

LEAF ANATOMICAL STUDY OF GYPSOPHILA (CARYOPHYLLACEAE) AND ALLIED GENERA IN IRAN AND ITS TAXONOMICAL IMPLICATION

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Received 2018. 05. 30; accepted for publication 2018. 10. 17

Amini, E., Zarre, Sh. & Assadi, M. 2018. 12. 30: Leaf anatomical study of *Gypsophila* (Caryophyllaceae) and allied genera in Iran and its taxonomical implication. -*Iran. J. Bot.* 24 (2): 138-155. Tehran.

Anatomical features as revealed from cross-sections of leaf blades and midribs in 21 taxa of *Gypsophila* representing its currently recognized seven sections distributed in Iran as well as four species of *Saponaria*, two species of *Allochrusa* and one species of *Ankyropetalum* as its closely related genera are examined. In total nine quantitative and five qualitative characters were selected and measured. The most important characters include general shape of leaves (assessed only for narrow leaves) in transverse section, type of mesophyll (dorsi-ventral vs. isobilateral), thickness of sclerenchyma surrounding the vascular bundles, shape of central vascular bundle, number of parenchyma layers in midrib, thickness (number of layers) and structure of mesophyll, density and distribution of druses. In general, leaf anatomy does not provide any unique feature supporting the separation of genera *Ankyropetalum* and *Allochrusa* from *Gypsophila*. The number of spongy layers provides support at least for separation of *Gypsophila* (more than two layers) from most species of *Saponaria* (only one layer). Our results show that leaf anatomical features provide reliable evidence for subgeneric classification of *Gypsophila* and could be taxonomically valuable.

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Key words: Crystal; *Gypsophila*; Subgeneric classification; Systematics; Taxonomy; Tribe Caryophylleae

مطالعه تشریحی برگ در سرده *Gypsophila* L. (تیره میخکیان) و خویشاوندانش در ایران و کاربرد آرایه شناختی آن
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خصوصیات تشریحی مربوط به پهنک و رگبرگ میانی ۲۱ گونه از سرده *Gypsophila* به عنوان نماینده هفت بخشه پراکنده در ایران، چهار گونه از *Saponaria*، دو گونه از *Allochrusa* و یک گونه از *Ankyropetalum* از سرده‌های خویشاوند آن، مورد بررسی قرار می‌گیرد. در مجموع نه صفت تشریحی کمی و پنج صفت کیفی انتخاب و بررسی شد که مهم‌ترین آنها عبارتند از: شکل کلی برش عرضی برگ‌ها (فقط در برگ‌های باریک)، نوع مزوفیل (بشتی-شکمی یا متقارن دوطرفی)، ضخامت اسکلرانشیم پیرامون دسته آوندی مرکزی، شکل دستجات آوندی مرکزی، ضخامت (تعداد لایه‌ها) و ساختار مزوفیل، شکل و تعداد ردیف پارانشیم اسفنجی و نردبانی، تعداد لایه‌های پارانشیم رگبرگ میانی، تراکم و توزیع بلورهای کریستال پروجهی در کل آناتومی برگ شواهدی برای جدایی سرده‌های *Allochrusa* و *Ankyropetalum* از *Gypsophila* ارائه نکرد. تعداد لایه‌های پارانشیم

اسفنجی جدایی *Gypsophila* (با مزوفیل اسفنجی بیش از دو لایه) از اکثر گونه‌های *Saponaria* (با یک لایه مزوفیل اسفنجی) را تأیید می‌کند. نتایج ما تأیید کرد که ویژگی‌های تشریحی برگ شواهد قوی برای رده‌بندی زیرسرده‌های *Gypsophila* فراهم کرده و از لحاظ آرایه شناختی می‌تواند مفید باشد.

INTRODUCTION

Gypsophila L. with approximately 150 species (Madhani & al. 2018) is ranked as the fourth largest genus of Caryophyllaceae (Bittrich 1993; Mabberley 2008) and the second largest genus of the tribe Caryophylleae. *Gypsophila* comprises annual as well as perennial herbs and cushion-like subshrubs that sometimes form prostrate and low growing plants occurring in dry mountain slopes, across all altitude zones, though chiefly at mid- and higher elevations, but some species grow in sandy deserts or directly on bare calcareous rocks (e. g. *G. aretioides* Boiss.), while some species are known as widely distributed weeds (e. g. *G. pilosa* Huds.) or as ruderal elements (e. g. *G. polyclada* Fenzl ex Boiss.), (Barkoudah 1962). In addition, members of *Gypsophila* are characterized by having annual or perennial habit, dichasial inflorescence, tubular-campanulate calyx with wide hyaline intervals, linear to spatulate or lanceolate leaves, bracteoles absent and numerous small flowers (Barkoudah 1962; Rechinger 1988). These species also show major variation in inflorescence type ranging from many-flowered lax thyrse or panicles (e. g., *G. elegans* M.Bieb., *G. paniculata* L., *G. pilosa* Huds.) to compact head-like cymes (e. g. *G. capitata* M. Bieb., *G. capituliflora* Rupr., *G. caricifolia* Boiss.), and few-(uni-) flowered raceme-like monochasia (e. g. *G. bazorganica* Rech.f., *G. saponarioides* Bornm. & Gauba), (Madhani & al. 2018).

Gypsophila is chiefly an Eurasian genus, distributed throughout temperate Asia and Europe, with few species in northeast Africa (Egypt), Australia, and North America, and 17 species (four endemics) growing in China (Barkoudah 1962). The main diversity centres of *Gypsophila* are the Black Sea region, Turkey, Caucasus, North Iraq and Iran (Barkoudah 1962). Iran (38 species) and Turkey (60 species) with about 98 species together, make a diversification hotspot for *Gypsophila* (Barkoudah 1962; Williams 1889; Sumaira & al. 2008; Korkmaz & Özçelik 2011). Almost half of the Iranian *Gypsophila* species are endemics (Barkoudah 1962; Rechinger 1988).

As established by Linnaeus (1753) *Gypsophila* included eight species, but today the genus is known as one of the most polymorphic genera of the subfamily Caryophylloideae (Bittrich 1993). *Gypsophila repens*

L., is the type species of the genus (Barkoudah 1962). Boissier (1867) in *Flora Orientalis* recognized 56 species of *Gypsophila* that were known to him into eight sections and several series (indicated as '§', and with no specified ranks) which have been ignored in later important taxonomic treatments of the genus (Williams 1889). A comprehensive classification within the genus using several morphological characters was performed by Barkoudah (1962), who classified 126 species of the genus in three subgenera and eight sections. Huber-Morath (1967) listed 46 species in nine sections in the *Flora of Turkey* and followed mainly the same classification as Barkoudah (1962). Rechinger (1988) recognized 47 species, 10 sections and 4 subgenera in *Flora Iranica*, of which 37 including 17 endemics were reported to occur in Iran. Recently, some new species of *Gypsophila* have also been described from Iran and Turkey, the focal area of this research (Mozaffarian 1991, 2008; Ataşlar 2005; Budak 2012; Kandemir & Ghazanfar 2012; Hamzaoglu 2012; Koç 2013; Armağan 2016; Nejad Falatoury & al. 2015a; 2016a; 2016b; 2016c).

Several biosystematic studies using non-molecular data including pollen morphology (Barkoudah 1962; Chanda 1962; Moore & al. 1991; Punt & Hoen 1995; Yıldız 2001; Ataşlar & al. 2009; Nejad Falatoury & al. 2017), seed micro-morphology (Fedotova & Ardjanova 1992; Kovtonyuk 1994; El Naggar 2004; Amini & al. 2011), hair micro-morphology (Nejad Falatoury & al. 2015b) and karyology (Siddique & Jeelani 2015) have been carried out on some members of *Gypsophila* focusing mainly on taxon boundaries in this genus.

Greenberg & Donoghue (2011), using nrDNA ITS sequences and five chloroplast genes and intergenic spacers (*matK*, *ndhF*, *trnL-trnF*, *trnQ-rps16*, and *trnS-trnfM*), retrieved *Gypsophila* and allied genera, with 23 species sampled, as a non-monophyletic group. Also, Pirani & al. (2014) mentioned the possible non-monophyly of this genus based on nrDNA ITS and *rps16* data. A recent molecular phylogenetic study has shown that *Gypsophila* in its current circumscription does not represent a natural group (Madhani & al. 2018) and considered major taxonomic changes in and around the genus. However, detailed phylogenetic analysis using multiple DNA sequence data and adequate taxon sampling of all recognized species on the genus is missed yet.

Regarding the anatomical studies in the genus, Metcalf (1957) showed some anatomical characters shared by most species of this genus. Comparative leaf anatomical studies has shown to be useful in delimitation of taxa in various genera of Caryophyllaceae (Zarrinkamar 2001; Schweingruber 2006; Mahmoudi Shamsabad & al. 2013; Al-Saadi & Al-Taie 2014; Nejati Edalatian & al. 2016; Maleki Sadabadi & al. 2017). *Gypsophila* has gained little attention in previous anatomical studies, where only a limited number of its species (*G. lepidioides* Boiss., *G. osmangaziensis* Ataşlar & Ocak, *G. paniculata* and *G. trichotoma* Wender.) have been included (Saidov & Niazov 2013; Özdemir & al. 2010; Ataşlar & al. 2017). This study presents the first comprehensive anatomical investigation of the genus in Iran. Trichome features and variation have also been rarely studied in Caryophyllaceae (Hoseini & al. 2016). A detailed study on trichome micromorphology and its systematic application is presented in *Gypsophila* and the presence or lacking of indumentum on various parts of it have been considered to have diagnostic importance on the genus. Size of trichomes has also been shown to be useful in delimitation between morphologically closed species (Nejad Falatoury & al. 2015b).

The current research presents the first

comprehensive issue on the systematic significance of leaf anatomy in Iranian species of *Gypsophila* discussed on the backgrounds of available molecular systematic results and traditional classifications. The objectives of our study are to: 1) evaluate the relevance of anatomical characters in discrimination of closely related species, 2) find additional informative characters of potential application in subgeneric classification of the genus and 3) to assess the value of anatomical characters in delimiting *Gypsophila* from its closest relative genera.

MATERIALS AND METHODS

Leaves of 21 species of *Gypsophila*, four species of *Saponaria* L., two species of *Allochrysa* Bunge ex Boiss. and one species of *Ankyropetalum* Fenzl distributed in Iran, were selected for leaf blade anatomical study. Fresh leaf materials of the second or third nodes of the stem were fixed in the field with formalin-acetic acid-alcohol (FAA), or removed from herbarium specimens at TUH and TARI. Four cross-sections were measured for each sample to assess the consistency of anatomical characters. A list of voucher specimens and the systematic positions of the corresponding species is given in table 1.

Table 1. Voucher specimens of *Gypsophila* and allied genera examined in this study and systematic placement of *Gypsophila* in some important classifications.

Species	Collection data (all samples are from Iran)	
	Subgenus	Section
<i>Ankyropetalum gypsophiloides</i> Fenzl		Kermanshah: Songhor to Bistoon, Nojehbaran, Hamzei and Asri 87786 (TARI)
<i>Allochrysa bungei</i> Boiss.		west Azarbaijan: between Maku and Khoy, Evaghli, Assadi and Mozaffarian, 30413 (TARI)
<i>A. persica</i> Boiss.		east Azarbayejan: Tabriz to Sperkhan to Sahand, Ghahreman and Mozaffarian 17366 (TUH)
<i>Gypsophila acantholimoides</i> Bornm.	<i>Gypsophila</i>	<i>Hoplites</i>
<i>G. aretioides</i> Boiss.	<i>Gypsophila</i>	<i>Exscapae</i>
<i>G. bellidifolia</i> Boiss.	<i>Gypsophila</i>	<i>Dichoglottis</i>
<i>G. bicolor</i> (Freyn & Sint.) Grossh.	<i>Gypsophila</i>	<i>Paniculaeformes</i>
<i>G. caricifolia</i> Boiss.	<i>Gypsophila</i>	<i>Ensifolia</i>
<i>G. elegans</i> M. Beib.	<i>Gypsophila</i>	<i>Dichoglottis</i>

Table 1. Continued.

Species	Collection data (all samples are from Iran)		
	Subgenus	Section	
<i>G. graminifolia</i> Barkoudah	<i>Gypsophila</i>	<i>Ensifolia</i>	west Azarbaijan: Urumieh, Gardan-e Ghoshchi, 1750-2000 m, Mozaffarian 70089 (TUH)
<i>G. iranica</i> Barkoudah	<i>Gypsophila</i>	<i>Dichoglottis</i>	Kurdistan: Sanandaj, 10 km to Divan-Darreh, Attar, Dadju, Mahdigholi and Okhovat17501 (TUH)
<i>G. leioclada</i> Rech. f.	<i>Gypsophila</i>	<i>Dichoglottis</i>	east Azarbaijan: Near at Darrehsi, ca 70 km southeast Bostamabad towards Mianeh, Podlech and Zarre 55219 (TUH)
<i>G. linearifolia</i> (Fisch. & C. A. Mey.) Boiss.	<i>Gypsophila</i>	<i>Dichoglottis</i>	Khuzestan: Masjed-Solayman Lahbari region between Abgah and Botevand, Mozaffarian 70207 (TUH)
<i>G. mozaffarianii</i> <i>Negaresh</i>	<i>Gypsophila</i>	<i>Exscapae</i>	Hamadan: Hamadan to Saveh, ca 5 km from Tajarak to Nubaran, Gardaneh Doukhan, Mozaffarian 64530 (TUH)
<i>G. mucronifolia</i> Rech. f.	<i>Gypsophila</i>	<i>Dichoglottis</i>	Semnan: Sorkh-e to Dahaghin, Ghahreman and Mozaffarian 5706 (TUH)
<i>G. perfoliata</i> L.	<i>Gypsophila</i>	<i>Paniculaeformes</i>	Semnan: Semnan, beginning of the road, Firouzkuh, 1500 m, Assadi 56649 (TARI)
<i>G. persica</i> Barkoudah	<i>Gypsophila</i>	<i>Dichoglottis</i>	Tehran: Assara on road from Karaj to Chalus, Ghahreman and Agustin 6159 (TUH)
<i>G. pilosa</i> Huds.	<i>Hagenia</i>	<i>Pseudosaponaria</i>	Semnan: Sorkh-e Dahaghin, Ghahreman and Mozaffarian, 5702 (TUH)
<i>G. platyphylla</i> Boiss.	<i>Hagenia</i>	<i>Hispidae</i>	Kurdistan: 50 km of north Sanandaj, between Sarab-e Ghamish village and kuh-e Chehelcheshmeh, 2100 m, Assadi 75259 (TARI)
<i>G. polyclada</i> var. <i>glandolusa</i> Barkoudah	<i>Gypsophila</i>	<i>Dichoglottis</i>	Mazandaran: Karaj-Chalus road, Harijan, Kuh-e Zard, 2100 m, Nazarian 33235 (TUH)
<i>G. polyclada</i> var. <i>polyclada</i> Fenzl ex Boiss.	<i>Gypsophila</i>	<i>Dichoglottis</i>	Tehran: Karaj, Shahrestanak, Rezvanian 23087 (TUH)
<i>G. ruscifolia</i> Boiss.	<i>Gypsophila</i>	<i>Paniculaeformes</i>	west Azarbaijan: 41 km Gharreh Chaman from Mianeh, 70782 (TUH)
<i>G. virgata</i> Boiss.	<i>Gypsophila</i>	<i>Paniculaeformes</i>	Tehran: Saveh, Gharghabad, Ghazemabad, 1700 m, Ghahreman and Mozaffarian 9919 (TUH)
<i>G. xanthochlora</i> Rech. f.	<i>Gypsophila</i>	<i>Dichoglottis</i>	Tehran: Firuzkuh, near Zarrindasht, 1700 m, Assadi, 56618 (TARI)
<i>Saponaria bodeana</i> Boiss.			Tehran: Firuzkuh, Gaduk, Ghahraman and Tarighi 6188 (TUH)
<i>S. floribunda</i> (Kar. & Kir.) Boiss.			Yazd: 50 km east Bafgh, mountains of Hamsuk village, Assadi and Bazgosha 56090 (TARI)
<i>S. orientalis</i> L.			Gilan: Ispili, Saidi 18748 (TUH)
<i>S. viscosa</i> C. A. Mey.			Qazvin: Mountains of Alamut, Ghaleh Gazorkhan, Attar and Mahdigholi 27639 (TUH)

Handmade cross-sections were obtained from leaf blades using commercial razor blades. The cross-sections were obtained using safranin-fast green double staining method (Gerlach 1977). Subsequently, the

leaves were dehydrated through an ethanol gradient (70% in three steps to 100%) with final incubation in xylol (99.5%). After dehydration, all slides were mounted in Canada Balsam.

All anatomical measurements and observations were done under light microscopy (VANOX AHBS3) at 125× to 412.5× magnifications. Micrographs were taken from the middle part of the leaf blades. Some of the most important anatomical characters of leaves in these genera are as follows: general shape of leaf cross-section (only for narrow leaves), type of mesophyll (dorsi-ventral vs. isobilateral), thickness of sclerenchyma surrounding the vascular bundles, shape of central vascular bundle, number of parenchyma layers in midrib, thickness (number of layers) and structure of mesophyll, number of vascular bundles in midrib (only for narrow leaves), density and distribution of druses (table 2).

RESULTS

The main anatomical features as outlined in the present study are shown in table 2. Selected LM micrographs of cross-sections of leaf blades are presented in figs. 1-28. Most characters show significant variability among different species but were constant among different specimens of each species studied.

The outline of the leaf blades is shown only for the narrow leaves, as for the broad leaves the shape was homogeneously linear. The cross-section of the narrow leaves can be subdivided into four basic types: orbicular (e. g. *G. mozaffarianii* Negaresh, fig. 5), triangular (e. g. *G. aretioides*, fig. 7), open V-shaped (e. g. *G. caricifolia*, fig. 17) and open sickle shaped (e. g. *G. acantholimoides*, fig. 15).

The length of ventral axis of the midrib (column 3 in table 2) varies from 285 μm in *G. elegans* to 700 μm in *G. acantholimoides* Bornm.. The dorsi-ventral axis of the midrib (column 4 in table 2) ranges from 295 μm in *G. elegans* to 800 μm in *G. acantholimoides*.

In general, the cross-sections are divided in two groups according to their plane of dorsi-ventral symmetry: bifacial and isobilateral. The former is characterized by palisade parenchyma arranged in 2-3 layers on the upper surface (or ventral side) and the lower mesophyll filled by spongy parenchyma (e. g. *G. pilosa*, *S. bodeana* Boiss., figs. 20, 24) while the latter comprises of 2-5 layers of palisade parenchyma at each side and 1-11 layers of spongy parenchyma between them (eg. *G. acantholimoides*, fig. 15 and *G. polyclada*, fig. 9). The spongy parenchyma consists of 2-4 layers of irregular cells in most species, but in *G. aretioides*

(fig. 8), *G. mucronifolia* Rech. f. (fig. 13) and *G. perfoliata* L. (fig. 3). Several layers of spongy parenchyma (4-11) fill the mesophyll of the midrib around the vascular cylinder. These cells are relatively compact and characterized by considerable intercellular spacing. In some species the spongy parenchyma fills the lamina around entirely (e. g. *G. perfoliata*, fig. 4), while in other species only few layers of spongy parenchyma can be observed in the ground tissue of the midrib (*G. polyclada*, fig. 9).

The shape of vascular bundles varies considerably among different species of *Gypsophila* and allied genera studied here from U-shaped (*Allochrysa bungei* Boiss, fig. 22 and *A. persica* Boiss.) through V-shaped (*Ankyropetalum gypsophyloides* Fenzl., fig. 2) and continuous arc median bundle often with curved ends (*G. polyclada*, fig. 9). Furthermore, most species of *Gypsophila* and *Saponaria* have continuous arc vascular bundle with curved ends. The median vascular bundle is usually curved (e. g. *G. acantholimoides*, fig. 16; *G. caricifolia*, fig. 18; *G. polyclada*, fig. 10), but rarely straight (e. g. *G. linearifolia* (Fisch. & C. A. Mey.) Boiss., fig. 12; *G. mucronifolia*, fig. 14). Each vascular bundle in the midrib is surrounded by a sclerenchymatous bundle sheath. For median vascular bundles, the thinnest bundle sheath is observed in *G. mucronifolia* (fig. 14), with only one layer of sclerenchymatous cells, and the thickest one is present in *G. acantholimoides* (fig. 16) and *G. ruscifolia* Boiss., with 11 layers of sclerenchymatous cells.

Most species possess inorganic deposits in their mesophyll most likely of calcium oxalate compounds. Druse crystals were mainly distributed in the mesophyll and around the vascular bundles of the leaf mostly in high density, but they were sparse near the epidermis. They were densely distributed in the mesophyll of some species (e. g. *G. mozaffarianii*, fig. 6), but sparsely in others (e. g. *G. mucronifolia*, fig. 13). Druse crystals near the epidermis were smaller than those near the vascular bundles (see *G. mozaffarianii*, fig. 6).

The majority of species show glabrous epidermis, but glandular multicellular trichomes can also be observed in some species studied (e. g. *G. acantholimoides*, fig. 26). A few species, for example *G. pilosa* shows simple multicellular non-glandular trichomes (fig. 27).

Table 2. Some anatomical features in selected species of *Gypsophila* and allied genera. Abbreviations: TS = transverse section, VA = ventral axis, DVA= dorsiventral axis, MB = median bundle, = not recognizable.

Species	TS shape	Mesophyll symmetry	VA (μm) in midrib	DVA (μm) in midrib	MB shape	Phloem thickness (μm)	Xylem thickness (μm)
<i>Ankyropetalum gypsophilooides</i>	Open V-shaped	Isobilateral	(370-) 390 \pm 4 (-410)	(440-) 450 \pm 3 (-460)	Open V-shaped	(8-) 9 \pm 1 (-10)	(18-) 20 \pm 2 (-22)
<i>Allochrusa bungei</i>	-	Isobilateral	(270-) 290 \pm 2 (-310)	(300-) 310 \pm 4 (-320)	Open U-shaped	(7-) 8 \pm 2 (-9)	(12-) 13 \pm 1 (-14)
<i>A. persica</i>	-	Isobilateral	(290-) 300 \pm 5 (-310)	(300-) 300 \pm 0 (-300)	Open U-shaped	(7-) 7 \pm 0 (-7)	(8-) 10 \pm 1 (-12)
<i>Gypsophila</i> Subg. <i>Gypsophila</i>							
Sect. <i>Dichoglottis</i>							
<i>G. bellidifolia</i>	-	Isobilateral	(285) 295 \pm 3 (-305)	(385-) 390 \pm 2 (-395)	Continuous arc with curved ends	(29-) 30 \pm 2 (-31)	(18-) 20 \pm 2 (-22)
<i>G. elegans</i>	-	Isobilateral	(275-) 285 \pm 4 (-295)	(285-) 295 \pm 4 (-305)	Continuous arc with curved ends	(38-) 40 \pm 3 (-42)	(23-) 25 \pm 1 (-27)
<i>G. iranica</i>	-	Isobilateral	(330-) 340 \pm 7 (-350)	(570-) 580 \pm 4 (-590)	Continuous arc with curved ends	(63-) 65 \pm 2 (-67)	(29-) 30 \pm 2 (-31)
<i>G. leioclada</i>	-	Isobilateral	(290-) 300 \pm 3 (-310)	(340-) 350 \pm 3 (-360)	Continuous arc with curved ends	(30-) 30 \pm 0 (-30)	(14-) 15 \pm 2 (-16)
<i>G. linearifolia</i>	Orbicular	Isobilateral	(540-) 550 \pm 4 (-560)	(435-) 450 \pm 4 (-465)	Continuous straight strand	(33-) 35 \pm 3 (-37)	(9-) 10 \pm 1 (-11)
<i>G. mucronifolia</i>	Orbicular	Isobilateral	(670-) 680 \pm 5 (-690)	(570-) 585 \pm 4 (-600)	Continuous straight strand	(67-) 70 \pm 2 (-73)	(10-) 10 \pm 0 (-10)
<i>G. persica</i>	-	Isobilateral	(390-) 400 \pm 4 (-410)	(540-) 550 \pm 4 (-560)	Continuous arc with curved ends	(48-) 50 \pm 3 (-52)	(25-) 25 \pm 0 (-25)
<i>G. polyclada</i> var. <i>glandolusa</i>	-	Isobilateral	(440-) 450 \pm 3 (-460)	(570-) 580 \pm 4 (-590)	Continuous arc with curved ends	(74-) 75 \pm 1 (-76)	(31-) 33 \pm 2 (-35)
<i>G. polyclada</i> var. <i>polyclada</i>	-	Isobilateral	(415-) 425 \pm 4 (-435)	(570-) 575 \pm 3 (-580)	Continuous arc with curved ends	(68-) 70 \pm 2 (-72)	(29-) 30 \pm 2 (-31)
<i>G. xanthochlora</i>	-	Isobilateral	(440-) 450 \pm 4 (-460)	(470-) 475 \pm 4 (-480)	Continuous arc with curved ends	(53-) 55 \pm 3 (-57)	(28-) 30 \pm 2 (-32)
Sect. <i>Ensifolia</i>							
<i>G. caricifolia</i>	Open V-shaped	Isobilateral	(570-) 580 \pm 5 (-590)	(650-) 650 \pm 0 (-650)	Continuous arc with curved ends	(96-) 100 \pm 4 (-104)	(19-) 20 \pm 1 (-21)
<i>G. graminifolia</i>	Open V-shaped	Isobilateral	(550-) 550 \pm 0 (-550)	(620-) 625 \pm 2 (-630)	Continuous arc with curved ends	(88-) 90 \pm 3 (-92)	(24-) 25 \pm 1 (-26)

Table 2. Continued.

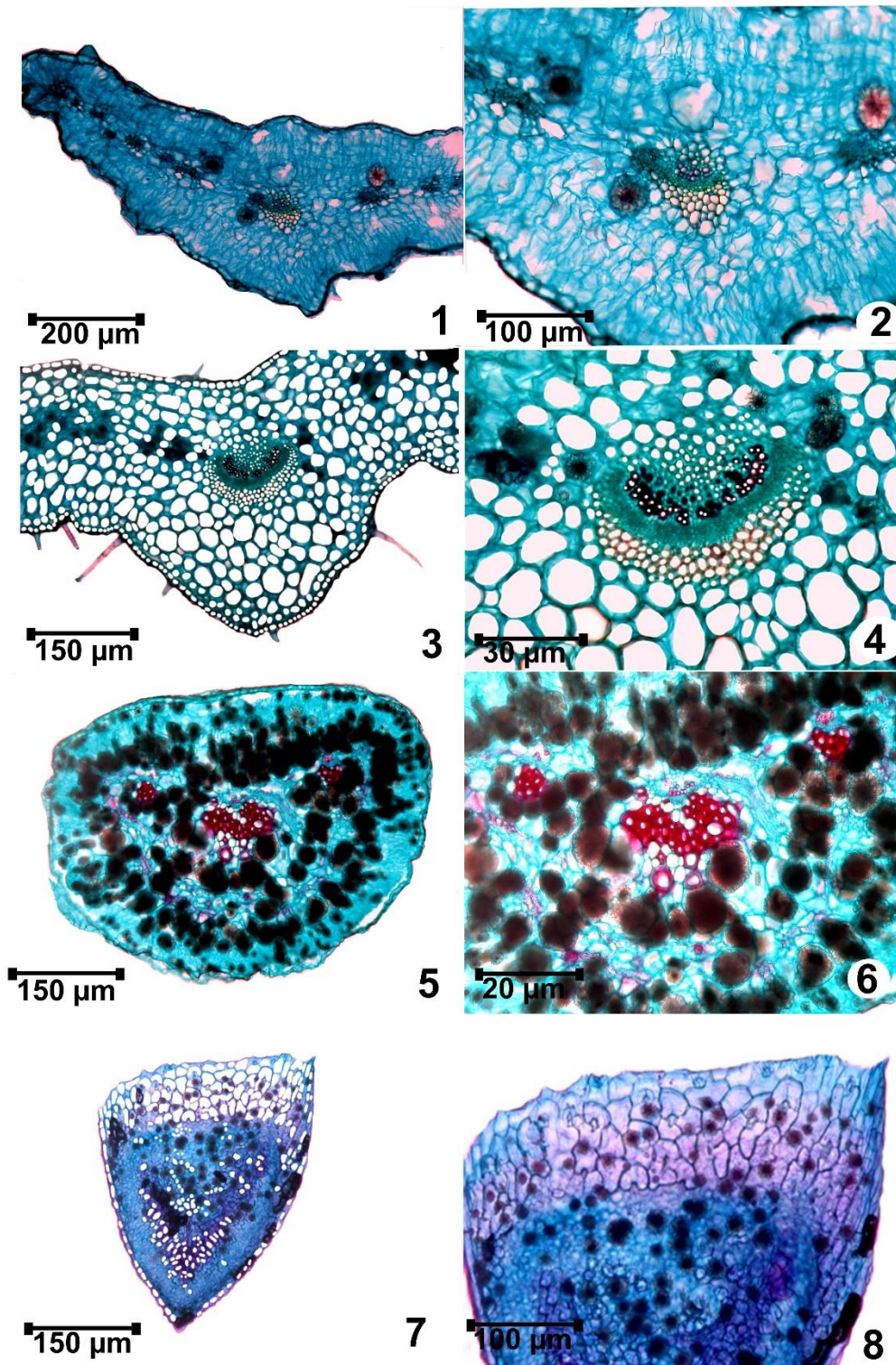
Species	TS shape	Mesophyll symmetry	VA (μm) in midrib	DVA (μm) in midrib	MB shape	Phloem thickness (μm)	Xylem thickness (μm)
Sect. <i>Hoplites</i>							
<i>G. acantholimoides</i>	Open sickle-shaped	Isobilateral	(700-) 700 ± 0 (-700)	(790-) 800 ± 4 (-810)	Continuous arc with curved ends	(68-) 70 ± 2 (-72)	(40-) 40 ± 0 (-40)
Sect. <i>Exscapae</i>							
<i>G. aretioides</i>	Triangular	Bifacial	(590-) 600 ± 4 (-610)	(680-) 700 ± 0 (-720)	Continuous arc with curved ends	(9-) 10 ± 1 (-11)	(18-) 20 ± 2 (-22)
<i>G. mozaffarianii</i>	Orbicular	Isobilateral	(635-) 650 ± 5 (-665)	(770-) 775 ± 2 (-780)	Continuous arc with curved ends	(19-) 20 ± 1 (-21)	(15-) 15 ± 0 (-15)
Sect. <i>Paniculaeformes</i>							
<i>G. bicolor</i>	-	Isobilateral	(440-) 450 ± 4 (-460)	(340-) 350 ± 4 (-360)	Continuous arc with curved ends	(23-) 25 ± 3 (-27)	(12-) 12 ± 0 (-12)
<i>G. perfoliata</i>	-	Isobilateral	(590-) 600 ± 4 (-610)	(520-) 530 ± 4 (-540)	Continuous arc with very much curved ends	(19-) 20 ± 2 (-21)	(14-) 15 ± 1 (-16)
<i>G. ruscifolia</i>	-	Isobilateral	(395-) 400 ± 2 (-405)	(370-) 375 ± 4 (-380)	Continuous arc with curved ends	(28-) 30 ± 3 (-32)	(18-) 20 ± 1 (-22)
<i>G. virgata</i>	-	Isobilateral	(415-) 425 ± 3 (-435)	(340-) 350 ± 4 (-360)	Continuous arc with very much curved ends	(23-) 25 ± 2 (-27)	(10-) 10 ± 0 (-10)
Subg. <i>Hagenia</i>							
Sect. <i>Hispidae</i>							
<i>G. platyphylla</i>	-	Isobilateral	(320-) 330 ± 4 (-340)	(310-) 315 ± 1 (-320)	Continuous arc with curved ends	(19-) 20 ± 1 (-21)	(10-) 10 ± 0 (-10)
Sect. <i>Pseudosaponaria</i>							
<i>G. pilosa</i>	-	Bifacial	(335-) 350 ± 5 (-365)	(315-) 325 ± 3 (-335)	Continuous arc with curved ends	(78-) 80 ± 3 (-82)	(19-) 20 ± 1 (-21)
<i>Saponaria</i>							
<i>S. bodeana</i>	-	Bifacial	(320-) 335 ± 4 (-350)	(310-) 320 ± 4 (-330)	Continuous arc with very much curved ends	(19-) 20 ± 1 (-21)	(12-) 14 ± 2 (-16)
<i>S. floribunda</i>	-	Isobilateral	(290-) 295 ± 4 (-300)	(330-) 340 ± 4 (-350)	Continuous arc with curved ends	(13-) 15 ± 2 (-17)	(8-) 10 ± 1 (-12)
<i>S. orientalis</i>	-	Isobilateral	(330-) 340 ± 2 (-350)	(335-) 355 ± 0 (-335)	Continuous arc with very much curved ends	(8-) 10 ± 2 (-12)	(15-) 16 ± 1 (-17)
<i>S. viscosa</i>	-	Isobilateral	(310-) 325 ± 2 (-340)	(340-) 345 ± 2 (-350)	Continuous arc with very much curved ends	(15-) 15 ± 0 (-15)	(14-) 14 ± 0 (-14)

Table 2. Continued.

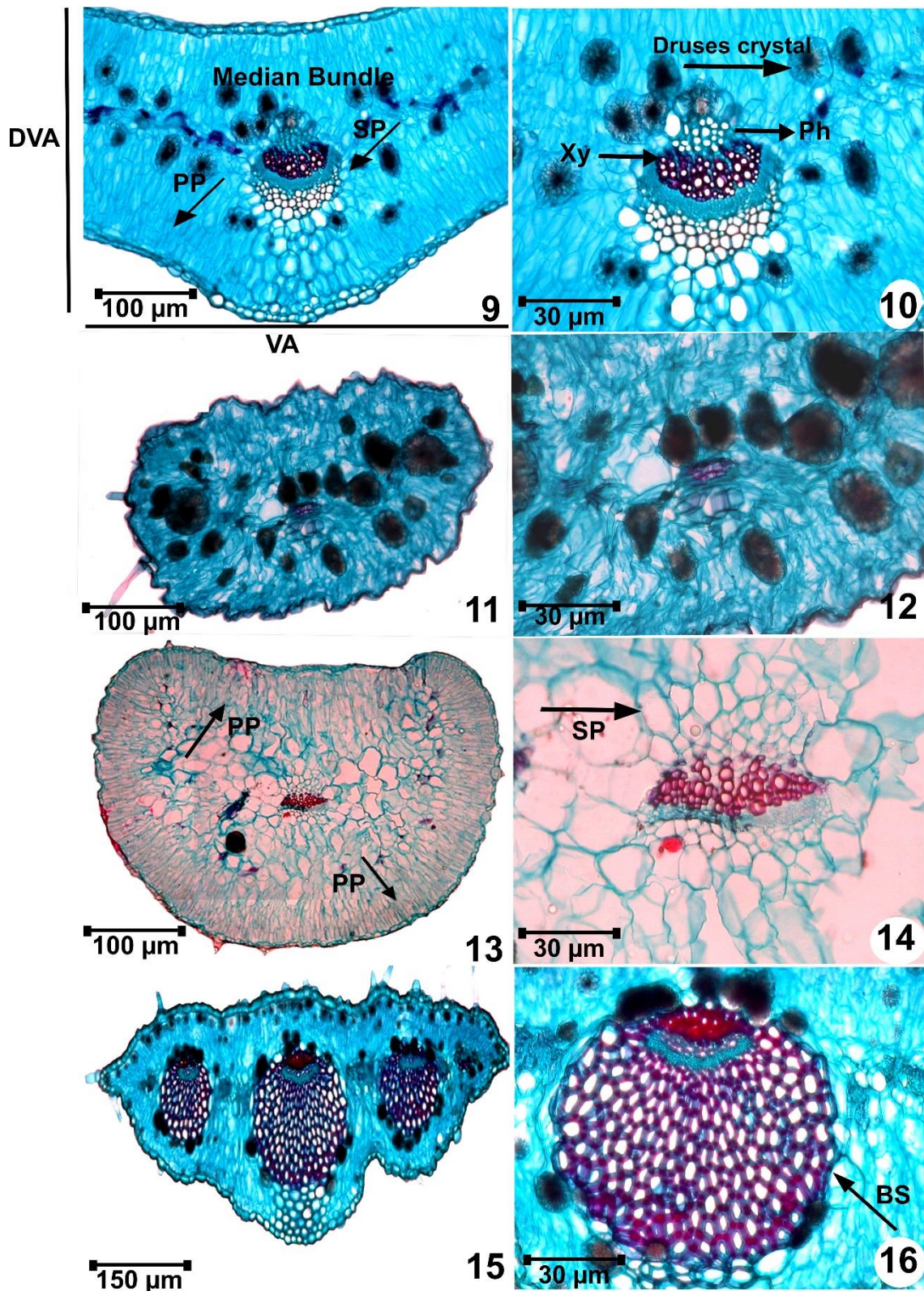
Species	Number of palisade layer	Number of spongy layer	Thickness of palisade tissue (μm)	Thickness of spongy tissue (μm)	Sclerenchyma thickness (μm)	Crystal druses in mesophyll	Hair like papillae
<i>Ankyropetalum gypsophiloides</i>	3	5	(78-) 80 ± 2 (-82)	(38-) 38 ± 0 (-38)	(88-) 90 ± 2 (-92)	Abundant	Absent
<i>Allochrysa bungei</i>	2	2	(64-) 65 ± 1 (-66)	(34-) 36 ± 2 (-38)	(78-) 80 ± 2 (-82)	Abundant	Absent
<i>A. persica</i>	2	2	(50-) 50 ± 0 (-50)	(34-) 35 ± 1 (-36)	(95-) 100 ± 4 (-105)	Abundant	Present-glandular
Gypsophila Subg. Gypsophila Sect. Dichoglottis							
<i>G. bellidifolia</i>	3	3	(68-) 70 ± 2 (-72)	(33-) 35 ± 2 (-37)	(77-) 80 ± 3 (-83)	Abundant	Absent
<i>G. elegans</i>	3	3	(65-) 65 ± 0 (-65)	(45-) 45 ± 0 (-45)	(69-) 70 ± 1 (-71)	Sparse	Absent
<i>G. iranica</i>	3	2	(78-) 80 ± 2 (-82)	(59-) 60 ± 1 (-61)	(75-) 75 ± 0 (-75)	Sparse	Absent
<i>G. leioclada</i>	3	3	(74-) 75 ± 3 (-76)	(38-) 40 ± 2 (-42)	(63-) 65 ± 3 (-67)	Sparse	Absent
<i>G. linearifolia</i>	3	2	(82-) 85 ± 4 (-88)	(33-) 35 ± 2 (-37)	Absent	Dense	Present-nonglandular
<i>G. mucronifolia</i>	3	5	(87-) 90 ± 4 (-93)	(54-) 55 ± 1 (-56)	(19-) 20 ± 1 (-21)	Sparse	Present-nonglandular
<i>G. persica</i>	3	3	(69-) 70 ± 1 (-71)	(40-) 40 ± 0 (-40)	(68-) 70 ± 2 (-72)	Sparse	Absent
<i>G. polyclada</i> var. <i>glandolusa</i>	3	2	(75-) 75 ± 0 (-75)	(33-) 35 ± 2 (-37)	(95-) 100 ± 5 (-105)	Abundant	Absent
<i>G. polyclada</i> var. <i>polyclada</i>	3	2	(78-) 80 ± 2 (-82)	(34-) 35 ± 1 (-36)	(97-) 100 ± 2 (-103)	Abundant	Absent
<i>G. xanthochlora</i>	3	2	(64-) 65 ± 1 (-66)	(30-) 30 ± 0 (-30)	(88-) 90 ± 3 (-92)	Sparse	Absent
Sect. Ensifolia							
<i>G. caricifolia</i>	3	4	(73-) 75 ± 3 (-77)	(38-) 40 ± 3 (-42)	(99-) 100 ± 1 (-101)	Abundant	Absent
<i>G. graminifolia</i>	3	3	(70-) 70 ± 0 (-70)	(44-) 45 ± 1 (-46)	(85-) 85 ± 0 (-85)	Abundant	Absent
Sect. Hoplites							
<i>G. acantholimoides</i>	5	2	(74-) 75 ± 1 (-76)	(48-) 50 ± 2 (-52)	(260-) 270 ± 7 (-280)	Abundant	Present-glandular
Sect. Exscapae							
<i>G. aretioides</i>	5	4	(54-) 55 ± 1 (-56)	(25-) 25 ± 0 (-25)	Absent	Dense	Absent
<i>G. mozzaffarianii</i>	3	5	(57-) 60 ± 2 (-63)	(30-) 30 ± 0 (-30)	(98-) 100 ± 2 (-102)	Dense	Absent
Sect. Paniculaeformes							
<i>G. bicolor</i>	3	2	(54-) 55 ± 1 (-56)	(33-) 35 ± 2 (-37)	(72-) 75 ± 3 (-78)	Sparse	Absent

Table 2. Continued.

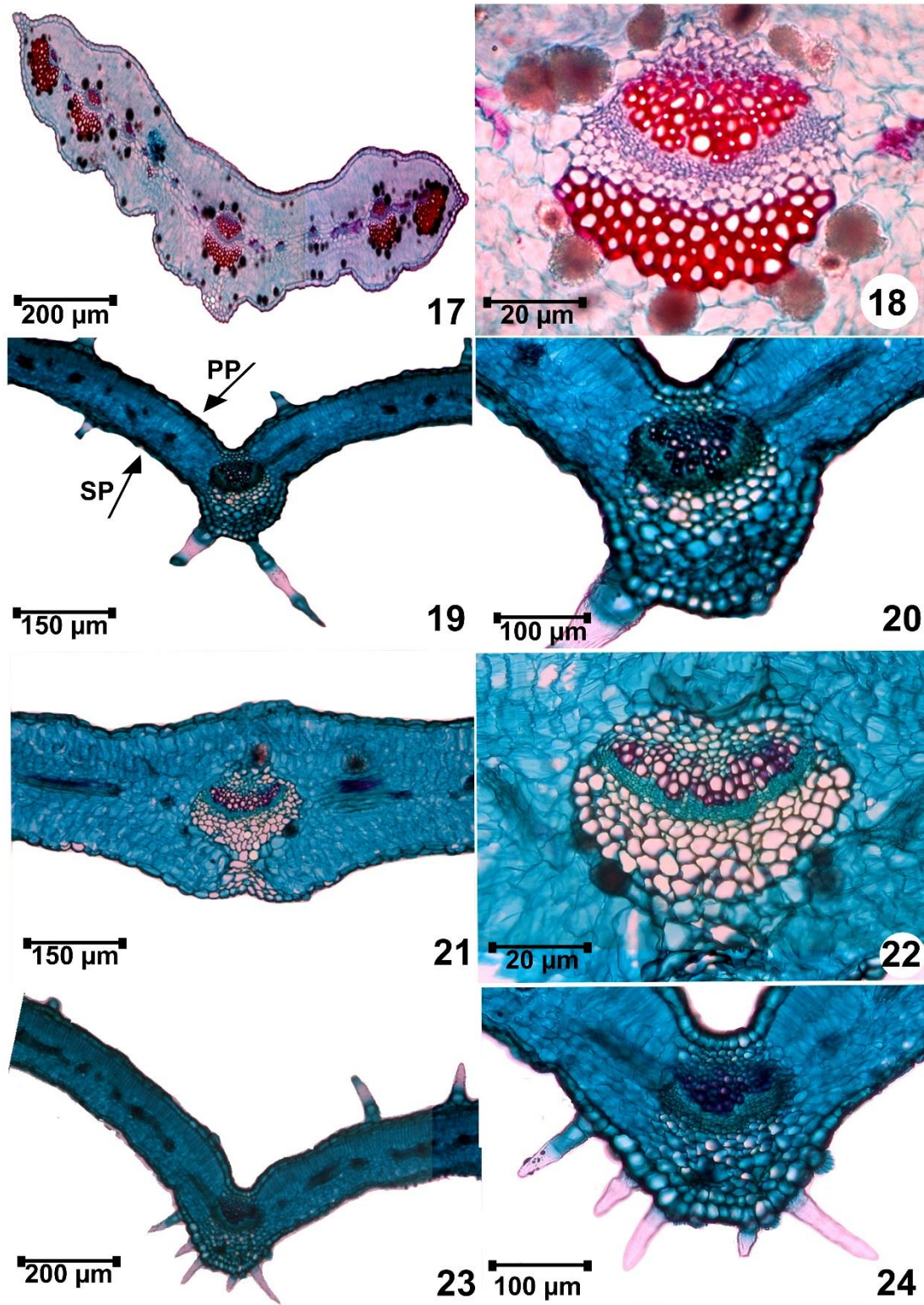
Species	Number of palisade layer	Number of spongy layer	Thickness of palisade tissue (μm)	Thickness of spongy tissue (μm)	Schlerenchyma thickness (μm)	Crystal druses in mesophyll	Hair like papillae
<i>G. perfoliata</i>	0	11	Absent	(54-) 55 ± 2 (-56)	(60-) 60 ± 0 (-60)	Sparse	Present-non glandular
<i>G. ruscifolia</i>	3	2	(39-) 40 ± 1 (-41)	(30-) 30 ± 0 (-30)	(190-) 200 ± 6 (-210)	Sparse	Absent
<i>G. virgata</i>	2	3	(49-) 50 ± 1 (-51)	(38-) 40 ± 2 (-42)	(97-) 100 ± 3 (-103)	Sparse	Absent
Subg. Hagenia							
Sect. Hispidae							
<i>G. platyphylla</i>	4	2	(57-) 60 ± 2 (-63)	(44-) 45 ± 3 (-46)	(80-) 85 ± 4 (-90)	Sparse	Absent
Sect. Pseudosaponaria							
<i>G. pilosa</i>	2	4	(78-) 80 ± 3 (-82)	(68-) 70 ± 3 (-72)	(105-) 110 ± 5 (-115)	Sparse	Present-non glandular
Saponaria							
<i>S. bodeana</i>	2	3	(53-) 55 ± 2 (-57)	(24-) 25 ± 1 (-26)	(70-) 75 ± 4 (-80)	Sparse	Present-non glandular
<i>S. floribunda</i>	2	1	(48-) 50 ± 3 (-52)	(30-) 30 ± 0 (-30)	(66-) 70 ± 3 (-74)	Sparse	Present-non glandular
<i>S. orientalis</i>	2	1	(57-) 60 ± 3 (-63)	(37-) 38 ± 1 (-39)	(60-) 60 ± 0 (-60)	Sparse	Absent
<i>S. viscosa</i>	2	1	(63-) 65 ± 2 (-67)	(44-) 45 ± 2 (-46)	(60-) 65 ± 3 (-70)	Sparse	Absent



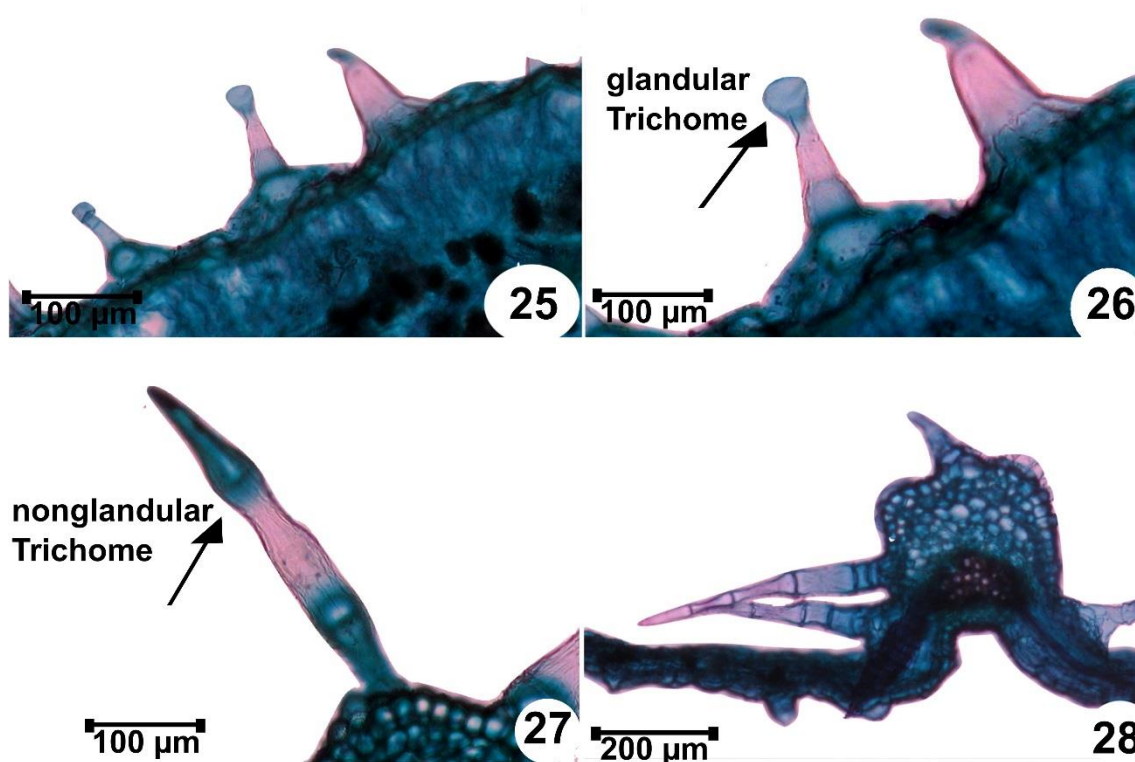
Figs. 1-8. Transverse section of midrib in selected species of *Ankyropetalum* and *Gypsophila*, 1-2. *Ankyropetalum gypsophylloides*; 3-4, *Gypsophila perfoliata*; 5-6, *G. mozaffarianii*; 7-8, *G. aretioides*. Abbreviations: Xy=Xylem, Ph=Phloem, PP=Palisade parenchyma, SP= spongy parenchyma, BS: Sclerenchymatous cap.



Figs. 9-16 Transverse section of midrib in selected species of *Gypsophila*, 9- 10, *G. polyclada* var. *polyclada*.; 11- 12, *G. linearifolia*; 13- 14, *G. mucronifolia*; 15- 16, *G. acantholimoides*.



Figs 17-24. C. Transverse section of midrib in selected species of *Gypsophila* and *Saponaria*, 17-18, *G. caricifolia*; 19- 20, *G. pilosa*; 21-22, *Allochrusa bungei*; 23-24, *Saponaria bodeana*.



Figs. 25-28. Trichome types in selected species of *Gypsophila*, 25-26, *G. acantholimoides*; 27-28, *G. pilosa*.

DISCUSSION

Due to the particular value of leaf anatomy, and because most of the surveyed characters were constant among different specimens of a given species, a comprehensive discussion is given below on the taxonomic importance of leaf blade anatomy at generic and subgeneric level. In general, leaf anatomy does not provide any unique feature supporting the separation of genera *Ankyropetalum* and *Allochrysa* from *Gypsophila*.

Circumscription of genera related to *Gypsophila*

The genera *Saponaria* and *Ankyropetalum* are closely related to *Gypsophila* (Barkoudah 1962; Harbaugh & al. 2010; Greenberg & Donoghue 2011; Pirani & al. 2014; Madhani & al. 2018). Although there are several morphological synapomorphies such as a high number of calyx veins and clawed petals (Bittrich 1993) supporting the monophyly of *Gypsophila* and separation of *Saponaria* from *Gypsophila*, but distinguishing *Ankyropetalum* from *Gypsophila* is much more a matter of doubt.

The four species of *Saponaria* studied here show

two different patterns of mesophyll symmetry. *Saponaria bodeana* is characterized by bifacial type, while *S. orientalis* L., *S. floribunda* (Kar. & Kir.) Boiss. and *S. viscosa* C. A. Mey. show isobilateral pattern. Also, druses are sparse in all species of *Saponaria*. It seems that the low number of spongy mesophyll layers (one-layer) and a thin sclerenchymatous bundle sheath around vascular bundle provide some support at least for separation of *Saponaria* from *Gypsophila*. Moreover, seed surface micro-sculpturing (Amini & al. 2011) and molecular phylogenetic data (Harbaugh & al. 2010; Greenberg & Donoghue 2011; Pirani & al. 2014; Madhani & al. 2018) can also be considered as additional evidence for separation of *Saponaria* from *Gypsophila*.

Leaf blade of *A. gypsophiloides* (fig. 1) is composed of five layers of spongy mesophyll with abundant crystals and isobilateral mesophyll symmetry. These characters (abundant crystals and isobilateral mesophyll symmetry) are found in *Gypsophila* too. The shape of vascular bundles in most of the studied species of *Gypsophila* is continuous arc with curved ends but it is V-shaped in *A. gypsophiloides* (fig. 2). Therefore,

leaf anatomy does not support separation of *Ankyropetalum* from *Gypsophila*. Moreover, seed micro-morphology does not provide supportive data for distinguishing these two genera (Amini & al. 2011). Also, the deeply incised petals and the exerted stamens in *Ankyropetalum* have been considered as strong morphological characters supporting the separation of these two genera (Barkoudah 1962). Molecular study (nrDNA ITS and *rps16*) conducted by Madhani & al. (2018), confirms inclusion of *Ankyropetalum* within *Gypsophila*.

Allochrusa was first proposed as distinct genus from *Acanthophyllum* C. A. Mey. (Boissier 1867) based on non-spiny leaves and enclosed stamens, but it was included in *Acanthophyllum* by Golenkin (1893) and Schischkin (1936). Barkoudah (1962) adopted Schischkin's treatment. However, *Allochrusa* was later accepted as a separate genus by some other taxonomists (Yukhananov 1974; Schiman-Czeika 1987; Bittrich 1993; Takhtajan 2009). Presence of bracteoles in *Allochrusa* reflects their phylogenetic position nested within *Acanthophyllum* (Madhani & al. 2018). In our study, shape of median bundle in two species of *Allochrusa* are U-shaped, while most species in *Gypsophila* indicated continuous arc with curved ends. Also, druses density is abundant in *Allochrusa* and glandular trichomes can also be observed in *A. persica*. Most species of *Gypsophila* have 3-5 layers of palisade parenchyma at each side and 2-11 layers of spongy parenchyma between them while the palisade parenchyma is arranged in 2 layers in *Allochrusa*. *Allochrusa* also shows a unique seed surface sculpturing composed of non- or indistinctly grooved anticlinal walls (Amini & al. 2011). Therefore, leaf blade anatomy and seed micro-morphology support this distant relationship. Mahmoudi Shamsabad & al. (2013) studied leaf anatomy of *Acanthophyllum* and found that mesophyll in *Acanthophyllum* is isolateral, sclerenchymatous layers are very thick and glandular and eglandular hairs are present. Leaf blade anatomy in *Allochrusa* shows thin sclerenchymatous layer. This character provide some support at least for the separation of *Acanthophyllum* from *Allochrusa*.

Subgeneric classification of *Gypsophila*

Gypsophila sect. *Dichoglottis* (Fisch. & C. A. Mey.) Boiss.

The members of this section are predominantly distributed in the eastern part of the Irano-Turanian region and endemic to Iran (Barkoudah 1962). Most species of this section are characterized by a lax or dichasium inflorescence, lanceolate leaves and rounded calyx lobes. Despite considerable morphological similarities among some species of the section, leaf

blade anatomy can provide support for distinguishing some species within this section. The shape of vascular bundles in the midrib seems to be useful in distinguishing sections within *Gypsophila*. *Gypsophila linearifolia* and *G. mucronifolia* are remarkable in having fleshy linear leaves, non-glandular hairs on the vegetative parts and small calyces. *Gypsophila mucronifolia* is the only species growing in gypsy soils restricted to Sorkheh, Semnan province, NE Iran (Rechinger 1988). The outline of the leaf blade in *G. linearifolia* and *G. mucronifolia* (fig. 12; fig. 13) (orbicular), provides further evidence for separation of these species from the rest of the section. Our results show that the shape of the central vascular bundle has diagnostic value in characterizing some species. For example, in most species of *G.* sect. *Dichoglottis* central vascular bundle is usually continuous arc with curved ends (e. g. *G. acantholimoides*, fig. 16; *G. caricifolia*, fig. 18; *G. polyclada*, fig. 10) but in *G. linearifolia* (fig. 12) and *G. mucronifolia* (fig. 14) it is continuous arc with straight ends. Leaves of *G. mucronifolia* (fig. 13) possess five layers of spongy mesophyll, sparse crystals and a thin sclerenchymatous bundle but in *G. linearifolia* druses crystals appear in high density and sclerenchymatous bundle is absent. *Gypsophila polyclada* var. *polyclada* Fenzl ex. Boiss. (fig. 9) and *G. polyclada* var. *glandolusa* Barkoudah have many morphological similarities: spatulate basal leaves and lanceolate cauline leaves with campanulate calyx as well as oblanceolate petals are characteristic for both varieties. They do not show any difference in leaf blade anatomy indicating the constancy of the mentioned characters at subspecific ranks. *Gypsophila bellidifolia* Boiss. is morphologically different from other species of the section based on its spatulate leaves and the presence of long hairs on the petals. Barkoudah (1962) placed this species in *Saponaria*, but Rechinger (1988) transferred it into *Gypsophila*. Results from leaf blade anatomy is in agreement with Rechinger's opinion. *Gypsophila persica* Barkoudah, *G. polyclada* and *G. xanthochlora* Rech. f. are three closely related species within sect. *Dichoglottis* which are recognized with minor morphological characters (Rechinger 1988). Sclerenchymatous bundle in *G. persica* is thinner than *G. xanthochlora* and *G. polyclada* and druses crystals are abundant in *G. polyclada* and sparse in *G. persica* and *G. xanthochlora*. *Gypsophila elegans* and *G. iranica* Barkoudah are other species of sect. *Dichoglottis* which are closely related to each other as indicated by their lanceolate and glabrous leaves. Two species show many similarities in leaf blade anatomy but phloem and xylem in *G. iranica* is thicker than *G. elegans*.

Madhani & al. (2018) demonstrated that three

species of this section including *G. elegans*, *G. leioclada* Rech. f. and *G. linearifolia* reside within different clades on ITS and *rps16* trees. For example, in the ITS tree, *G. linearifolia* was placed in a clade including *G. pilosa* (sect. *pseudosaponaria*), whereas, these two species in *rps16* tree nested in distinct clades (Madhani & al. 2018). Druses density in *G. linearifolia* is dense and mesophyll symmetry is isobilateral, while in *G. pilosa* are sparse and bifacial. In conclusion, leaf blade anatomy in agreement with morphological characters, seed micro-morphology and molecular data, and confirms the heterogeneity and the probable polyphyletic nature of sect. *Dichoglottis* as currently circumscribed.

Gypsophila* sect. *Ensifoliae Barkoudah and sect. ***Hoplites*** Rech. f.

Two species from section *Ensifoliae* were included in leaf blade anatomy in this study, *G. caricifolia* and *G. graminifolia*. Barkoudah (1962) placed three species (*G. caricifolia*, *G. graminifolia* Barkoudah and *G. acantholimoides*) in *Ensifoliae* but Rechinger (1988) separated *G. acantholimoides* as a monotypic sect. *Hoplites* mainly based on its cushion-form habit. In addition, the leaf cross-sections exhibit considerable difference in thickness of the sclerenchymatous bundle sheath (table 2) among the mentioned three species. The main vascular bundle in most species studied is surrounded by a bundle sheath which is mostly sclerenchymatous. The xylem and sclerenchyma thickness in *G. acantholimoides* (fig. 16) are more than the members of sect. *Ensifoliae*. Moreover, glandular trichomes can also be observed in *G. acantholimoides*, but they are absent in species of sect. *Ensifoliae*. Therefore, leaf anatomy as well as seed micro-morphology provides further evidence for separating sect. *Hoplites* from sect. *Ensifoliae*. The expansion of bundle sheath provides mechanical support for the leaf and is a xeromorphic characteristic (Rudall 2007). The number of palisade layers also have taxonomic value in Caryophyllaceae (Metcalfe & Chalk 1979). It has been proposed that low water can raise development of palisade tissue, and consequently increase photosynthetic activity (Fahn 1982). Accordingly, the mesophyll in *G. acantholimoides* (fig. 15), growing in center of Iran (Karkas Mountains) at an altitude between 2300 and 3300 m (Rechinger 1988), and exposed to high light intensity is mostly filled with palisade parenchyma (5-layers)..

Gypsophila* sect. *Exscapae Williams

The species of this section are mountain plants and mostly grow on bare calcareous rocks or in rock crevices. They are both morphologically and

ecologically well-characterized through their dense cushion-form habit, accompanied with very small leaves and reduced internodes (Barkoudah 1962). Size and shape of the leaves of *Gypsophila* and allied genera show considerable variation allowing a number of novel morphological features useful in the taxonomy of the group. In our study, the shape of the transverse section seems to be useful in distinguishing some groups or species (regarding the narrow leaves). As an example, *G. aretioides* is different from *G. mozaffarianii* by triangular transverse section (figs.7) although, both species have been identified as members of sect. *Exscapae*. In the former, mesophyll symmetry can be seen bifacial while in the latter is isobilateral. Druses density are similar in *G. aretioides* (fig. 8) and *G. mozaffarianii* (fig. 6). In these species, dense druse crystals were mainly distributed in the mesophyll and around the vascular bundles of the leaf, but detected near the epidermis scarcely. These species grow at an altitude between 1100 and 3700 m and are exposed to high light intensity. However, it is known that density of druse crystals is an environmentally influenced anatomical character and we cannot use it as a strong taxonomic character for grouping species (Kilic 2009). Regardless to distinct differences in morphology and anatomy of *G. aretioides* and *G. elegans*, analysis of ITS data by Madhani & al. (2018) indicated a sister group relationship between these two taxa (Madhani & al. 2018). Therefore, leaf blade anatomy support the heterogeneous composition and probably non-monophyly of this section.

Gypsophila* sect. *Paniculaeformes Williams

The members of this section have paniculate inflorescence, rigid stem with woody base. Seed micromorphological studies of Amini & al. (2011) showed that sect. *Paniculaeformes* is too heterogeneous, but anatomical characters were constant within this section. Mesophyll symmetry in all examined species from this section is isobilateral and druse crystals are sparse. All species of this section comprise of 2-3 layers of palisade parenchyma at each side and 2-11 layers of spongy parenchyma between them, except in *G. perfoliata* (fig. 4) which grows on sandy soils and prefers low altitudes and moisture (Barkoudah 1962). Mesophyll in *G. perfoliata* is totally filled with spongy parenchyma (11 layers). Madhani & al. (2018) indicated *G. bicolor* (Freyn & Sint.) Grossh. and *G. perfoliata*, both belonging to the section *Paniculaeformes*, reside in different clades. They clearly showed that the two species (*G. bicolor* and *G. perfoliata*) have independently derived within the section. The results from our study on leaf blade anatomy indicates that this section is homogeneous.

***Gypsophila* sect. *Hispidae* (Schischk.) Rech. f.**

As members of *G.* subgen. *Hagenia* (Moench) Fenzl, the species of this section are characterized by turbinate-tubiform long calyces covered with glandular hairs, linear and long petals, ovate leaves and lax inflorescence. The species in this section resemble *Saponaria* to some extent, but leaf blade anatomy as well as seed micro-morphology suggest that this species are more closely related to *Gypsophila*. Sclerenchymatous bundle in *G. platyphylla* Boiss. is thicker than *Saponaria* and *G. platyphylla* Boiss. shows 4-layers of palisade mesophyll, but *Saponaria* has 2-layers.

***Gypsophila* sect. *Pseudosaponaria* Williams**

We examined the only species of this section in Iran, *G. pilosa*, which prefers ruderal places and field margins. It is superficially very similar to the species of *Saponaria*. It possesses simple multicellular non-glandular trichomes (fig. 27) and based on the key to the genera provided by Barkoudah, it is clearly placed within *Gypsophila*. Unlike other species of *Gypsophila*, leaf symmetry in this species can be seen as bifacial. In general, the leaf blade anatomy as well as the type of seed microsculpturing (Amini & al. 2011) discriminates this species from *Saponaria*. Based on the molecular analyses of ITS data by Madhani & al. (2018), *G. pilosa* and *G. linearifolia* are sister taxa, while, on the *rps16* tree they resolve on different clades. *Gypsophila pilosa* and *G. linearifolia* are morphologically and anatomically distinct from each other.

Conclusions

The infrageneric classification of *Gypsophila* and intergeneric relationships in Caryophyllaceae need to be revised based on evidence from different sources, especially molecular systematics. Leaf blade anatomical data presented here, indicate a heterogeneous composition of some sections and provide some support for most sections defined in previous taxonomic treatments of the genus. Based on morphological and anatomical data it seems that *Ankyropetalum* should be included in *Gypsophila*. *Gypsophila polyclada* var. *glandolusa* is closely related to *G. polyclada* var. *polyclada* and differs from it by having hairs on pedicel. These two species do not have any difference in leaf blade anatomy as well as seed surface micro-sculpturing which suggests their probable synonymy.

ACKNOWLEDGEMENT

We are grateful to Research Institute of Forests and Rangelands (Tehran, TARI) and Tehran University

Herbaria (TUH) for their supports and permission to use the herbarium specimens..

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