TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2020) 44: 281-294

doi:10.3906/bot-1912-32

Comparative anatomical studies in relation to taxonomy of *Sedum s.l.* (Crassulaceae) in Iran

Maryam MOHAMMADI SHAHRESTANI[1,*], Marzieh BEYGOM FAGHIR[1], Mostafa ASSADI[2]
[1]Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
[2]Research Institute of Forests and Rangelands, Tehran, Iran

Received: 24.12.2019 • **Accepted/Published Online:** 18.03.2020 • **Final Version:** 06.05.2020

Abstract: This study aimed to characterize the stem, peduncle, and leaf anatomy of 22 species of *Sedum senso lato* distributed in Iran. The results showed that the presence of tanniniferous storage cells in stems and leaves, distinct xylem vessels in stems, and the shape of peduncle cross sections, were taxonomically informative evidence, and isolated *Phedimus* from the other studied taxa of *Sedum s.l.*, whereas hairy leaves and peduncles containing starch storage cells were identified as *Prometheum* and *Hylotelephium* respectively. In addition, the current anatomical evidence confirmed the alliance of 2 sections of *Sedum* and *Epeteium* within *Sedum sensu stricto*. The result of numerical analysis (including 31 qualitative and quantitative anatomical characters) supported complete separation of the fourallied genera and revealed significant influence of anatomical traitsin taxonomy of *Sedum s.l.*

Key words: *Sedum s.l.,* anatomy, taxonomy, *Phedimus, Prometheum*, numerical analysis

1.Introduction

Sedum L., comprising ca. 420 species, is the most species-rich member of the family Crassulaceae (Thiede and Eggli, 2007; Nikulin et al., 2016). The genus is almost entirely confined to the subtropical and temperate regions of the Northern Hemisphere (t Hart, 1991; Mifsud et al., 2015). With its extreme morphological diversity and homoplasy of phenotypic features, several hybrid species, and current ploidy, *Sedum* has a confused systematic position (t Hart and Koek-Noorman, 1989; Nikulin et al., 2016), influencing the current classifications. Several previous authors (Paraeger, 1921;Froderstrom, 1936; Clausen, 1975) considered *Sedum* as a hold-all taxon, containing only a few additional genera within the subfamily Sedoideae, whereas others (Borissova, 1969; Ohba, 1977a, 1978b; Grulich, 1984) divided the genusinto controversial genera (Van Ham and t Hart, 1998; Mort et al., 2001; Giuliani,2017), among which the Asian *Hylotelephium* Ohba, *Phedimus* Rafin, *Rhodiola* L., and *Umblicus* Dc. gained wide taxonomic recognition and were completely separated from *Sedum* based on molecular data (Mauyzumi and Ohba, 2004;Gontcharova and Gontcharov, 2009; Nikulin et al., 2016). Despite these separations, *Sedum* encompasses 1/3 of family diversity (Nikulin et al., 2016). *Sedum* is a polyphyletic genus placed in four major crown clades (including *Acre, Aeonium*, *Leucosedum* and *Sempervivum*) of the crassulacean tree (t Hart, 1995; Van Ham, 1995; Van Ham and t Hart, 1998; Mort et al., 2001; Mauyzumi and Ohba, 2004; Gontcharova and Gontcharov, 2009; Carrillo-Reyes et al., 2009). According to Jansson and Rechinger (1970), in Iran, the genus is represented by 16 species arranged into 2 sections: Sect. *Sedum* (Syn.: *Seda Genuina* Koch) and *Epeteium* Boiss. Akhiani (2000) reported 22 representatives of *Sedum* for the flora of Iran, mainly growing in semiarid and mountainous regions. The systematic delimitation of Iranian representatives of *Sedum* shows many contradictions, more precisely in the following species: *Sedum pallidum, S. pentapetalum; Prometheum sempervivoides, P. pilosum; Phedimus stoloniferus, Ph. spurius*, and *Ph. obtusifolious* (Jansson and Rechinger, 1970; Akhiani, 2000). In spite of a number of previous studies, anatomical characteristics of the genus were poorly understood. These include the general anatomical study of family Crassulaceae (Metcalf and Chalk, 1950), and the stem anatomy of selected species of *Sedum* (Zheng et al.,2016). The current survey aims to describe the anatomical structure of the stem, peduncle, and leaf of Iranian representatives of *Sedum s.l.* to determine diagnostic characters and to evaluate the extent to which these evidences can be used for separating the taxonomic ranks.

2. Materials and methods

Plant specimens were obtained from both field and herbarium materials. Measurements and observations

* Correspondence: maryammohammadi666@gmail.com

were carried out for at least 5 cross-sections, selected from 2 to 3 populations per species. A list of voucher specimens is given in Table 1. Leaf samples were selected from the 2nd to 6th nodes of the stems. Stem samples were cut from about 1cm in the middle of the 2nd to 4th nodes. Fresh materials were fixed in 50% FAA (formaldehyde/acetic acid/ethanol) for 48 h, then washed with distilled water, dehydrated in an ethanol series (30, 50, 70, 95 and 100%), and transferred to 70% ethanol for long-term preservation. Herbarium specimens were rehydrated in water before fixing in FAA. Stem peduncle and leaf cross-sections were prepared by hand cutting and then stained with methyleneblue and Congo red. Observations were carried out using an Olympus BX-51 light microscope under 100× and 400× magnifications. For cluster analysis (CA), NTSYS software (version 2.02) (Rohlf, 1997) was used to construct the dendrogram using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973). Cophenetic correlation coefficient was calculated to find out to what extent cophenetic matrix fits the original similarity matrix. In this analysis, a total of 46 samples (Table 1) and 31 anatomical characteristics comprising 20 qualitative (Tables 2 and 3) and 11 quantitative characteristics (i.e. diameter of tanniniferous storage cells in stem; thickness of stem cuticle, endodermis and parenchyma layer; ratio of xylem thickness to phloem in stem; number of collenchyma layers in stem and peduncle; thickness of epidermal layer and cortex in peduncle, ratio of xylem thickness to phloem and ratio of pith thickness to cross section in peduncle) were involved.

3. Results

3.1. Cross-section of stem (Table 2, Figure 1, Figure 2)

Cross-sections were undulating circular to circular in shape with 2 lateral wings. There was a single layer of epidermal cells surrounded by a layer of cuticle on the surface. Stomata were present on the epidermis of *Ph. obtusifolious* (Figure 1e), *S. tenellum, S. sabulatum* and *S. annum* but absent in the other studied taxa. Trichomes were observed in the epidermis of the representatives of the genus *Prometheum* (Figures 1f–1h) and 2 taxa of *Sedum s.s.: S.tenellum* (Figure 1l) and *S. elbursense* (Figure 2o). Underneath the epidermis was a collenchymatous region consisting of 1–3 cell layers. This region was absent in *S. caespitosum* (Figure 2j) A parenchymatous cortex comprising circular compact cells was located between the collenchyma and the endodermis. Cortical bundles were observed in the parenchymatous tissue of *S. tetramerum* (Figure 2a) and *S. pallidum* (Figure 2c). One layer of endodermis separating the cortex from vascular cylinder was recognized in the stem of all examined taxa, except in *Prometheum* taxa Vascular cylinder consisted of a poorly developed phloem (which were not easily recognized) and a well-developed xylem. Xylem vessels were in a continuous ring in all studied species except in *Ph. stoloniferus* (Figure 1a), *Ph. spurius* (Figure 1c) and *Ph. obtusifolius* (Figure 1d), which exhibitedcluster vessels of unequal size. The pith region was observed in the central part of the stem of all examined taxa, except *S. callichroum* (Figure 2l). In some species, parenchymatous tissue of cortex and pithcontainedtanniniferous and starch storage cells that varied in size and density among the studied taxa (Figures 1a–1d, 2e, 2g–2i).

3.2. Cross-section of peduncle (Table3, Figure3, Figure4)

Cross-sections had different shapes including circular, undulating circular, circular with 2 wings, semicircular with 3 wings and stellate. The outer surface was covered by 1 layer of cutinized epidermal cells and in some species with uni- and multicellular glandular trichomes (Figures 3d–3e, 3n–3o, 4a–4b, 4i). The cortex consisted of 1–3 layers of collenchyma and strongly developed parenchyma layers (especially in the representatives of the genus *Prometheum*). Some storage cells were observed in the parenchymatous region of *H. caucasicum* (Figure 3g).The vascular cylinder consisted of xylem, which was found in a closed ring or in clusters, a poorly developed phloem, and a pith zone.

3.3. Cross-section of leaf (Table 3, Figure 5, Figure 6)

The shapes of transverse section were convex, flat and semicircular/undulating semicircular. In some of the studied taxa of *Sedum s.s*., e.g. *S. album* (Figure 5j), *S. tetramerum* (Figure 6a), *S. pallidum* (Figure 6b), *S. hispanicum* (Figure 6c) and *S. pentapetalum* (Figure 6d), adaxial and abaxial sides were indistinguishable. In all taxa, there was a single layer of ovate-circular epidermal cells on both leaf surfaces. Trichomewas observed on the epidermis of *P. pilosum* and *P. sempervivoides*. *Prometheum pilosum* had multicellular glandular hairs on the lower epidermis (Figures 5e–5f), while *P. sempervivoides* showed a higher density of trichomes on both lower and upper epidermises (Figures 5g–5h). In some species (e.g. *S. lenkoranicum* and *S. gracile*), lower epidermal cells were larger than upper cells (Figures 5m–5o). Hypoderm was observed in the studied taxa of *Phedimus, Prometheum, Hylotelephium* and 3species of *Sedum s.s*. (*S. lenkoranicum, S. rubens* and *S. annum*). In all examined taxa, mesophyll was isobilateral and not differentiated into palisade and spongy layers. There were some large mucilaginous cells associated with parenchymatus cells in some species (e.g. *S. hispanicum*, *S. pentapetalum, S. album* etc.), but these cells were not constant in the different populations of the same species. In the representatives of *Phedimus*, storage cells were found in the mesophyll (especially around the vascular bundles) (Figures 5a–5d). The midrib region was not clearly recognized in some species of *Sedum s.s.*, while it was obvious and prominent in the examined taxa

Table 1. Voucher specimens of the examined species.R represents the examined individuals in the numerical taxonomy.

Species	Collection data
Ph. stoloniferus (S.G.Gmel.) 't Hart, 1995	**Guilan**: 25 km of Masal South, Olesbelanga, 1500 m, Mohammadi & Saeidi , GUH-8299 (R1);**Mazandaran**: 40km of Ramsar Southwest, Garesmasar village, 1800 m, Hosaini, GUH-8300 (R2); **Golestan:** Azad-Shahr, Farsian village, 500 m, Mohammadi, GUH-8301 (R3)
Ph. Spurius (M. Bieb.) 't Hart, 1995	**Guilan**: Rezvan-Shahr, Paresar, Arde village, 1000 m, Mohammadi, GUH-8302 (R4); **Mazandaran**: Ghalus, Kuhestan, Dalir village, 2100 m, Mohammadi & Alizadeh, GUH-8303 (R5)
Ph. Obtusifolium (C. A. Mey.) 't Hart, 1995	**Azerbaijan**: Salavat, Golidaragh village, Golidaragh mountain, 1500-1850 m, Mozafariyan & Noruzi, TARI-35065 (R6)
P. pilosum (Fischer ex M. Bieb.) H. Ohba, 1978	**Azerbaijan**:Majarshin village, Uriyan mountain, unknown, GUH-8296 (R7);**Azerbaijan:** Pashtab village, 2000-2500m, Ghahremani-Nejad, GUH-8334 (R8)
P.sempervivoides (Fischer ex M. Bieb.) H. Ohba, 1978	**Azerbaijan**: 17 km of Kale bar, Pashtab village, 2000-2500 m, Ghahremani-Nejad, GUH-8297 (R9); **Azerbaijan:** Kale bar, Rudi, GUH-8335 (R10)
H. caucasicum (Grossh.) H. Ohba, 1977	**Azerbaijan**: 17 km of Kale bar to Khoda-Afarin, 2000-2100 m, Mozafariyan & Mohammadi, TARI-37661 (R11); **Azerbaijan**: Arasbaran protected area. Southeast of Makedi, 1700m, Runemark and Assadi, TARI-22091 (R12)
S. album L., 1753	**Guilan**: Eshkevarat, Garmabdasht, 550 m, Naser, GUH-8304 (R13); **Guilan**: Amlash, Guraj village, 1600 m, Mohammadi, GUH-8305 (R14); **Mazandaran**: Ghalus, Kelardasht, 1000 m, Soleymanpour, GUH-8306 (R15)
S. tenellum M. von Bieberstein, 1819	**Azerbaijan**: Sabalan mountain , 2500 m, unknown, GUH-8298 (R16); **Azerbaijan:** Sabalan mountain, Faridi, GUH-8333 (R17)
S. sabulatum (C.A. Mey.) Boiss., 1872	**Ardebil**: Khalkhal, Sardal West, 2300 m, Mohammadi & Nabizadeh, GUH-8307 (R18)
S. gracile C. M. Mey., 1831	**Guilan:** Asalem road to Khalkhal, unknown,GUH- 8332 (R19) **Mazandaran**: Noor, Chamestan, Vaz village, 900-1000 m, Mohammadi, GUH-8308 (R20)
S. lenkoranicum Grossh., 1915	**Guilan**: Asalem road to Khalkhal, 1800 m, Mohammadi & Nabizadeh, GUH-8309 (R21);**Arbebil**: Heyran pass, 500 m, Mohammadi & Nabizadeh, GUH-8310 (R22)
S. tetramerum Trautv., 1881	**Fars**: 15 km of Noorabad Southeast , Hrayerz, 950-1000 m, Mohammadi& Nabizadeh, GUH-8311 (R23); **Fars**: 23 km of Lar, Grash, 800 m, Mohammadi& Nabizadeh, GUH-8312 (R24); **Kerman**: Sirjan road to Bandar-Abbas, 70 m, Mohammadi& Jalali, GUH-8313 (R25)
S. pallidum M. B., 1808	**Guilan**: Fuman road to Masuleh, 1000 m, Mohammadi&Saeidi, GUH-8314 (R26); **Guilan**: Talesh, Arasbaran, Moradi and Gholami, GRC-847 (R27); **Mazandaran**: Noor, Chamestan, Vaz, 970 m, Mohammadi. GUH-8315 (R28)
S. hispanicum L., 1755 ***S. pentapetalum*** Boiss., 1939	**Guilan:** South of Chaboksar, Sarvelat village, 1200-2100 m, Mohammadi&Fahmideh, GUH-8316 (R29); **Khorasan**: Amiri, GUH-8317 (R30); **Golestan**: Ziyarat, 1560 m, Mohammadi, GUH-8318 (R31) **Guilan:** South of Chaboksar, Sarvelat village, 800 m, Mohammadi&Fahmideh, GUH-8319 9 (R32); **Mazandaran**: Noshahr, Sisangan park, 100 m, Mohammadi, GUH-8320 (R33);**Guilan:** Rudbar, Dogahe, 1293 m, Moradi&Ladani, GRC-429 (R34)
S. rubens L., 1753	**Hormozgan**:Bander-Abbas, Geno mountain, 1500 m, Mohammadi, GUH-8321 (R35);**Guilan**: Talesh, Dokhalekuh, 1700 m, Mohammadi, GUH-8322 (R36)
S. caespitosum (Cav.) DC., 1828	**Guilan**: Rudsar, Eshkevarat, 900 m, Shahi, GUH-8323 (R37); **Fars**: 9 km of Shiraz road to Noorabad, Mohammadi&Nabizadeh, GUH-8324 (R38)
S. kotschyanum Boiss., 1845	**Lorestan**: Sefid–Kuh mountain, 2300 m, Mohammadi&Roudi, GUH-8325 (R39);**Fars**: Safashahr, 2400 m, Khosravan & Mohammadi, GUH-8332 (R40)
S. callichroum Boiss., 1845	**Fars**:Noorabad, Harayerz, 900-1000 m, Mohammadi&Nabizadeh, GUH-8331 (R41)
S. nanum Boiss., 1845	**Lorestan**: Sefid-Kuh mountain, 2000 m, Mohammadi&Roudi, GUH-8327 (R42); **Lorastan**: Makhmal-Kuh mountain, 500 m, Mohammadi& Rudi, GUH-8328 (R43)
S. annum L., 1753	**Guilan**: Jirandeh, 1850 m, Mohammadi, GUH-8329 (R44); **Fars**: Rostam city, Shahi, GUH-8330 (R45)
S. elbursense Akhiani&Assadi	**Zanjan**: Tarom, Sandestan, 1900m, Mohammadi & Saeidi GUH-8326 (R46)

Table 2. Selected qualitative characters of stems for anatomical comparison of the examined species.+represents presence of character, - represents absence of character; numbers indicate cell layers. CS = cross-section, T = trichome, TSH = trichome shape, St = stomata, TSC = tanniniferous storage cell, CB = cortical bundle, SSC = starch storage cell, En = endodermis, XyV = xylem vessel, Pi = pith region, Cl = collenchyma.

Species	CS	T	TSh	St	TSC	CB	SSC	En	XyV	Pi	Cl
Ph. stoloniferus	Undulating circular	_	_	_	+	_	+	+	Distinct	+	1
Ph. spurius	Undulating circular	_	_	_	+	_	_	+	Distinct	+	1
Ph. obtusifolium	Undulating circular	_	_	+	+	_	_	+	Distinct	+	1
P. pilosum	Circular	+	Papillae, glandular	_	_	_	_	_	Closed ring	+	2
P. sempervivoides	Circular	+	Papillae, glandular	_	_	_	_	_	Closed ring	+	2
H. caucasicum	Circular	_	_	_	_	_	_	+	Closed ring	+	2–3
S. album	Circular	_	_	_	_	_	_	+	Closed ring	+	1
S. tenellum	Circular	+	Glandular	+	_	_	_	+	Closed ring	+	1
S. sabulatum	Undulating circular	_	_	+	_	_	_	+	Closed ring	+	1
S. gracile	Undulating circular	_	_	_	_	_	_	+	Closed ring	+	1
S. lenkoranicum	Undulating circular	_	_	_	_	_	+	+	Closed ring	+	1
S. tetramerum	Circular with 2 wings	_	_	_	_	+	_	+	Closed ring	+	1
S. pallidum	Undulating circular	_	_	_	_	+	_	+	Closed ring	+	1
S. hispanicum	Circular with 2 wings	_	_	_	_	_	+	+	Closed ring	+	1
S. pentapetalum	Circular with 2 wings	_	_	_	_	_	+	+	Closed ring	+	1
S. rubens	Undulating circular	_	_	_	_	_	_	+	Closed ring	+	1
S. caespitosum	Undulating circular	_	_	_	_	_	_	+	Closed ring	+	0
S. kotschyanum	Undulating circular	_	_	_	_	_	_	+	Closed ring	+	1
S. callichroum	Circular	_	_	_	_	_	_	+	Closed ring	_	1
S. nanum	Circular	_	_	_	_	_	_	+	Closed ring	+	1
S. annum	Circular	_	_	+	_	_	_	+	Closed ring	+	1
S. elbursense	Undulating circular	+	Glandular	_	_	_	_	+	Closed ring	+	1

of *Phedimus, Prometheum* and *Hylotelephium*. Vascular bundles were crescent-shaped or approximately circular.

The result of the UPGMA analysis (Figure 7) showed a high cophenetic correlation (98%), indicating a great proportion between the dendrogram and original matrix, and a complete separation of the genera into2 majorclusters; the 1st cluster contained representatives of *Phedimus, Promethium* and *Sedum s.s.*, and the 2nd comprised *Hylotelephium* species. Two sections of *Sedum* and *Epeteium* could not be delimitated in this dendrogram.

4. Discussion

Environmental influences can simulate the appearance of various adaptations in plants and thus strongly affect their morphoanatomical differentiation (Anacker, 2014). These differences usually represent an adaptive response to certain habitat conditions, but in cases of highly distinct differences may have important taxonomic significances (kruckeberg, 1951;Anacker et al., 2011). Herein we used different accessions for each taxon and selected features that were constant in them.

4.1. Important anatomical characteristicss used for the intergeneric delimitation and taxonomic relationships

The characteristics that contributed most to the separation of genera were the presence or absence of hairs and tanniniferous secretory cells in the stem and leaf; the number of collenchyma layers; the presence or absence of endodermis and types of xylemvessels (distinct or continuous) in the stem; the shape of the cross-section; and the presence or absence of starch storage cells in peduncles. Based on Metcalf and Chalk (1950), tanniniferous secretory cells are commonly present in stemnonlignified tissues, especially the cortex, pith, and phloemof the family Crassulaceae. In majority of these plants, distinct vascular bundles are rare, and the xylem forms a continuous cylinder. Our finding revealed the importance of stem and peduncle anatomical charactersin the separation of *Phedimus* from *Prometheum*, *Hylotelephium* and *Sedum*

Table 3. Selected qualitative characters of peduncle and leaf for anatomical comparison of the examined species. + represents presence of character, - represents absence of character, numbers indicate cell layers. CS = cross-section, T = trichome, SSC = starch storage cells, XyV = xylem vessel, Cl = collenchyma, Hy = hypodermis, TSC = tanniniferous storage cell, Mid = midrib region.

Peduncle						Leaf					
species	CS	T	SSC	XyV	Cl	CS	Hy	T	TSC	Mid	XyV
Ph. stoloniferus	Semicircular with 3 wings	_	_	Closed ring	1-2	Convex	+	_	+	+	Crescent
Ph. spurius	Semicircular with 3 wings	_	_	Closed ring	1-2	Convex	+	_	+	+	Crescent
Ph. obtusifolium	Semicircular with 3 wings	_	_	Closed ring	1-2	Convex	+	_	+	+	Crescent
P. pilosum	Circular	+	_	Closed ring	1	Flat	+	+	_	+	Crescent
P. sempervivoides	Circular	+	_	Closed ring	1	Flat	+	+	_	+	Crescent
H. caucasicum	Circular with 2 wings	_	+	Closed ring	2-3	Convex- flat	+	_	_	+	Crescent
S. album	Circular with 2 wings	_	_	Closed ring	1	Semi-circular	_	_	_	_	Crescent
S. tenellum	Circular	_	_	Closed ring	1	Convex	_	_	_	+	Crescent
S. sabulatum	Circular	_	_	Closed ring	1	Convex	_	_	_	+	Crescent
S. gracile	Undulating circular	_	_	Distinct	1	Convex	_	_	_	+	Circular
S. lenkoranicum	Undulating circular	_	_	Distinct	1	Convex	+	_	_	+	Circular
S. tetramerum	Circular	_	_	Distinct	1	Semi-circular	_	_	_	_	Crescent
S. pallidum	Undulating circular	+	_	Distinct	1	Flat	_	_	_	_	Circular
S. hispanicum	Circular with 2 wings	+	_	Distinct	1	Flat	_	_	_	_	Circular
S. pentapetalum	Circular with 2 wings	+	_	Distinct	1	Flat	_	_	_	_	Circular
S. rubens	Undulating circular	+	_	Distinct	1	Convex	+	_	_	+	Circular
S. caespitosum	Undulating circular	_	_	Distinct	1	Convex	_	_	_	+	Circular
S. kotschyanum	Undulating circular	_	_	Distinct	1	Flat	_	_	_	_	Circular
S. callichroum	Undulating circular	_	_	Distinct	1	Flat	_	_	_	_	Circular
S. nanum	Undulating circular	_	_	Distinct	1	Convex	_	_	_	+	Circular
S. annum	Stellate	_	_	Distinct	0	Flat	+	_	_	+	Circular
S. elbursense	Circular	+	_	Distinct	1	Flat	_	_	_	+	Circular

s.s. The exclusive characters enabling the separation of the examined species of *Phedimus* from the others were the presence of tanniniferous storage cells in the cortex and pith zone of the stem; distinct type of xylem vessels; the 3-winged cross section shape of the peduncle (Figures 3a–3c), and the presence of secretory cellsin the leaf. The current results support the classification proposed by 't Hart (1995) and transfer of 3 species of *Sedum* (i.e. *S. stoloniferum, S. spurium* and *S. obtusifolium*) to the genus Phedimus. However, 2 Iranian floras (Jansson and Rechinger, 1970; Akhiani, 2000) and the flora of Turkey (Chamberlain, 1972) have treated these species under the genus *Sedum*.

The present result supports the phylogenetic distance between the 2 genera *Phedimus* and *Sedums.s.* According to phylogenetic results ('t Hart, 1992; Van Ham, 1995;Mayuzumi and Ohba, 2004;Thiede and Eggli, 2007),the 1st *genus* isnested in the tribe Umbliceae, clade Telephium and subclade Phedimus, while the 2nd is placed in tribe Sedae, clades Acre, and Leucosedum. In addition, further morphological traits (especially the shape of leaf blade and margin) (Moran, 2009), palynological data (e.g. pollen size, shape and infolding patterns), and micro morphological evidences of the reproductive structures (Giauni, 2017) confirm this segregation.

Hairy leaves (Figures 5e–5h), the absence of endodermis, and 2 collenchyma layers in the stem and peduncle are the most useful traits for characterizing *Prometheum*. This result supports Ohba's treatment (1978) in which *Prometheum pilosum* (Syn.: *Sedum pilosum* Bieb.) and *P. sempervivoides* (Syn.: *Sedum sempervivoides*) were excluded from *Sedum*. This result is also in accordance with morphological (Aminirad et al, 2017) and cytological evidence ('t Hart, 1995; 't Hart, 2003).

The presence of starch storage cells in the cortex of the peduncle (Figure 3g) and 2–3 collenchyma layers in the

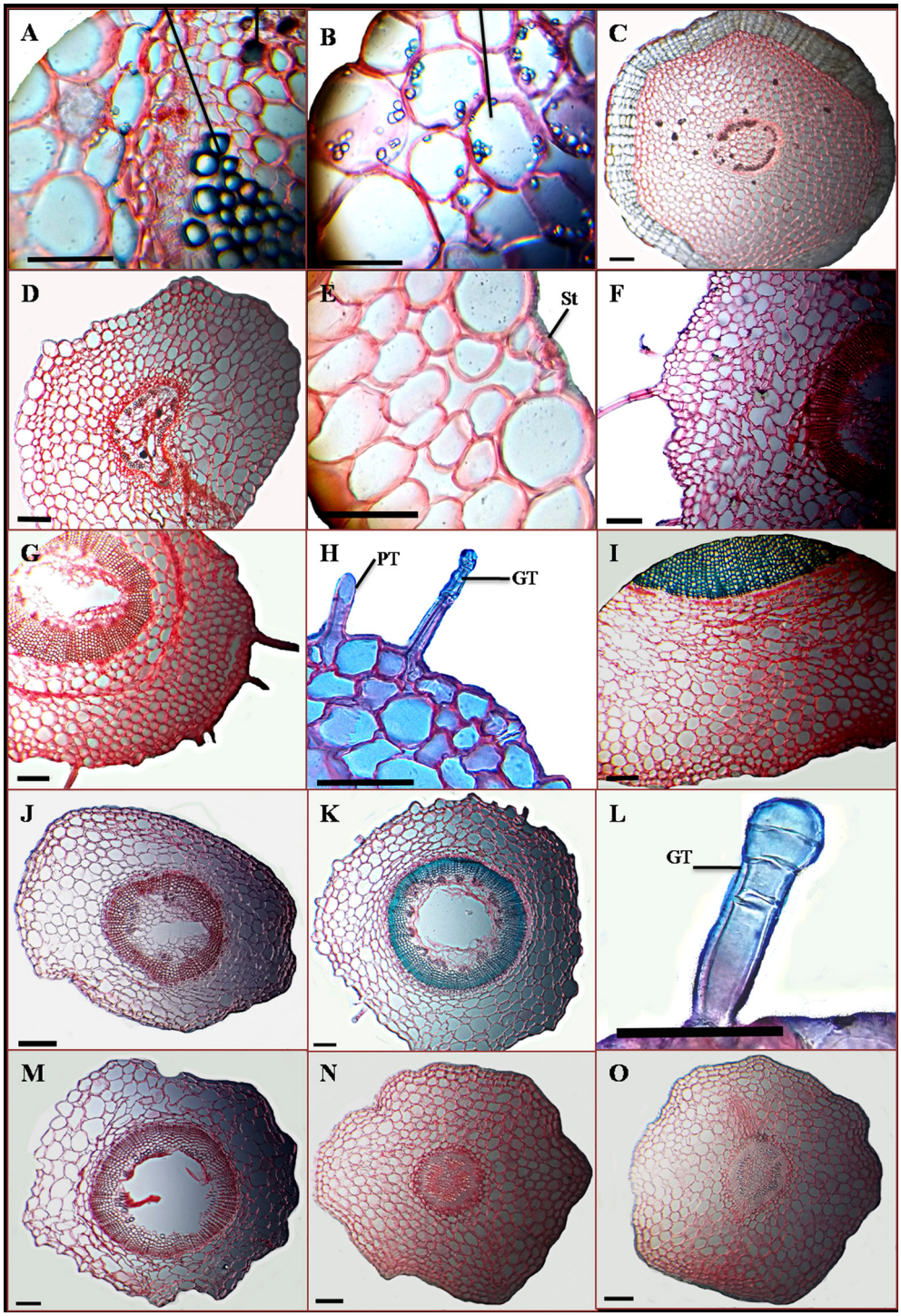

Figure 1. Cross-sections of stem. (a-b) *Ph. stoloniferus*, (c) *Ph. spurius*, (d-e) *Ph. obtusifolium*, (f) *P. pilosum*, (g-h) *P. sempervivoides*, (i) *H. caucasicum*, (j) *S. album*, (k-l) *S. tenellum*, (m) *S. sabulatum*, (n) *S. gracile*, (o) *S. lenkoranicum*. DXyV = distinct xylem vessel, TSC = tanniniferous storage cell, SSC = starch storage cell, ST = stomata, GT = glandular trichome, PT = papillae trichome. Scale bars 100 µm.

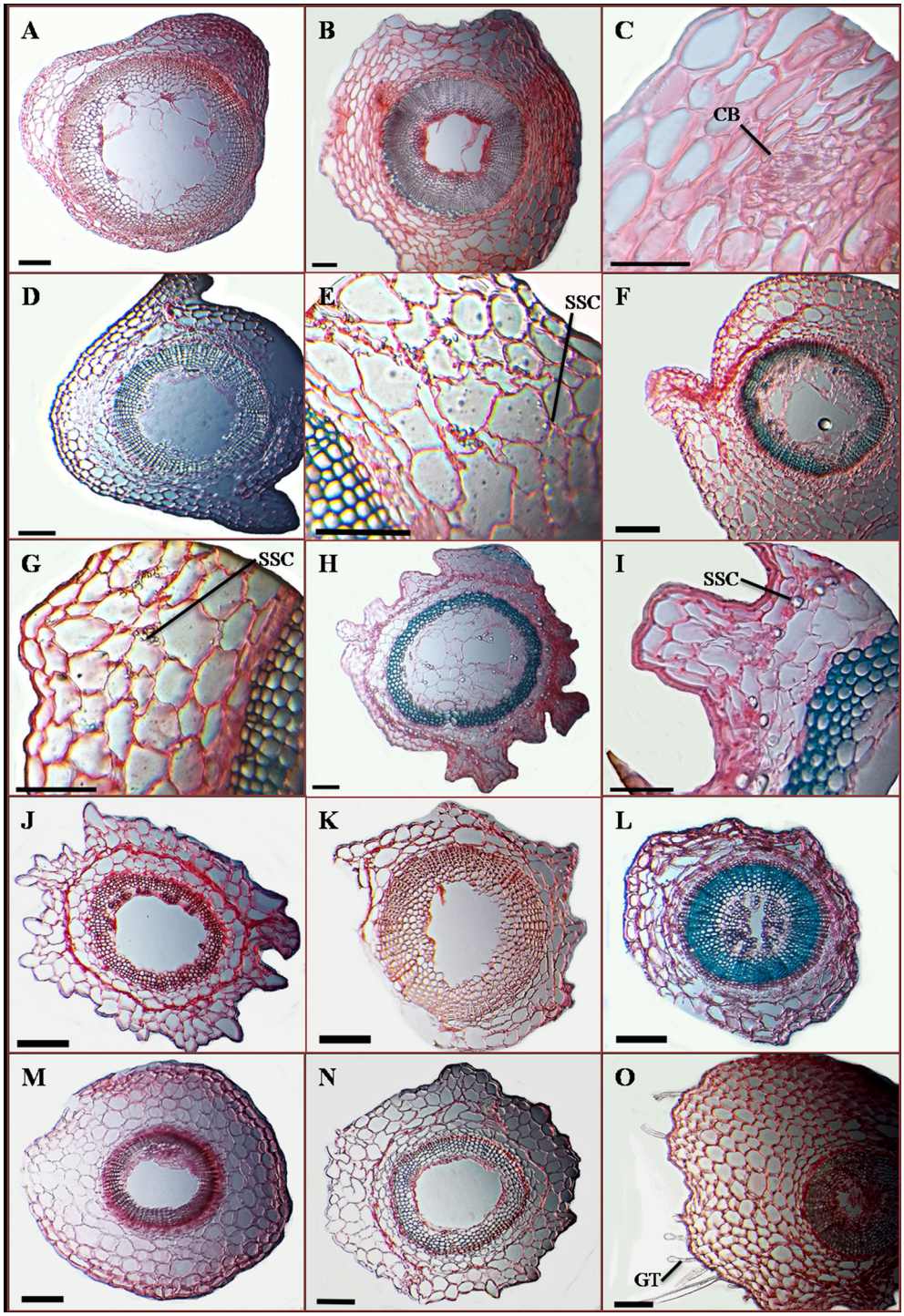

Figure 2. Cross-sections of stem. (a) *S. tetramerum*, (b-c) *S. pallidum*, (d-e) *S. hispanicum*, (f-g) *S. pentapetalum*, (h-i) *S. rubens*, (j) *S. caespitosum*, (k) *S. kotschyanum*, (l) *S. callichroum*, (m) *S. nanum*, (n) *S. annum*, (o) *S. elbursense*. CB = cortical bundle, SSC = starch storage cell, GT = glandular trichome. Scale bares 100 µm

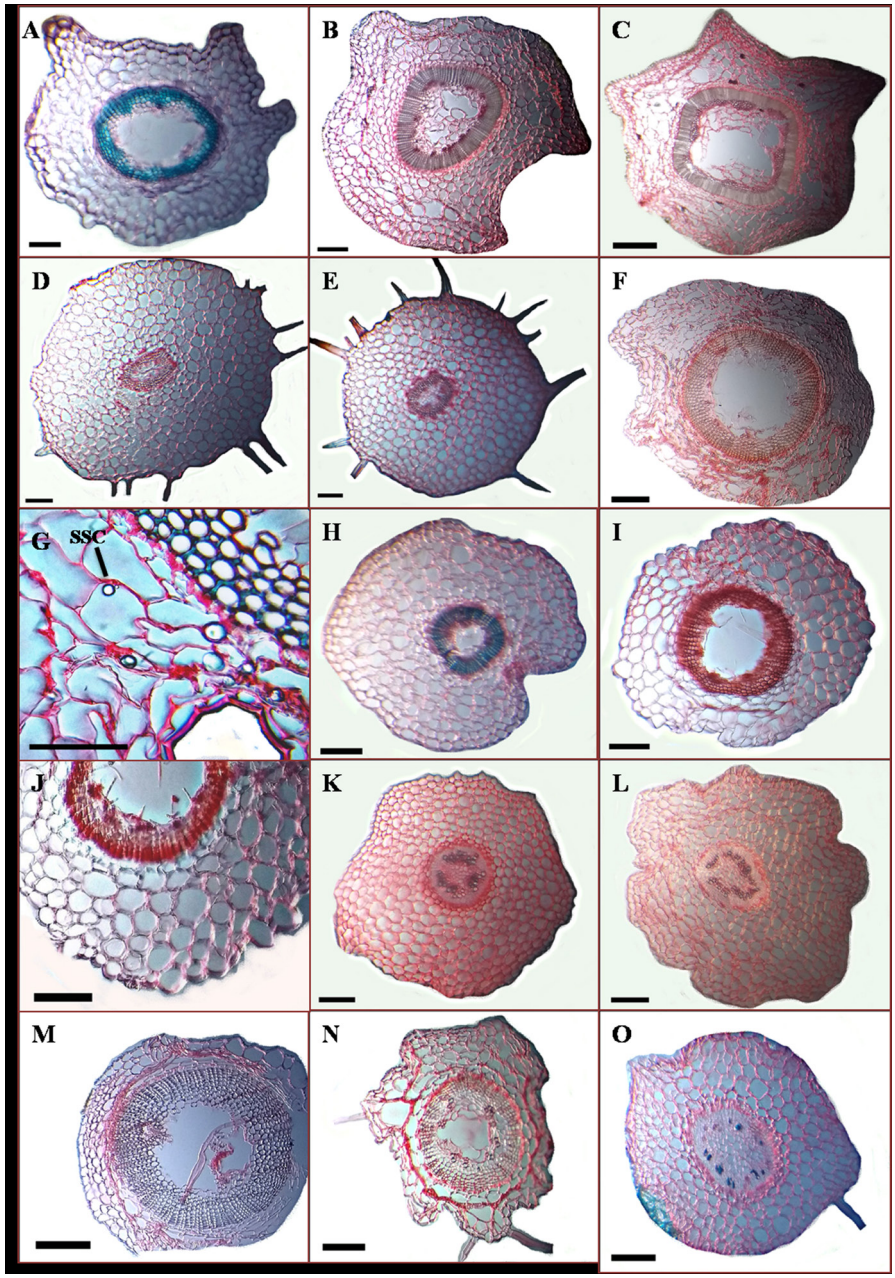

Figure3. Cross-sections of peduncle. (a) *Ph. stoloniferus*, (b) *Ph. spurius*, (c) *Ph. obtusifolium*, (d) *P. pilosum*, (e) *P. sempervivoides*, (f-g) *H. caucasicum*, (h) *S. album*, (i) *S. tenellum*, (j) *S. sabulatum*, (k) *S. gracile*, (l) *S. lenkoranicum*, (m) *S. tetramerum*, (n) *S. pallidum*, (o) *S. hispanicum*. SSC = starch storage cell. Scale bars 100 µm.

stem and peduncle identify *H. caucasicum* (classified in the genus *Sedum*) (Akhiani 2000), the only representative of the genus *Hylotelephium* in Iran. These results confirm the position of *Hylotelephium* in clade Telephium, subclade Hylotelephium.

The studied anatomical characters present a strong variation within *Sedum s.s.*; however, these traits do not show obvious synapomorphies in the genus.

4.2. Importantanatomical characters used for infragenericdelimitation and taxonomic relationships

Within the genus *Phedimus, Ph. stoloniferus* was distinguished by its starch storage cells (Figure 1b), and *Ph. obtusifolious* was differentiated from the other 2 examined taxa in having stomata in its stem cross section (Figure 1e).

Following Berger's classification (1930), some local flora, such as Flora Europea (Webb, 1964), Flora Iranica

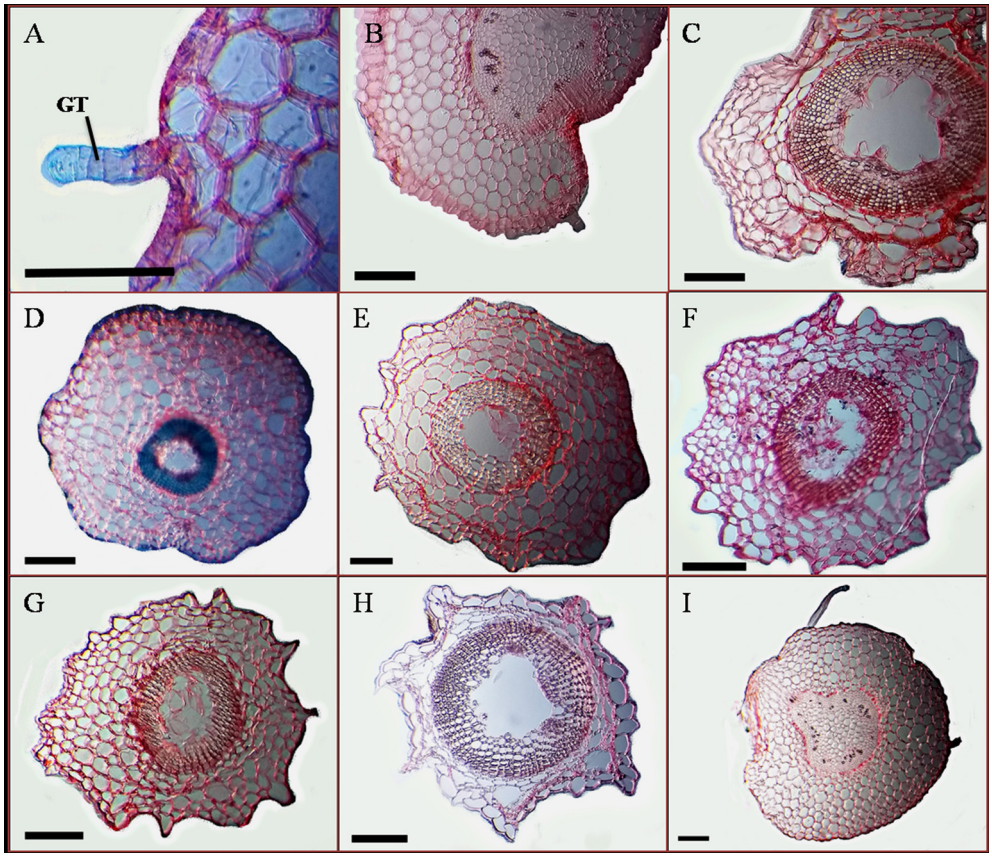

Figure 4. Cross-sections of peduncle. (a) *S. hispanicum*, (b) *S. pentapetalum*, (c) *S. rubens,* (d) *S. caespitosum*, (e) *S. kotschyanum*, (f) *S. callichroum*, (g) *S. nanum*, (h) *S. annum*, (i) *S.elbursense*. GT = glandular trichome. Scale bares 100 µm.

(Jansson and Rechinger, 1970) and flora of Turkey (Chamberlain, 1972) arranged *Sedums.s.* into 2 sections, *Sedum* and *Epeteium*. However, the 2 sections were merged by recent studies (Clausen, 1975; Ohba, 1978; ’t Hart, 1982; Akhiani, 2000). Our anatomical evidences support the later investigation concerning the alliance of 2 sections. The 2 sections have several common anatomical traits, e.g. the shape of the stem cross-section (circular, undulating circular, and circular with 2 wings); number of (0–1) collenchyma layer in the stem and peduncle; the absence of storage cells in the stem, peduncle, and leaf; the presence or absence of trichome in the stem and peduncle; the presence of endodermis in the stem; the shape ofxylem vessels in the stem (a ring) and leaf (circular/crescent), and the presence of pith in the stem and peduncle. According to Berger’s classification (1930), the *Sedum rubens* group (including *S. hispanicum, S. pentapetalum*, and *S. rubens*) has a controversial systematic position and their delimitation is not clearly understood (’t Hart, 1985). Some previous authors (Froderstrum, 1932; Jansson and Rechinger, 1970) considered *S. pallidum* as a synonym of *S. hispanicum*, while others (Borrisova, 1939; Webb, 1964; Chamberlain, 1972; Akhiani, 2000) treated it as a distinct species. Furthermore, Zaffran (1976) considered *S. pallidum* to be conspecific with *S. rubens* (’t Hart 1985). *Sedum pallidum* and *S. hispanicum* have identical morphological characters,such asterete leaves; 10 stamens; triangular sepals with equal size; and white petals with a reddish median line. However, based on palynological (Giuliani, 2017) and phylogenetic evidence (Nikulin, 2016), *S. rubens, S. hispanicum,* and *S. pallidum* are distinct species occurring in the Leucosedum clade. According to our anatomical data, these 3 closely related taxa can be readily isolated by the cross-section shape of their stem and peduncle (circular with 2 wings in *S. hispanicum* (Figures 2d and 3o), but undulating circular in *S. pallidum* (Figure 2b, Figure 3n) and *S. rubens* (Figures 2h and 4c). In addition, *S. pallidum* is characterized by the presence of cortical bundles in the stem (Figure 2c). Metcalf and Chalk (1950) and Abdel-Raouf (2012) have reported this character in some other taxa of Crassulaceae. The absence of starch storage cells in the stem of *S. pallidum* and a convex cross-section of the leaf with a defined midrib region in *S. rubens* (Figure 6E) are other important differentiating characters in this group.

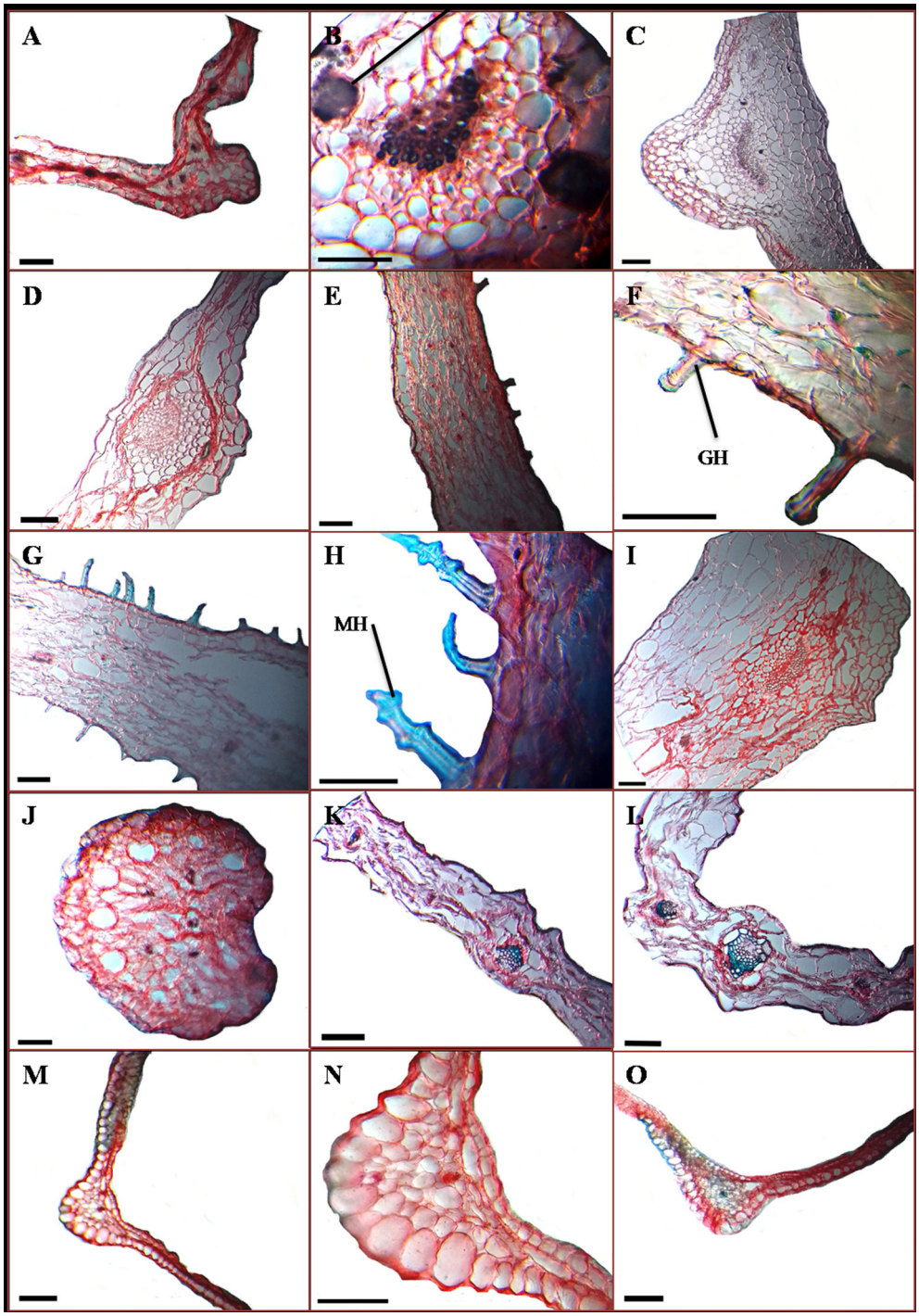

Figure 5. Cross-sections of leaf. (a-b) *Ph. stoloniferus*, (c) *Ph. spurius*, (d) *Ph. obtusifolius*, (e-f) *P. pilosum*, (g-h) *P. sempervivoides*, (i) *H. caucasicum*, (j) *S. album*, (k) *S. tenellum,* (l) *S. sabulatum*, (m-n) *S. gracile*, (o) *S. lenkoranicum*. TSC = tanniniferous storage cell, MH = multicellular hair, GH = glandular hair. Scale bars 100 μm.

S. hispanicum and *S. pentapetalum* have an ambiguous taxonomic position. They were treated as 2 distinct species in both Flora Iranica (Jansson and Rechinger, 1970) and flora of Iran (Akhiani, 2000),whereas Sarvar (2004) combined the 2 later species and considered *S. pentapetalum* as a synonym of *S. hispanicum*. These species have several palynological and morphological similarities (Sarvar, 2004). However, the result of present study did not

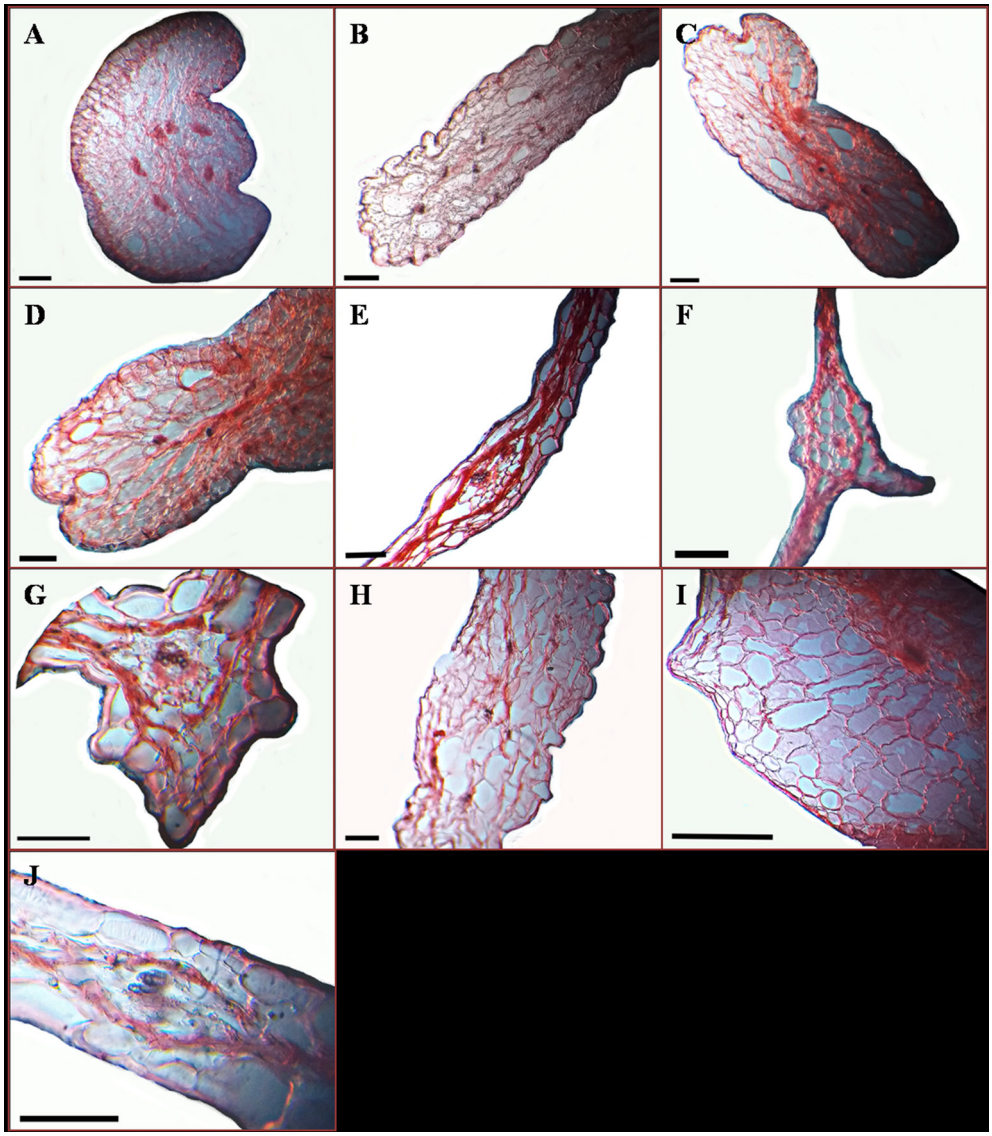

Figure 6. Cross-sections of leaf. (a) *S. tetramerum*, (b) *S. pallidum*, (c) *S. hispanicum*, (d) *S. pentapetalum*, (e) *S. rubens*, (f-g) *S. caespitosum*, (h) *S. kotschyanum*, (i) *S. annum*, (j) *S. elbursense*. Scale bares 100 µm.

show significant anatomical differences between the 2 later species.

5. Conclusion

Our study shows anatomical traits can provide helpful data in solving the current problem of the taxonomy and nomenclature of Sedums.l. in Iran. The representatives of the genera *Phedimus, Prometheum*, and *Hylotelephium* can be easily delimitated from the species of *Sedum s.s.* using anatomical features. The results also support the alliance of 2 sections *Sedum* and *Epeteium* due to several identical anatomical characters described within the sections. An identification key was provided based on the most important diagnostic characters:

1. Tanniniferous storage cells in stem and leafarepresent; xylem vessels in stem aredistinct; peduncle cross-section is circular with 3 wings 2
- Tanniniferous storage cells in stem and leaf areabsent; xylem vessels in stem arecontinuous; peduncle cross-section is not circular with 3 wings 4
2. Starch storage cells in stem arepresent .... *Ph. stoloniferus*
- Starch storage cells in stem areabsent 3
3. Stem with stomata ..... *Ph. obtusifolius*
- Stem without stomata ..... *Ph. spurius*
4. Stem and peduncle have 2 to 3 collenchyma layers; starch storage cells are present in peduncle .... *H. caucasicum*

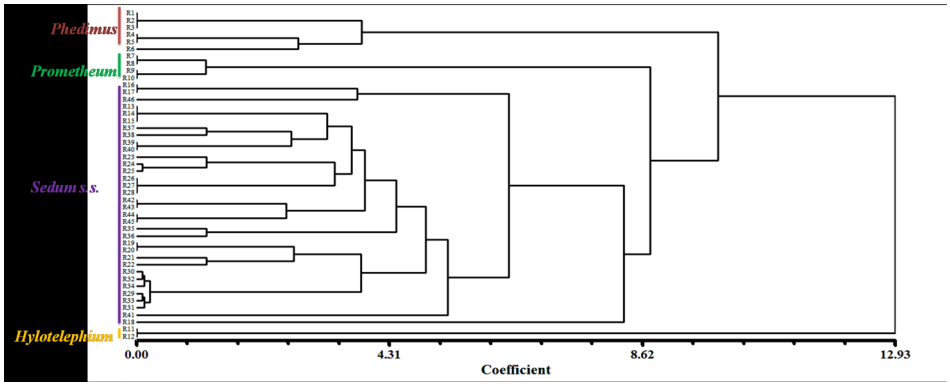

Figure 7. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram. R represents the examined individuals R1–R3 = *Ph. stoloniferus*, R4–R5 = *Ph. spurius*, R6 = *Ph. obtusifolium*, R7–R8 = *P. pilosum*, R9–R10 = *P. sempervivoides*, R11–R12 = *H. caucasicum*, R13–R15 = *S. album*, R16–R17 = *S. tenellum*, R18 = *S. sabulatum*, R19–R20 = *S. gracile*, R21–R22 = *S. lenkoranicum*, R23–R25 = *S. tetramerum*, R26–R28 = *S. pallidum*, R29–R31 = *S. hispanicum*, R32–R34 = *S. pentapetalum*, R35–R36 = *S. Rubens*, R37–R38 = *S. caespitosum*, R39–R40 = *S. kotschyanum*, R41 = *S. callichroum*, R42–R43 = *S. nanum*, R44–R45 = *S. annum*, R46 = *S. elbursense*; (for more details see Table 1).

- Stem and peduncle have 1 collenchyma layer; starch storage cells are absent in peduncle 5
5. Stem without endodermis, stem have 2 collenchyma layers 6
- Stem with endodermis, having 1 collenchyma layer 7
6. Peduncle with thick cortex (380–470 µm) .... *P. pilosum*
- Peduncle with thin cortex (320.4–453 µm) .... *P. sempervivoides*
7. Collenchyma layer in peduncle isabsent, peduncle cross-section is stellate ..... *S. annum*
- Collenchyma layer in peduncle arepresent, peduncle cross-section is circular/undulating circular or circular with 2 wings 8
8. Midrib region is clear 9
- Midrib region is unclear 16
9. Leaf cross-sectionis flat, stem endodermis layer 16.9–20.6 µm ..... *S. elbursense*
- Leaf cross-section isconvex; stem endodermis layer thinner (11.6–14.5 µm) 10
10. Stem contains stomata; peduncle cross-section iscircular in shape 11
- Stem lacks stomata; peduncle cross-section is undulating circular in shape 13
11. Stem cross-section is undulating circular in shape ..... *S. sabulatum*
- Stem cross-section circular in shape 12
12. Stem glabrous; leaf vascular bundles are circular in shape ..... *S. nanum*
- Stem hairy; leaf vascular bundles are crescent in shape ..... *S. tenellum*
13. Leaf with hypodermis; stem contains starch storage cells 14
- Leaf without hypodermis; stem does not contain starch storage cells 15
14. Pedunclegla brousand with distinct vascular bundles ..... *S. lenkoranicum*
- Peduncle hairy and with continuous vascular bundles ..... *S. rubens*
15. Stem without collenchyma; adaxial epidermal cells = abaxial ..... *S. caespitosum*
- Stem with collenchyma; abaxial epidermal cells > adaxial ..... *S. gracile*
16. Leaf cross-section is semicircular, leaf vascular bundle is crescent in shape 17
- Leaf cross-sectionis flat, leaf vascular bundle is circular in shape 18
17. Stem cross-section is circular with 2 wings, cortical bundles are present in stem ..... *S. tetramerum*
- Stem cross-section is circular, cortical bundles are absent in stem ..... *S. album*
18. Peduncle cross-section is circular with 2 wings; stem contains starch storage cells .... *S. hispanicum / S. pentapetalum*
- Peduncle cross-section is undulating circular; stem lacks starch storage cells 19
19. Stem lacks pith region, endodermis layer is thin (4.5–6.8 µm in thickness) ..... *S. callichroum*
- Stem with pith region; endodermal layer is thick (12.5–14.1µm in thickness) 20
20. Stem with cortical bundles; pedunclehairy .... *S. pallidum*
- Stem without cortical bundles; peduncle glabrous ..... *S. kotschyanum*

Acknowledgment
We would like to thank Dr. Moradi (from herbarium of Guilan Research Center, Iran) for his cooperation. The authors commemorate Dr. Shahryar Saeidi Mehrvarz, who unfortunately did not have the opportunity to see the study completed.

References

Abdel-Raouf HS (2012). Anatomical traits of some species of *Kalanchoe* (Crassulaceae) and their taxonomic value. Annals of Agricultural Science 57:73-79.

Akhiani KH (2000). Crassulaceae In: Assadi M (editor.), Flora of Iran, Vol. 32. 1st ed. Tehran, Iran: Research Institute Forest Rangelands, pp. 32-72.

Aminirad M, Eggli U, Gholipour A(2017). *Prometheum rechingeri*, a new report from Iran. Rostaniha 18: 142-149.

Anacker BL, Whittall JB, Goldberg EE, Harrison SP (2011).Origins and consequences of serpentine endemism in the California flora.Evolution 65: 365-376.

Anacker BL(2014). The nature of serpentine endemism. American Journal of Botany 101: 219-224.

Berger A (1930). Crassulaceae. In: Engler A, Prantl KL (editors), Die natürlichen Pflanzenfamilien. Leipzig, Germany: Wilhelm Engelmann, pp. 352-485.

Borissova AG(1969). Conspectus systematic family Crassulaceae florae URSS. Novit Systematic Vascular Academy URSS 6: 112-121.

Carrillo-Reyes P, Sosa V, Mort ME (2009). Molecular phylogeny of the Acre clade (Crassulaceae): dealing with the lack of definitions for *Echeveria* and *Sedum*. Molecular Phylogenetic Evolution 53: 267-276.

Chamberlain DF (1972), *Sedum*. – In: Davis PH. (editor), Flora of Turkey and East Eagean Islands, Vol. 4. Edinburgh, UK: Edinburgh University Press, pp. 224-243.

Clausen RT (1975). Sedum of North America and North of the Mexican Plateau. Ithaca, NY, USA: Cornell University Press.

Froderstrom H(1936). The genus *Sedum* L.A systematic essay. Part 4. Acta *Horticulture* Gothoburgensis 10: 1-262.

Giuliani C, Foggi B, Mariotti Lippi M(2017). Floral morphology, micromorphology and palinology of selected *Sedum s.l.* species (Crassulaceae). Plant Biosystematic. 16-32. doi: 10.1080/11263504.2016.1271056

Gontcharova SB, Gontcharov AA (2009). Molecular phylogeny and systematic of flowering plants of the family Crassulaceae DC. Molecular. Biology 43: 794-803.

Grulich V (1984).Generic division of Sedoideae in Europe and adjacent regions. Preslia 56: 29-45.

Jansson CA (1970). Crassulaceae. In: Rechinger KH (editor), Flora Iranica. Vol. 72. Graz, Austria: Akademische Druck- und Verlagsanstalt, pp. 4-18.

Kruckeberg AR (1951). Intraspecific variability in the response of certain native plant species to serpentine soil. American Journal of Botany 38: 408-419.

Mayuzumi S,Ohba H (2004). The phylogenetic position of easternAsian Sedoideae (Crassulaceae) as inferred from chloroplast and nuclear DNA sequences. Systematic. Botany 29: 587-598.

Metcalfe CR, Chal L (1950). Anatomy of the Dicotyledons I. Oxford, UK: Oxford University Press.

Mifsud S, Stephenson R, Thiede J (2015). *Sedum album* subsp. *rupi-melitense* (Crassulaceae), a new vegetatively reproducing subspecies from Malta (Maltese Islands, Central Mediterranean). Phytotaxa 227: 135-146

Moran RV(2009).Crassulaceae. Flora of North America. 8: 147

Mort ME, Soltis DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A(2001). Phylogenetic relationships and evolution of the Crassulaceae inferred from *mat*K sequence data. American Journal of Botany 88: 76-91.

Nikulin VY, Gontcharova SB, Stephenson R, Gontcharov AA (2016). Phylogenetic relationships between *Sedum* L. and related genera (Crassulaceae) based on ITS rDNA sequence comparisons. Flora 224: 218-229.

Ohba H (1977). The taxonomic status of *Sedum telephium* and its allied species (Crassulaceae). Botanical Magazine. (Tokyo) 90: 41-56.

Ohba H (1978). Generic and infrageneric classification of the Old World Sedoideae (Crassulaceae). Journal of the Faculty of Science, University of Tokyo 12: 139-198.

Praeger RL(1921). An account of the genus *Sedum* as found in cultivation. Royal Horticultural Society 46: 1-314.

Rohlf FJ(1997). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, version 2.0, 2.02h edition. New York, NY, USA: Exeter Software, Setauket.

Sarvar GR(2002). Crassulaceae. In: Ali SI, Gaiser M (editors) Flora of Pakistan, Vol. 209. Karachi, Pakistan: Karachi University Press (Department of Botany), pp. 1-69.

Sneath PA, Sokal RR (1973). Numerical Taxonomy: the Principles and Practice of Numerical Classification. San Francisco, CA, USA: W.H. Freeman.

Thiede J, Eggli U (2007). Crassulaceae DC. In: Kubitzki K (editor). The families and genera of vascular plants. Berlin, Germany: Springer, pp. 83-118.

't Hart H, Koek-Noorman J (1989). The origin of the woody Sedoideae (Crassulaceae). Taxon 38: 535-544.

't Hart H(1991). Evolution and classification of European *Sedum* species (Crassulaceae). Flora Mediterranea 1: 31-61.

't Hart H (1995).Infrafamilial and generic classification of the Crassulaceae. In: Eggli U (editor). Evolution and systematic of the Crassulaceae. Leiden, Netherlands: Backhuys Publisher, pp.159-172.

't Hart H (2003).*Prometheum*. In: Eggli U. (editor). Illustrated handbook of succulent plant: Crassulaceae. Berlin, Germany: Springer- Verlag, pp. 204-207.

Van Ham RCHJ. 1995. Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA variation. In: 't Hart, H., Eggli, U., (eds). Evolution and systematic of the Crassulaceae. Leiden, Netherlands: Backhuys Publisher. pp. 16-29.

Van Ham RCHJ, 't Hart H. 1998. Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA restrictionsite variation. American Journal of Botany 85: 123-134.

Webb DA (1964). Crassulaceae. In: Tutin TG. (editor). Flora Europaea, Vol.1. Cambridge, UK: Cambridge University Press, pp. 356-363.

Zaffran J (1976). Contributions a la floret a la vegetation de la Crete. 1: Floristique. Marseille, France: University of Provence.

Zheng Y, Gong J, Liu D, Jiang Y, Xu Y (2016). Anatomical studies on stem of *Sedum* from Anhui province. Journal of Anhui Normal University 24 (3): 239-242 (in Chinese) doi: 10.14182/j.enki.1001-2443.2001.08.01