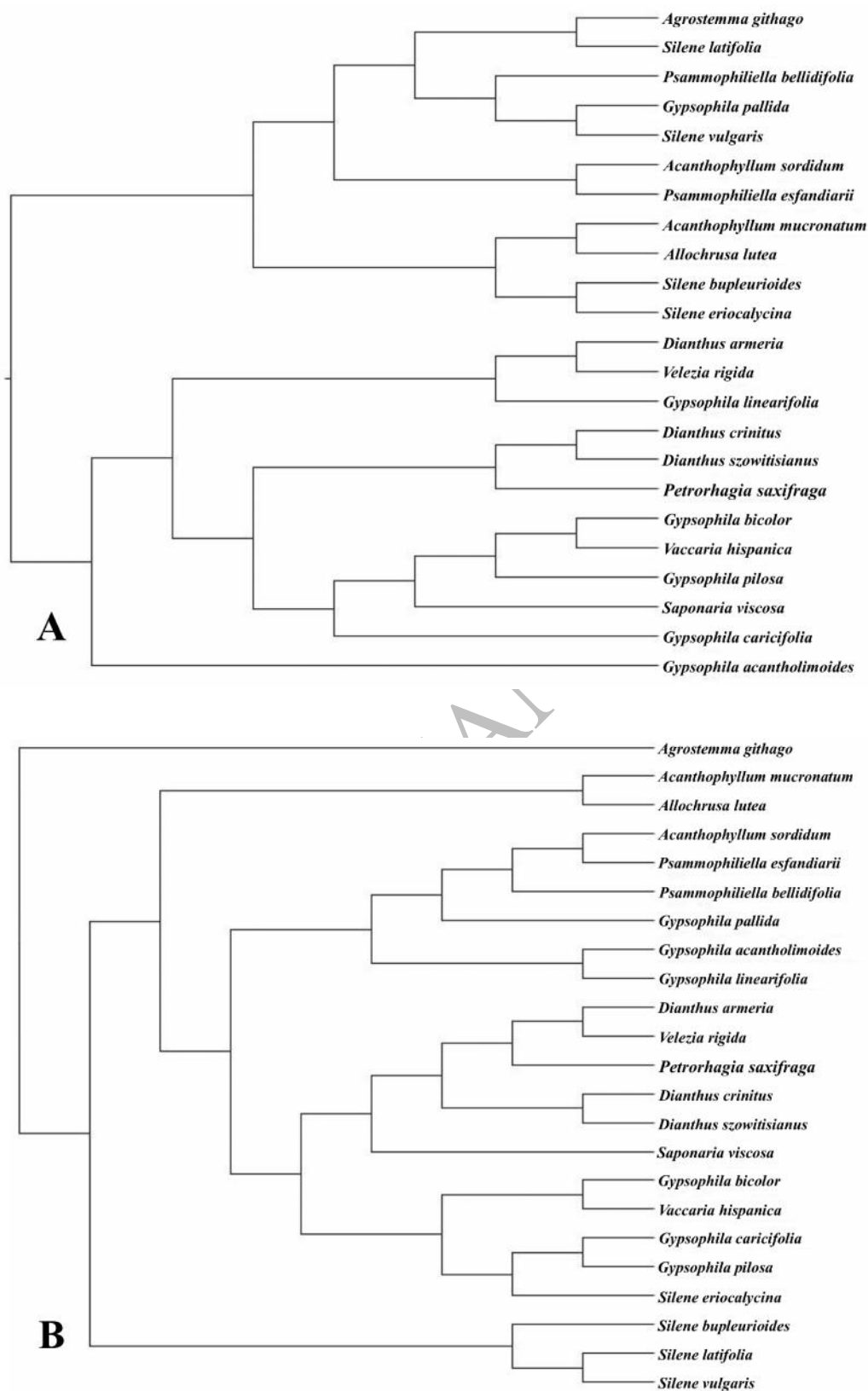


Fig. 8. A. UPGMA phenogram constructed from results of anatomical studies of taxa, B. Neighbor-joining phenogram constructed from results of anatomical studies of taxa.

### An updated key for the genera of *Caryophylloideae* in Iran

Due to the addition of *Psammophiliella* to the flora of Iran here, the following simplified key for *Caryophylloideae* is presented based on morphological traits using available sources including Flora Iranica (Rechinger 1988).

1. Calyx with commissural veins.....2  
 - Calyx without commissural veins.....4
2. Calyx teeth foliaceous, (11–)15–35 mm, exceeding the petals.....*Agrostemma*  
 - Calyx teeth not foliaceous, less than 10 mm, shorter than the petals.....3
3. Capsule teeth as many as styles; styles 5, flowers hermaphrodite.....*Lychnis*  
 - Capsule teeth twice as many as styles; styles 3 or if 5 then flowers unisexual.....*Silene*
4. Calyx well developed, with relatively expanded base and tapering at the base of calyx dents.....5  
 - Calyx not as above.....6
5. Annual plant, calyx with 5 well-developed wings.....*Vaccaria*  
 - Suffruticose, multi stems, calyx with prominent nerve .....*Diaphanoptera*
6. Calyx without membranous hyaline intervals between the nerves.....7  
 - Calyx with membranous hyaline intervals between the nerves.....9
7. Bracteoles closely adpressed to calyx.....*Dianthus*  
 - Bracteoles not adpressed to calyx.....8
8. Seeds peltate with facial hilum; petals without or with small coronal scales.....*Velezia*  
 - Seeds reniform with lateral hilum; petals with or without coronal scales.....*Saponaria*
9. Seeds peltate with facial hilum.....*Petrorrhagia*  
 - Seeds reniform or comma-shaped, with lateral hilum.....10
10. Fruit 1–4-seeded, indehiscent or rupturing irregularly at base.....11  
 - Fruit 4–36-seeded, dehiscing by valves or teeth.....13
11. Petals trifold.....*Ankyropetalum*  
 - Petals at most notched.....12
12. Leaves, bracts and calyx teeth  $\pm$  spiny; stamens exserted.....*Acanthophyllum*  
 - Plant not spiny; stamens not exserted.....*Allochrusa*
13. Hyaline intervals distinctive; stigma capitate.....*Gypsophila*  
 - Narrow hyaline intervals; stigmatic surface along the whole inner side of the styles.....*Psammophiliella*

Table 4. Basic data matrix of taxa. Taxa's code (T) according to table 1, descriptions and coding of character states (Ch) and states from table 2

T Ch	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	0	1	0	1	3	1	0	1	0	2	0	0	1	2	1	1	1	1	1	1	1	0	1	0	0
2	0	0	0	0	1	1	1	1	1	1	0	1	1	1	2	0	2	2	0	1	2	0	1	0	0
3	0	0	0	0	1	1	0	1	2	2	3	1	2	2	1	1	1	1	0	1	1	0	1	0	0
4	0	0	0	0	3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0
5	0	0	0	0	2	1	1	0	0	1	0	0	1	1	1	1	0	1	1	1	1	0	1	1	0
6	0	0	0	0	2	2	0	1	1	1	1	1	1	1	0	1	0	0	0	1	1	0	2	1	1
7	0	0	0	0	2	1	0	1	1	1	0	0	1	1	0	1	0	1	0	1	1	0	1	1	0
8	0	0	0	0	2	2	1	2	1	1	3	1	1	1	2	0	0	0	0	0	1	0	0	2	1
9	0	0	0	0	2	0	0	1	1	2	1	0	1	2	2	1	0	0	1	1	0	0	0	1	0
10	0	1	0	2	2	0	1	1	2	0	1	1	2	1	2	0	0	1	0	1	0	1	0	0	0
11	0	2	0	2	2	2	1	2	1	2	2	2	1	2	2	1	0	0	0	0	1	0	2	2	1
12	0	0	0	0	2	1	1	2	0	1	3	3	0	1	0	1	0	1	1	1	1	0	0	0	0
13	0	0	0	0	2	1	1	1	1	2	1	1	1	2	1	1	0	0	1	0	1	0	0	1	0
14	0	1	0	2	2	0	1	1	2	2	0	2	1	2	1	1	0	0	0	1	1	0	1	0	0
15	0	0	0	0	3	2	0	1	1	1	1	0	1	1	1	1	0	1	1	0	1	0	2	1	1
16	0	0	0	0	0	2	0	1	2	2	3	0	1	2	1	1	0	0	1	0	0	0	0	0	0
17	0	0	0	0	2	2	2	1	2	1	0	3	1	1	1	1	0	0	1	0	1	0	1	0	0
18	1	1	1	2	3	1	1	1	2	3	1	2	1	2	3	1	1	2	1	0	1	0	1	1	0
19	1	0	1	0	3	1	1	1	2	2	1	2	1	2	2	1	2	2	1	1	1	1	1	0	0
20	1	1	1	1	3	0	1	1	1	2	0	3	1	2	1	1	1	1	1	0	2	0	1	1	0
21	1	1	1	1	2	0	1	1	2	2	0	2	1	2	2	1	0	0	1	0	2	0	1	1	0
22	0	1	0	1	2	0	1	1	1	1	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0
23	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	1	1	0

Table 4. (contd)

T Ch	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
1	0	2	1	0	0	0	1	1	4	4	0	0	1	1	1	1	1	1	0	0	1	0	0	0
2	0	2	1	0	0	1	0	0	2	2	0	1	1	2	1	1	1	1	0	1	0	0	0	0
3	0	3	1	0	0	1	0	0	0	0	1	1	1	2	1	1	1	1	0	0	1	1	1	1
4	0	2	1	0	0	1	0	0	2	2	1	1	1	0	0	0	0	0	1	0	1	1	0	0
5	0	2	0	0	1	0	1	1	4	4	0	0	1	0	0	1	0	0	1	0	1	1	1	1
6	1	2	1	0	0	1	0	0	2	2	0	0	2	1	1	0	2	0	0	1	0	1	1	1
7	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1
8	1	3	2	0	0	2	1	1	0	0	0	0	1	1	1	1	0	0	0	0	1	0	1	1
9	0	3	0	2	0	1	0	1	0	4	1	1	1	2	1	1	0	1	1	1	1	1	1	1
10	0	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1	1	1
11	1	2	1	2	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1
12	0	3	2	2	0	1	1	1	4	4	0	0	1	1	1	0	1	0	0	1	1	1	0	0
13	0	2	1	2	0	1	1	1	0	0	0	0	1	1	1	1	1	2	1	0	1	1	1	1
14	0	2	1	0	0	1	0	0	3	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0
15	0	2	0	0	1	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	1	1	0	1
16	1	3	2	0	0	1	0	1	0	0	1	1	1	1	1	0	1	1	0	1	2	2	0	0
17	0	2	1	1	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0	1	1	1	1	1
18	0	2	1	1	0	1	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	1	0	0
19	0	2	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	1	1	1	0	1	1
20	0	1	0	1	1	0	0	1	0	4	0	0	1	1	1	1	0	1	0	0	1	1	0	0
21	0	2	1	2	0	0	0	1	0	4	0	0	1	1	1	1	0	1	0	1	0	1	1	1
22	0	2	1	2	0	0	1	1	4	4	0	0	1	0	0	0	0	0	1	1	0	0	1	1
23	0	1	0	0	1	0	1	1	4	4	0	0	0	0	0	0	0	0	0	0	1	1	1	1

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