The significance of morphological and anatomical investigation of leaves and stems for the identification of Zygophyllaceae.

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Abstract

Background

In this study we examined morphological and anatomical variation in stem and leaf tissue of three genera in the Zygophyllaceae: *Fagonia* (4 taxa), *Tetraena* (4 taxa), and *Tribulus* (4 taxa) were investigated.

Results

The twelve examined taxa included nine species that grow in eastern region of Saudi Arabia. Additional features for taxon identification and characterization were developed as a result of our observations. We recorded stem diagram, epidermal cell shape, cortex, pith, the number of vascular bundles, petiole and blade outline in cross section, mesophyll type, and secretory and crystal distribution.

Conclusions

We conclude that anatomical features, particularly the petiole and blade outlines and the type of vasculature in the stem and petiole, are significant for identifying and distinguishing between the species examined here. A bracketed key is provided for the identification of the investigated taxa based on the characters analyzed here. The characters assessed in this study agreed with previous classifications of Zygophyllaceae species.

Introduction

Zygophyllaceae (caltrops) is a large, widespread family of plants adapted to semi-desert and Mediterranean climates (Hammoda et al., 2013). This family is found in parts of Europe, Asia, Australia, Africa, and the Americas (Sheahan, 2007), and consists of approximately 285 species belonging to 22 genera (Mabberley, 2008). The family itself has been recently divided into five subfamilies (Zygophylloideae, Seetzenioideae, Tribuloideae, Morkillioideae, and Larreoideae) according to (Sheahan & Chase, 2000), (Beier et al., 2003) and (Bellstedt et al., 2008).

Which taxa have been allocated to the Zygophyllaceae has changed through time, since there is substantial structural diversity in associated species, chiefly in the genera *Balanites, Nitraria, Peganum*, and *Tetradiclis.* Engler, (1931) originally included all of these species in the Zygophyllaceae but under different subfamilies. However, (Takhtajan 1969) separated species from *Balanites*, *Nitraria*, and *Peganum* from the Zygophyllaceae and allocated them to distinct families (i.e., the Nitrariaceae, Balanitaceae, and Peganaceae). More recently, (Sheahan and Cutler 1993) examined the stem and leaf anatomy of 37 species belonging to 19 genera. They found that there was sufficient micro-morphological evidence to separate *Balanites* into a distinct family, and that the genera *Tribulus, Kallstroemia*, and *Kelleronia* did not belong in the Zygophylloideae subfamily. Later, (El-Hadidi and Fayed 1995) suggesting adding species from the genera *Peganum, Tetradiclis, Seetzenia; Zygophylum*, and *Fagonia* to the Zygophyllaceae and considered *Tribulus, Nitraria*, and *Balanites* to be distinct families (i.e., the Tribulaceae, Nitrariaceae and Balanitaceae).

Later studies, such as (Sheahan and Chase 1996) also considered the *Nitraria* and *Peganum to be distinct* from Zygophyllaceae based on morphological, anatomical, and DNA data and were found close to members of Sapindales. Boulos, (2009) followed Sheahan and Chase, (1996) showed that Zygophyllaceae include *Fagonia, Zygophyllum, Tribulus, Balanites* and *Seetzenia*, but *Nitraria* and *Peganum* recorded under distinct families Nitrariaceae and Peganaceae. Then Sheahan (2011) placed *Zygophyllum, Fagonia, Tribulus, Balanites, Seetzenia* and *Guaiacum* in the family Zygophyllaceae.

Migahid (1978) reported that five genera and 17 species from the Zygophyllaceae were present in Saudi Arabia. In contrast, (Chaudhary 2001) recorded seven genera *(Zygophyllum, Fagonia, Tribulus, Balanites, Seetzenia, Peganum*, and *Nitraria)* 24 species, 2 sub-species, and 12 varieties. More recently, (Thomas 2011) identified 6 genera from the Zygophyllaceae *(Balanites, Fagonia, Seetzenia, Tetraena, Tribulus*, and *Zygophyllum)*, which included 22 species and 9 varieties. In eastern Saudi Arabia, only 15 species, belonging to 6 genera *(Zygophyllum, Fagonia, Tribulus, Seetzenia, Peganum*, and *Nitraria)* were present according to (Mandaville 1990).

Of the Zygophyllaceae species present in Saudi Arabia belong to the Zygophylloideae subfamily. Zygophylloideae are the largest subfamily in the Zygophyllaceae, consisting of about 180 species of shrubs, subshrubs, and herbs. These are currently grouped in four genera, monotypic *Augea, Fagonia, Tetraena*, and *Zygophyllum* as per (Sheahan and Chase 1996 and 2000). Recently, many phylogenetic studies have been performed on the Zygophylloideae. For example, (Beier et al. 2003) investigated the phylogeny of the Zygophylloideae by combining morphological characters and noncoding *trn*L plastid data. Using this dataset they proposed moving 35 species from *Zygophyllum* to *Tetraena*. This classification scheme was validated by subsequent studies, including (Norton et al. 2009), (Louhaichi et al. 2011), (Mosti *et al.* 2012), (Sakkir et al. 2012), (Symanczik et al. 2014), and (Ghazanfar and Osborne, 2015).

The main characters used to distinguish between the different taxa of Zygophyllaceae is foliate number, foliate shape, the presence or absence of calyx in fruit, the number of stamen, and fruit characters. Ahmed (1991) studied the petiole vasculature of *Fagonia* species in Egypt, while (Sheahan and Cutler 1993) described the micro-morphology of 37 Zygophyllaceae species from 19 genera, and (Ahmed and Khafagi 1997) investigated both macro- and micro-morphological leaf characters of *Fagonia* species found in Egypt. Another study by Khafagi (2004) examined the macro- and micro-morphological characters of spiny stipules in *Fagonia* species. Finally, (Waly et al. 2011) studied the petiole and blade anatomy of eleven *Zygophyllum* species from Saudi Arabia, and (Elkamali et al. 2016) studied the stem and leaflet anatomy of *Tribulus longipetalous, T. pentandrus*, and *T. terrestris* found in Khartoum State (Central Sudan). Given the difficulty involved in the classification of Zygophyllaceae species, the main objective of this study is to identify additional characters that would be useful for the identification and differentiation between different Zygophyllaceae species by studying the morphological and anatomical characters of the vegetative organs of Zygophyllaceae taxa from eastern Saudi Arabia.

Material & Methods

Twelve samples belonging to nine species and representing three genera of the family Zygophyllaceae were collected fresh from different locations in eastern Saudi Arabia (Table 1). The plants used in this study were identified using the plant key used by (Mandaville 1990), (Chaudhary 2001), (Thomas 2011), and (Alzahran and Albokhari 2017). Foliar details were examined using a binocular stereo microscope under incident light. For micro-morphological investigation, each specimen was fixed in formalin:glacial acetic acid: 70% alcohol (FAA) at a ratio of 5:5:90 by volume (Nassar and El-Sahhar, 1998). Sections of stem and leaf specimens (i.e., petioles and blades) approximately 20–30 [jm in thickness were produced, then stained with safranin (1% solution in 50% ethanol) and light green (1% solution in 96% ethanol) for microscopic observation (Dilcher, 1974)]. Stomata were determined by stripping and by fixing the lower leaf epidermis in 70% ethanol before clearing in 1% warm lactic acid; samples were then examined by light microscopy (Nassar and El-Sahhar, 1998). All terminology concerning mesophyll type is given according to previous reports (Fahn, 1974; Metcalfe & Chalk, 1979).

Table 1
Collection data of the species included in the present study, nomenclature according to Chaudhary 1999, Beier et al. 2003 and Alzahran & Albokhari 2017.

Species	Locality and date
Fagonia olivieri DC.	Rayan – Dammam, 3/2017
Fagonia indica Burm.f.	Rayan – Dammam, 3/2017
Fagonia ovalifolia Hadidi. f.	Rayan – Dammam, 3/2017
Fagonia bruguieri DC.	Rayan – Dammam, 3/2017
Tetraena migahidii (Hadidi) Beier & Thulin.	Rawda – Dammam, 5/2017
Tetraena hamiensis (Scweinf) Beier & Thulin var. *hamiensis.*	Rayan – Dammam, 3/2018
Tetraena hamiensis (Scweinf) Beier & Thulin var. *qatarensis* (Hadidi) Alzahrani & Albokhari.	Rayan – Dammam, 3/2018
Tetraena hamiensis (Scweinf) Beier & Thulin var. *mandavillei* (Hadidi) Alzahrani & Albokhari.	Rayan – Dammam, 3/2018
Tribulus pentandrus Forssk var. *pentandrus.*	Rawda – Dammam, 4/2017
Tribulus macropterus Boiss var. *arabicus* (Hosni) Al-Hemaid & J. Thomas.	Second Industrial area in Dammam, 4/2017
Tribulus terrestris L. var. *terrestris.*	Rayan – Dammam, 3/2018
Tribulus terrestris L. var. *parvispinus* (Presl) Al-Hemaid & J. Thomas.	Rawda – Dammam, 4/2017

Results

In this study, we conducted morphological and anatomical examinations of 12 taxa belonging to the Zygophyllaceae that were collected in eastern Saudi Arabia. Our aim was to identify the most important characters for plant identification and to permit effective discrimination between studied taxa. The different morphological and anatomical characters we used are summarized in Tables 2–4 and Plate 1–4.

I- Macro-morphological character:

Growth form: The growth forms of the studied taxa ranged between annual herbs (found in *Tribulus* spp.), perennial shrubs (found in *Tetraena* spp.), and annual to perennial herbs found in *Fagonia* spp.). We found that all plants showed one of two textures: hairy (*Tribulus*) or glabrous (all others). We also found that only *Tetraena* contained succulent plants.
Stem: Stems are erect in *Tetraena* taxa, prostrate in *Tribulus* taxa, and procumbent in *Fagonia* taxa. The internode length is short (1.5–2 cm) in most of the taxa studied here but long (2.1–2.5 cm) in *Tetraena migahidii, Tribulus macropterus* var. *arabicus*, and *Tribulus terrestris* var. *parvispinus*.

Leaf: We observed a high degree of variability in the leaves of the studied taxa with respect to shape, type, texture, apex, and the number of leaflets. Most of the focal taxa have petiolate leaves except *Fagonia olivieri, Fagonia indica*, and *Fagonia ovalifolia*, which have subsessile leaves. Moreover, in *Fagonia bruguieri* lower leaves are petiolate and upper leaves are subsessile. The leaves of most of the studied taxa are compound and unifoliate. One exception is *Tetraena migahidii*, which has compound and bifoliate leaves. Another clade containing exceptions was *Tribulus*, which had species with multifoliate (> 3) leaves. We also observed two types of leaves in *Fagonia bruguieri*, the lower leaves are compound and trifoliate and the upper leaves are unifoliate.

The leaves of all species are entire and exstipulate except in *Fagonia* spp., where they are spiny-stipulate. With respect to blade outline (leaflet shape) we recorded six main types: oblong with three sub-types, found in *Tetraena hamiensis* var. *hamiensis, Fagonia indica, Fagonia ovalifolia* and *Tribulus* spp.; elliptic lanceolate, found in *Fagonia olivieri* and *Fagonia bruguieri*; obovate, found in *Tetraena migahidii*; globose, found in *Tetraena hamiensis* var. *qatarensis*; and sub-globose, found in *Tetraena hamiensis* var. *mandavillei.* Leaflet apices ranged from acute in *Tribulus* spp. and *Fagonia* spp. to obtuse in *Tetraena* spp. We also observed a truncate apex in *Tetraena hamiensis* var. *qatarensis*. Leaflets are glabrous in most of the studied taxa except in *Tribulus* spp., where they are pubescent. The leaflet length is short (0.5–0.9 cm.) in most of the species studied, but was longer i.e., 1–1.5 cm.) in *Fagonia bruguieri* and *Tetraena migahidii.*
II- Micro-morphological characters:

A. Stem Anatomy:

The anatomical characters of stems of the Zygophyllaceae are recorded in Table 3 and Plate 2.

Stem Cross Sections: The outer shape of the stem cross section was useful for distinguishing between studied taxa. It varies from terete in *Tribulus terrestris.* var. *terrestris* and *Tribulus terrestris* var. *parvispinus*, ± terete with a wavy margin in *Tetraena hamiensis* var. *hamiensis, Tetraena hamiensis* var. *qatarensis, Tetraena hamiensis* var. *mandavillei* and *Tribulus pentandrus.* var. *pentandrus*, ovoid with a wavy margin in *Tribulus macropterus* var. *arabicus*, rectangular with many ridges in *Tetraena migahidii*, ±obtriangle with many ridges in *Fagonia olivieri* and *Fagonia bruguieri*, ± hexagonal with many ridges in *Fagonia indica* and *Fagonia ovalifolia.*

Cortex: The cortex also has value for distinguishing between different taxa. In *Fagonia*, spp., it consists of palisade tissue followed by parenchyma but consists of only parenchyma in all other species. The number of layers varies as well. We observed 3–5 layers of palisade followed by two layers of irregular parenchyma in *Fagonia olivieri* and *Fagonia indica* or round parenchyma in *Fagonia ovalifolia* and *Fagonia bruguieri*, 4–6 layers of round parenchyma in

Tribulus terrestris var. *parvispinus*, 4–6 layers of polygonal parenchyma in *Tribulus terrestris* var. *terrestri*, 5–6 layers of polygonal parenchyma in *Tetraena hamiensis* var. *hamiensis, Tetraena hamiensis* var. *qatarensis* and *Tetraena hamiensis* var. *mandavillei* 7–8 layers of round parenchyma in *Tribulus macropterus* var. *arabicus*, 7–9 layers of polygonal parenchyma in *Tribulus pentandrus* var. *pentandrus*, and or with irregular parenchyma in *Tetraena migahidii.* Patches of stone cells are also present in the cortex of some *Fagonia* spp.

Vascular System: The vascular system differs significantly between the species examined here. In most species it consists of an ectopholic siphonostele with complete phloem and xylem rings. However in *Tribulus* spp. the vascular system is eustele with 15–20 vascular bundles. An ill-defined phloem and a xylem with wide vessels is present in most studied taxa except in *Tetraena hamiensis* var. *hamiensis, Tetraena hamiensis* var. *qatarensis* and *Tetraena hamiensis* var. *mandavillei*, all of which have well defined phloems and narrow xylem vessels.

Pith: Thin-walled parenchymatic cells are the main type of pith, and the shape of the parenchyma ranged from round in *Tetraena hamiensis* var. *qatarensis* and *Tribulus terrestris* var. *parvispinus*, irregular in *Tetraena migahidii* and *Tribulus terrestris* var. *terrestri*, round to polygonal in *Fagonia olivieri, Tetraena hamiensis* var. *hamiensis, Tetraena hamiensis* var. *mandavillei* and *Tribulus pentandrus* var. *pentandrus* and elongated to irregular in all other species. Pith widths are narrow in most of the studied taxa except in *Tribulus pentandrus* var. *pentandrus, Tribulus terrestris* var. *terrestri*, and *Tribulus terrestris* var. *parvispinus*, where the pith width is wide. Schizogenous canals are present in all of the studied taxa except *Tetraena migahidii.* Druses are absent in *Fagonia* spp. and *Tetraena migahidii* but are present in all other species.

B- Petiole Anatomy:

The anatomical characters associated with Zygophyllaceae leaf petioles are presented in Table 4 and Plate 3.

Petiole outlines in cross section: The shape of the petiole in cross section was valuable for characterizing the taxa studied here. It is oblong in *Tetraena hamiensis* var. *hamiensis* and *Tetraena hamiensis* var. *mandavillei*, a half circle with a wavy margin in *Fagonia bruguieri* and *Tetraena migahidii*, terete with two ridges in *Tribulus pentandrus* var. *pentandrus, Tribulus macropterus* var. *arabicus*, and *Tribulus terrestris* var. *parvispinus*, and ± terete in all other species.

Ground tissue: The ground tissue consists of only parenchyma in *Fagonia olivieri, Fagonia indica, Fagonia ovalifolia, Tetraena migahidii*, and *Tribulus terrestris* var. *terrestri*, of palisade followed by parenchyma in *Fagonia bruguieri, Tetraena hamiensis* var. *hamiensis, Tetraena hamiensis* var. *qatarensis*, and *Tetraena hamiensis* var. *mandavillei*, and of parenchyma and palisade on the ridge in other species. The stone cell is present only in *Fagonia bruguieri.* Schizogenous canal and druses are absent in most of the studied taxa except *Tetraena hamiensis* var. *qatarensis* and *Tribulus* spp., where druses are present.

Vascular System: The number of vascular bundles in the petiole varies from: (1) one main vascular bundle in the middle as in *Fagonia olivieri, Fagonia indica*, and *Fagonia ovalifolia;* (2) three vascular bundles including one large bundle in the middle and two smaller lateral bundles as in *Fagonia bruguieri*; (3) five vascular bundles including one large bundle in the middle and four (2,2) smaller lateral bundles as in *Tetraena migahidii;* (4) six vascular bundles including four

main bundles in the middle and two smaller lateral bundles as in *Tribulus* spp.; (5) more than 10 vascular bundles, including one main bundle in the middle and many peripheral vascular bundles, as in *Tetraena hamiensis* var. *qatarensis* and *Tetraena hamiensis* var. *mandavillei*, or one main bundle with two lateral and many peripheral vascular bundles as in *Tetraena hamiensis* var. *hamiensis.*

C- Blade anatomy:

The anatomical characters of the blades of the examined Zygophyllaceae species are shown in Table 4 and Plate 4.

Blade Shape in Cross Section: The outline of the cross section of the blade was recorded as having one of two types: oblong in *Tetraena hamiensis* var. *hamiensis, Tetraena hamiensis* var. *qatarensis*, and *Tetraena hamiensis* var. *mandavillei*, and duplicate in all other species.

Mesophyll: The blades of Zygophyllaceae species were one of three types: isolateral, as in *Tetraena migahidii, Tribulus pentandrus* var. *pentandrus, Tribulus macropterus* var. *arabicus, Tribulus terrestris* var. *terrestris*, and *Tribulus terrestris* var. *parvispinus*; isobilateral in *Fagonia* spp.; and centric in the reminder. The mesophyll is continuous in all studied taxa except in *Tetraena migahidii*, where it is discontinuous.

Vascular System in the Midrib: The number of vascular bundles in the midrib vary from: (1) one collateral vascular bundle, as in *Fagonia olivieri, Fagonia ovalifolia, Fagonia bruguieri, Tribulus pentandrus* var. *pentandrus, Tribulus macropterus* var. *arabicus, Tribulus terrestris* var. *terrestri*, and *Tribulus terrestris* var. *parvispinus;* (2) two collateral vascular bundles, as in *Fagonia indica*, and (3) three collateral vascular bundles with peripheral vascular bundles, as in *Tetraena* spp.

Table 2
Macro- morphological characters of vegetative parts for 12 taxa of Zygophyllacea.

Characters			Taxa	*Fagonia olivieri*	*Fagonia indica*	*Fagonia ovalifolia*	*Fagonia bruguieri*	*Tetraena migahidii*	*Tetraena hamiensis* var. *hamiensis.*	*Tetraena hamiensis* var. *qatarensis*	*Tetraena hamiensis* var. *mandavillei*	*Tribulus pentandrus* var. *pentandrus*
Whole Plant	**Duration**: 1- Annual, 2- Perennial, 3- Annual to perennial			3	3	3	3	2	2	2	2	1
	Habit: 1- Herb, 2- Shrub			1	1	1	1	2	2	2	2	1
	Texture:1- Glabrous, 2- Hairy			1	1	1	1	1	1	1	1	2
	Nature: 1- Succulent, 2- Non-succulent			2	2	2	2	1	1	1	1	2
Stem	**Nature**:1- Erect, 2- Prostrate, 3- Procumbent			3	3	3	3	1	1	1	1	2
	Internode length(Cm.)	Mean		1.96	1.98	1.74	1.56	2.12	1.26	1.74	1.7	1.68
		Std.		± 0.71	± 0.75	± 0.56	± 0.33	0.87	± 0.40	± 0.55	± 0.53	± 0.53
	Internode width Cm.)	Mean		0.15	0.15	0.15	0.15	0.2	0.2	0.23	0.19	0.15
		Std.		± 0.05	± 0.05	± 0.05	± 0.05	± 0.07	± 0.07	± 0.08	± 0.07	± 0.05
Leaf	**Petiole**: 1- Petiolate, 2- Subsessile			2	2	2	1&2	1	1	1	1	1
	Petiole length (Cm.)	Mean		0.08	0.08	0.1	0.64	1.2	0.74	0.52	0.64	0.42
		Std.		± 0.02	± 0.02	± 0	± 0.26	± 0.57	± 0.21	± 0.15	± 0.15	± 0.12
	Leaf length (Cm.)	Mean		0.86	0.94	0.66	1.92	2.26	1.48	1.02	1.28	2.16
		Std.		± 0.21	± 0.22	± 0.14	± 0.43	± 0.78	± 0.22	± 0.23	± 0.23	± 0.39
	Base:1- Stipular, 2- Normal			1	1	1	1	2	2	2	2	2
	Number of leaflets: 1- One, 2- Two, 3- Three, 4-more than three			1	1	1	3	2	1	1	1	4
	Leaflet	**Shape**: 1- Oblong, 2- Oblong lanceolate, 3- Elliptic, 4- Oblong ovate, 5- Obovate, 6- Globose or sub-globose		2	3	3	5	5	1	6	6	4
		Apex: 1-Acute, 2- Obtuse, 3-Truncate		1	1	1	1	2	2	3	2	1
		Texture: 1- Glabrous, 2- Pubescent		1	1	1	1	1	1	1	1	2

Characters	Taxa	*Fagonia olivieri*	*Fagonia indica*	*Fagonia ovalifolia*	*Fagonia bruguieri*	*Tetraena migahidii*	*Tetraena hamiensis* var. *hamiensis.*	*Tetraena hamiensis* var. *qatarensis*	*Tetraena hamiensis* var. *mandavillei*	*Tribulus pentandrus* var. *pentandrus*
Leaflet length(Cm.)	Mean	0.72	0.84	0.56	1.26	1.28	0.72	0.51	0.64	0.53
	Std.	± 0.21	± 0.22	± 0.14	± 0.31	± 0.37	± 0.15	± 0.10	± 0.10	± 0.07
Leaflet Width(Cm.)	Mean	0.3	0.26	0.18	0.25	0.37	0.39	0.46	0.53	0.26
	Std.	± 0.07	± 0.06	± 0.05	± 0.06	± 0.11	± 0.08	± 0.12	± 0.07	± 0.06

Table 3
Micro- morphological characters of stem for 12 taxa of Zygophyllaceae.

Characters	Taxa	*Fagonia olivieri*	*Fagonia indica*	*Fagonia ovalifolia*	*Fagonia bruguieri*	*Tetraena migahidii*	*Tetraena hamiensis* var. *hamiensis*	*Tetraena hamiensis* var. *qatarensis*	*Tetraena hamiensis* var. *mandavillei*	*Tribulus pentandrus* var. *pentandrus*	*Tr ma va ar*
Stem outline:	1- terete	5	6	6	5	4	2	2	2	2	3
	2- ±Terete with wavy margin										
	3-Ovoid with wavy margin										
	4- rectangular with many ridges										
	5-±obtriangle with many ridges										
	6-± hexagonal with many ridges										
Cuticle	Thickening: 1- Thin 2- Thick 3- Very thick	2	3	3	2	2	1	2	1	2	2
	Surface: 1- Smooth 2- Warty	1	2	1	1	1	1	1	1	1	2
Epidermis:1- Tangential 2- Radial 3- Mixed (Tangential & radial)		3	3	3	3	2	2	2	2	1	2
Cortex:	Types:1- Parenchyma 2- Palisade follow by parenchyma	2	2	2	2	1	1	1	1	1	1
	No. of layer: 1–3:6 layers 2- more than 6 layers	1	1	1	1	1	1	1	1	2	2
	Types of parenchyma: 1- Irregular 2- Round 3- Round to polygonal	1	1	2	2	1	3	3	3	3	2
Stone cell in cortex: 1- Present 2- Absent		1	1	1	1	2	2	2	2	2	2
Pericycle	Form:1- cycle 2- Patches	2	2	2	2	1	2	2	2	2	2
	Type: 1- Parenchyma 2- Sclerenchyma	2	2	2	2	1	2	2	2	2	2
Type of stele:1- siphonostele 2- Eustele		1	1	1	1	1	1	1	1	2	2
Phloem: 1- define 2- Ill-defined		2	2	2	2	2	1	1	1	2	2
Xylem vessel:1- Wide 2- Narrow		1	1	1	1	1	2	2	2	1	1

Characters	Taxa	*Fagonia olivieri*	*Fagonia indica*	*Fagonia ovalifolia*	*Fagonia bruguieri*	*Tetraena migahidii*	*Tetraena hamiensis* var. *hamiensis*	*Tetraena hamiensis* var. *qatarensis*	*Tetraena hamiensis* var. *mandavillei*	*Tribulus pentandrus* var. *pentandrus*	*Tr ma* va *ar*
No. of vascular bundles:1-Cycle 2- 15-20		1	1	1	1	1	1	1	1	2	2
Pith	1-Narrow 2-Wide	1	1	1	1	1	1	1	1	2	1
	Type:1-Round parenchyma 2- Irregular parenchyma 3- Round to polygonal 4- Elongated to irregular	3	4	4	4	2	3	1	3	3	4
Schizogenous canals:	1- Present in cortex only	2	2	2	2	4	3	3	3	3	3
	2- Present in Pith only										
	3- Present in cortex and pith										
	4- Absent										
Druses: 1- Present 2- Absent		2	2	2	2	2	1	1	1	1	1

Table 4
Micro- morphological characters of petiole for 12 taxa of Zygophyllaceae.

Characters				Taxa	*Fagonia olivieri*	*Fagonia indica*	*Fagonia ovalifolia*	*Fagonia bruguieri*	*Tetraena migahidii*	*Tetraena hamiensis* var. *hamiensis.*	*Tetraena hamiensis* var. *qatarensis*	*Tetraena hamiensis* var. *mandavill*
petiole	Petiole outline:	1- ± terete			2	1	1	5	5	4	1	4
		2- Pentagonal										
		3-± Pentagonal with 2 ridges										
		4- Ovoid										
		5- Half circle with wavy margin										
	Cuticle	Thickening	1- Thin		2	2	2	2	2	1	1	1
			2- Thick									
		Surface:	1- Smooth		1	1	1	1	1	1	1	1
			2- Warty									
	Epidermis:	1- Tangential			3	3	3	3	3	1	3	1
		2- Radial										
		3- Mixed										
	Ground tissue	Type:	1- Parenchyma		1	1	1	2	1	2	2	2
			2-Palisade follow by parenchyma									
			3-Parenchyma and palisade at ridges									
		Stone cell:	1- Present		2	2	2	1	2	2	2	2
			2- Absent									
	Vascular bundles	Main V. B.:1- One			1	1	1	1	1	1	1	1
		2- Four										
		Lateral V B.	1- Absent		1	1	1	2	3	2	1	1
			2- Two									
			3- Four (2,2)									
		Peripheral V. B.	1- Present		2	2	2	2	2	1	1	1
			2- Absent									
	Schizogenous canals		1- Present		2	2	2	2	2	2	1	2
			2- Absent									
	Druses		1- Present		2	2	2	2	2	2	1	2
			2- Absent									
Leaflet blade	Outline		1- Duplicate		1	1	1	1	1	2	2	2
			2- Oblong									
	Cuticle	Thickening	: 1- Thin		2	3	2	2	3	2	2	2
			2- Thick									
			3- Very thick									
		Surface	1- Smooth		1	1	1	1	1	1	1	1
			2- Warty									
	Epidermis:		1- Tangential		2	2	1	1	2	2	2	2
			2- Mixed									
	Mesophyll	Type	1- Isolateral		2	2	2	2	1	3	3	3

Characters			Taxa	*Fagonia olivieri*	*Fagonia indica*	*Fagonia ovalifolia*	*Fagonia bruguieri*	*Tetraena migahidii*	*Tetraena hamiensis* var. *hamiensis.*	*Tetraena hamiensis* var. *qatarensis*	*Tetraena hamiensis* var. *mandavill*
			2-Isobilateral								
			3-Centeric								
		Midrib	1- Continuous	1	1	1	1	2	1	1	1
			2- Discontinuous								
	vascular bundles	Main V B	1- one	1	2	1	1	1	1	1	1
			2- Two								
		Lateral V B	1- Absent	1	1	1	1	2	2	2	2
			2- Two								
		Peripheral V B	1- Present	2	2	2	2	1	1	1	1
			2- Absent								
		Bundle sheath:	1- Present	2	2	2	2	2	2	2	2
			2- Absent								
	Schizogenous canals:		1- Present	2	2	2	2	1	1	1	1
			2- Absent								
	Druses:		1- Present	2	2	2	2	1	1	1	1
			2- Absent								
	Stomata	Level:	1- Superficial	1	1	2	1	1	2	3	3
			2- At level								
			3- Depressed								

II- The constructed key:

The recorded data in Tables 2-4 were used to construct the following bracketed key to the twelve taxa of Zygophyllaceae that could be helpful in the confirmation of their identity.

1- The plant succulent perennial shrub with erect stem……..**2**

1- The plant not succulent annual herb with prostrate or procumbent stem ……..**5**

2- Leaf bifoliate with obovate shape and duplicate outline in cross section, stem outline rectangular with irregular parenchyma cortex, pericycle cycle of parenchyma tissue and peripheral vascular bundles absent …….. ***Tetraena migahidii***

2- Leaf one-foliate with other obovate shape and oblong outline in cross section, stem outline ± terete with wavy margin and round to polygonal parenchyma cortex, pericycle patches of sclerenchyma tissue and peripheral vascular bundles present……**3**

3- Leaflet globose with truncate apex and± terete petiole outline…… …….. ***Tetraena hamiensis* var. *qatarensis***

3- Leaflet not globose with obtuse apex and petiole outline ± oblong……..**4**

4- Leaflet oblong and the number of vascular bundles in petiole are three…… …… ***Tetraena hamiensis* var. *hamiensis***

4- Leaflet subglobose and the number of vascular bundles in petiole are one…… …… ***Tetraena hamiensis* var. *mandavillei***

5- Stem procumbent and not hairy, leaf uni - trifoliate, stem cortical palisade present, siphonostele stem, leaf bundle sheath absent and druses absent……**6**

5- Stem prostrate and hairy, leaf paripinnate, stem cortical palisade absent, euostele stem, leaf bundle sheath present and druses present ……..**9**

6- Leaf uni - trifoliate; lower petiolate, trifoliate and upper subsessile, unifoliate, petiole outline half circle with wavy margin, ground tissue two types, stone cell present on petiole and the number of vasculatures in petiole are three…… ***Fagonia bruguieri***

6- Leaf unifoliate, subsessile, one-foliate, petiole outline ± terete, ground tissue one type, stone cell absent in petiole and the number of vasculatures in petiole are one ……**7**

7- Leaflet elliptic lanceolate shape and stem outline ± obtriangle with wavy margin …… ***Fagonia olivieri***

7- Leaflet oblong lanceolate or linear ovate in shape and stem outline ± hexagonal with wavy margin ……**8**

8- Leaflet oblong lanceolate and the main vascular bundle in Madrid region is two……***Fagonia indica***

8- Leaflet linear ovate and the main vascular bundle in midriff region is one……***Fagonia ovalifolia***

9- Stem outline is ovoid with wavy margin with warty cuticle and round to irregular parenchyma cortex, pith elongated to irregular parenchyma…… …… ***Tribulus macropterus*** **var.** ***arabicus***

9- Stem outline terete or ± terete with smooth cuticle and round or round to polygonal parenchyma……**10**

10- Stem outline ± terete with wavy margin and pith have round to polygonal parenchyma…… ***Tribulus pentandrus*** **var.** ***pentandrus***

10- Stem outline terete and pith tissue irregular parenchyma or round parenchyma……..**11**

11- Cortex round to polygonal, pith irregular parenchyma, schizoncanal present in Cortex only, petiole outline terete, ground tissue one Type.. ***Tribulus terrestris*** **var.** ***terrestri***

11- Cortex round, pith round parenchyma, schizoncanal present in Cortex and pith, petiole outline terete with 2 ridges and ground tissue two types…… …… ***Tribulus terrestris*** **var.** ***parvispinus***

Discussion

The twelve Zygophyllaceae species analyzed here showed substantial morphological and anatomical differences. Identification and classification of taxa depends greatly on morphological characteristics, and these species showed variation in growth form, stem characters, and leaf characters (including leaf shape, type, texture, apex, and the number of leaflets).

With respect to micro-morphological characters, our data shows that for most of the studied species, the stem has an epidermal layer that is covered by a thick or very thick cuticle, the cortex has parenchyma associated with mechanical tissue, and the vascular system has an ill-defined phloem and a narrow xylem vessel.

Fagonia spp. are characterized by the presence of stone cells in the cortex and the vascular system is an ectopholic siphonostele with a complete phloem and xylem ring. This finding agrees with the report of (Taia et al. 2017). *Fagonia bruguieri* is distinguished from congeneric species by the uni-trifoliate, obovate shape of its leaves. Similarly, *Fagonia ovalifolia* is distinguished by possessing simple leaves.

For *Tribulus* spp., the cortex consists only of parenchyma and the vascular system is eustele. This classification agrees with that of (Nikolova and Vassilev 2011). Moreover, the presence of druses in some of the studied taxa agrees with the findings of (Abd Elhalim et al. 2016).

Tetraena spp. are characterized by their succulent and erect plant form. Moreover, their leaflet shape is oblong, the apex acute to obtuse, and they possess a greater number of leaflets. Within this genus, *Tetraena migahidii* differs from *Tetraena hamiensis* by its leaves, which are bifoliate. It also has a petiole length of ~ 1.2 cm, a leaf length of ~ 2.26 cm, isolateral leaf mesophyll, and both druses and schizogenous canal were present.

We identified a variety of leaf characters among the Zygophyllaceae spp. studied here. We found four types of petiole outline (oblong, half circle with a wavy margin, ±terete, and terete with two ridges). The ground tissue consisted of either parenchyma only or palisade and parenchyma together. The number of vascular bundles in the petiole ranged from: one to more than ten. We also found three types of mesophyll: isolateral, isobilateral, and centric. The number of vascular bundles in the midrib ranged from: one main bundle, two collateral vascular bundles, and three collateral vascular bundles with druses. We conclude that the characters assessed in this study agreed with previous classifications of Zygophyllaceae species (Nikolova & Vassilev, (2011), Waly et al. (2011), Abd Elhalim et al. (2016), Elkamali et al. (2016), and Taia et al. (2017)).

Conclusions

We conclude that anatomical features, particularly the petiole and blade outlines and the type of vasculature in the stem and petiole, are significant for identifying and distinguishing between the species examined here. A bracketed key is provided for the identification of the investigated taxa based on the characters analyzed here. The characters assessed in this study agreed with previous classifications of Zygophyllaceae species.

Declarations

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Author contributions

All the authors have contributed to the work, and they have agreed to submit the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

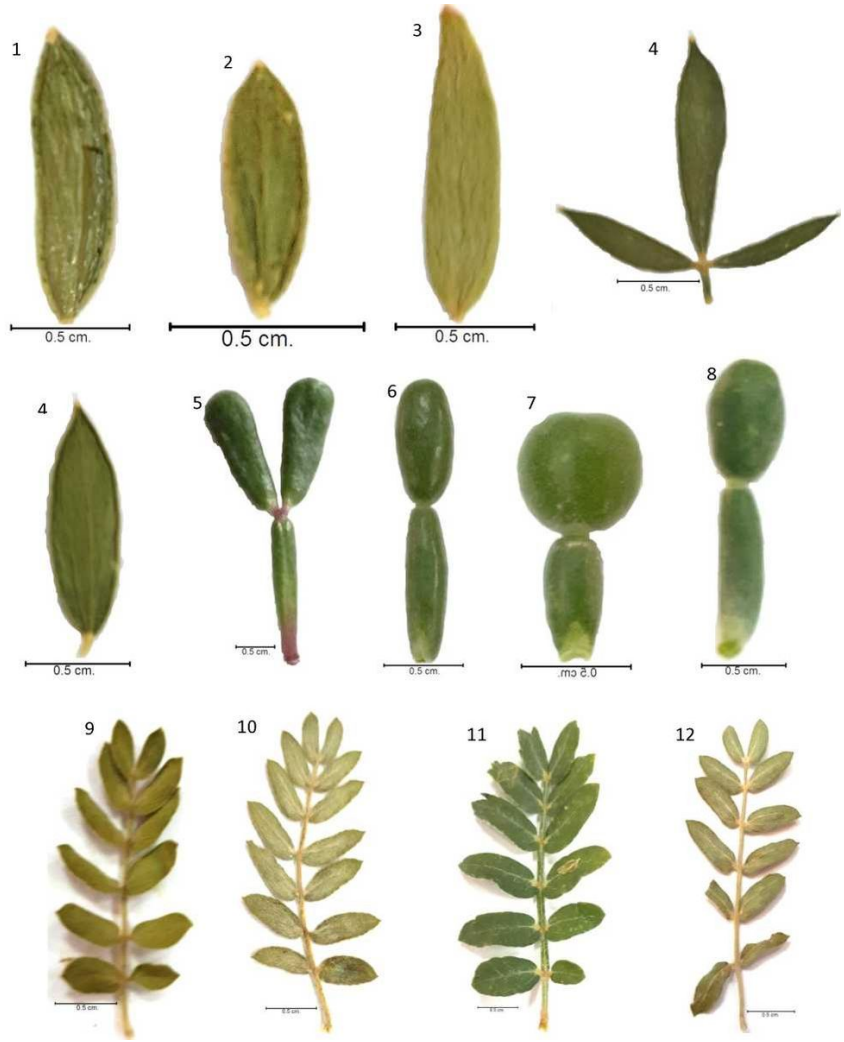

Figure 1

Plate 1 Leaf morphology of 12 taxa of Zygophyllaceae: 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Fagonia ovalifolia*; 4. *Fagonia bruguieri*; 5. *Tetraena migahidii*; 6. *Tetraena hamiensis* var. *hamiensis*; 7. *Tetraena hamiensis* var. *qatarensis*; 8. *Tetraena hamiensis* var. *mandavillei*; 9. *Tribulus pentandrus* var. *pentandrus*;

10. *Tribulus macropterus* var. *arabicus*; 11. *Tribulus terrestris* var. *terrestris*; 12. *Tribulus terrestris* var. *parvispinus*.

a- Lower leaf b-Upper leaf

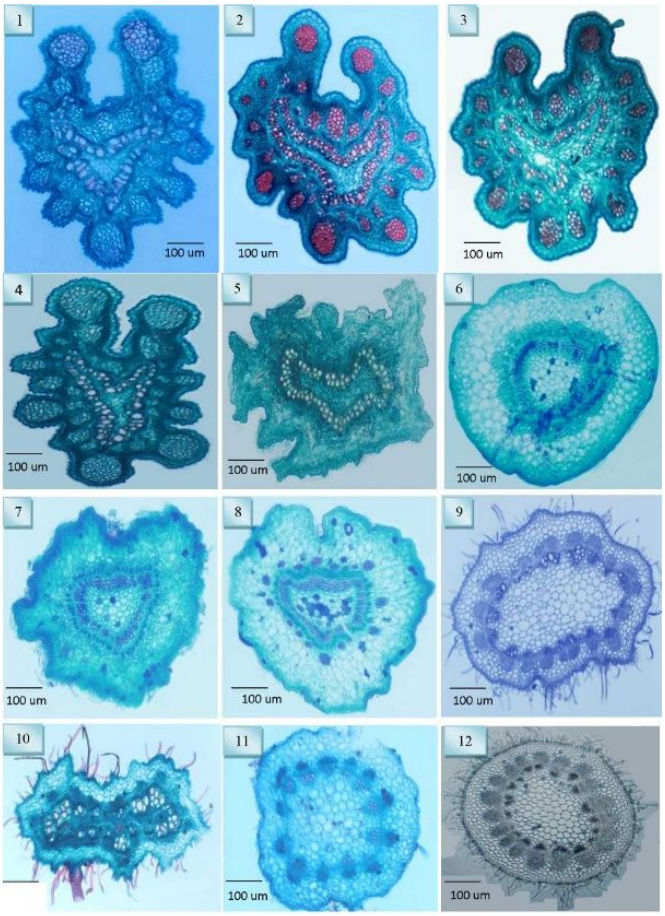

Plate 2 Stem anatomy of 12 taxa of Zygophyllacea: 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Fagonia ovalifolia*; 4. *Fagonia bruguieri*; 5. *Tetraena migahidii*; 6. *Tetraena hamiensis* var. *hamiensis*; 7. *Tetraena hamiensis* var. *qatarensis*; 8. *Tetraena hamiensis* var. *mandavillei*; 9. *Tribulus pentandrus* var. *pentandrus*; 10. *Tribulus macropterus* var. *arabicus*; 11. *Tribulus terrestris* var. *terrestris*; 12. *Tribulus terrestris* var. *parvispinus*.

Figure 2

See image above for figure legend.

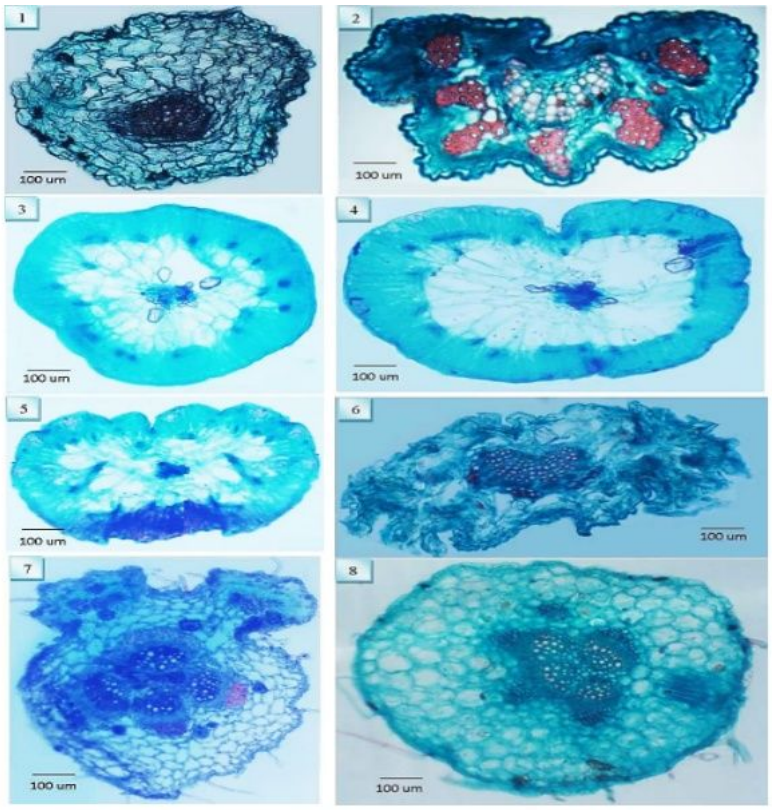

Plate 3 Main different petiole anatomy of studied taxa of Zygophyllaceae: 1. *Fagonia olivieri*; 2. *Fagonia bruguieri*; 3. *Tetraena hamiensis* var. *qatarensis*; 4. *Tetraena hamiensis* var. *mandavillei*; 5. *Tetraena hamiensis* var. *hamiensis*; 6. *Tetraena migahidii*; 7. *Tribulus terrestris* var. *parvispinus*; 8. *Tribulus terrestris* var. *terrestris*.

Figure 3

See image above for figure legend.

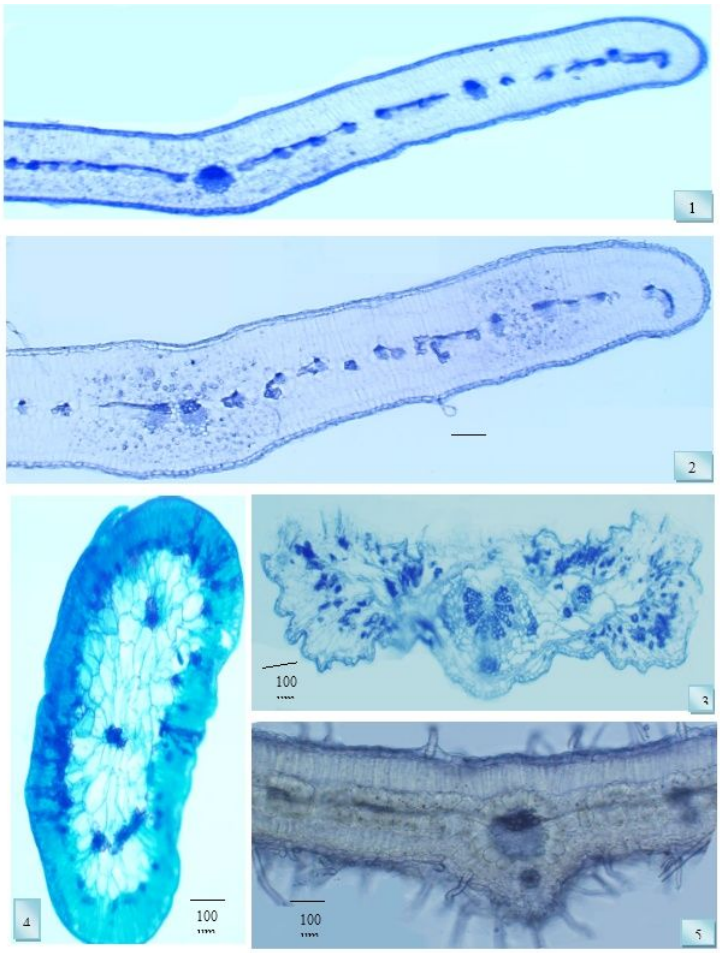

Plate 3. Main different leaflet blade anatomy of studied taxa of Zygophyllacea. 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Tetraena migahidii*; 4. *Tetraena hamiensis* var. *mandavillei*; 5. *Tribulus terrestris* var. *parvispinus*.

Figure 4

See image above for figure legend.