Rostaniha 22(2): 259–272 (2021) – Research Article
رستنیها ۲۲(۲): ۲۷۲–۲۵۹ (۱۴۰۰) – مقاله پژوهشی

DOI: 10.22092/BOTANY.2022.356773.1283

Taxonomic significance based on palynological data of the genus *Sedum* in Iran

Received: 20.11.2021 / Accepted: 14.12.2021

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Abstract

In the current survey, a detailed account in pollen morphology of 22 species of *Sedum s.l.* in Iran are presented using light (LM) and scanning electron microscopy (SEM). The pollen grains are radially symmetrical, isopolar, mostly trizonocolporate and rarely tetrazonocolporate, small-to-medium sized, prolate, subprolate, and prolate-spheroidal in shape. Two types of exine sculpturing (regulate-striate and regulate-striate-perforate) were recognized. Among the examined characters, pollen shape and size were taxonomically useful in the intergeneric classification and segregation of the genera *Phedimus* and *Hylotelephium* from *Sedum s.s.*. Numerical analysis (unweighted pair-group method with arithmetic averages and principal components analysis) of 15 qualitative and quantitative characters also confirmed the delimitation of these genera. The PCA analysis of the selected pollen morphological characters indicated the alliance of two sections *Sedum* and *Epeteium*. Pollen morphological characters are also taxonomically informative to provide good data for species delimitation.

Keywords: Colpi, *Crassulaceae*, heteromorphism, isopolar, ornamentation, *Prometheum*

اهمیت آرایه‌شناختی داده‌های گرده‌شناسی در جنس *Sedum* از ایران*

دریافت: ۱۴۰۰/۰۸/۲۹ / پذیرش: ۱۴۰۰/۰۹/۲۳

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خلاصه

در این مطالعه، دانه‌های گرده ۲۲ گونه از جنس *Sedum s.l.* متعلق به تیره گل‌نازیان از ایران با استفاده از میکروسکوپ نوری (LM) و میکروسکوپ الکترونی نگاره (SEM) مورد بررسی قرار گرفتند. نتایج نشان داد که دانه‌های گرده متقارن شعاعی، جور قطب، عمدتا دارای سه شیار منفذی (به ندرت چهار شیار)، کوچک تا متوسط و در شکل‌های کشیده، تقریبا کشیده و کشیده-کروی بودند. در بررسی سطح دانه گرده، دو نوع مختلف از تزیینات اگزین در نمونه‌ها مشاهده شد (چروکیده-مخطط و چروکیده-مخطط-سوراخدار). از میان ویژگی‌های بررسی شده، اندازه و شکل دانه گرده دو صفت مهم آرایه‌شناختی در سطح جنس محسوب شده که در تفکیک اعضای دو جنس *Phedimus* و *Hylotelephium* از جنس *Sedum s.s.* مؤثر بودند. نتایج آنالیز عددی براساس ۱۵ صفت کمّی و کیفی نیز تاییدکننده تعیین حدود این جنس‌ها از یکدیگر بود. آنالیز صفات ریخت‌شناسی انتخاب شده نزدیکی دو بخش *Sedum* و *Epeteium* را در جنس *Sedum s.s.* نشان داد. همچنین، صفات ریخت‌شناسی گرده، از نظر آرایه‌شناختی در تعیین حدود گونه‌های نزدیک بسیار مفید بود.

واژه‌های کلیدی: تزیینات، تیره گل‌نازیان، جورقطب، چندشکلی، شیار، *Prometheum*

* مستخرج از رساله دکتری نگارنده نخست به راهنمایی دکتر مرضیه بیگم فقیر ارایه شده به دانشگاه گیلان

Introduction

The large genus, *Sedum* L. belongs to the family *Crassulaceae* and subfamily *Sedoideae* (Thiede & Eggli 2007, Giuliani *et al*. 2017, Messerschmid *et al*. 2020). It comprises 463 species and constituting a third of the family diversity (Nikulin *et al*. 2016, PoWO 2021). The circumscription of the genus has been a matter of controversy (Mort *et al*. 2001), and the genus concept has changed by separating a number of infrageneric taxa into distinct genera [e.g., *Orostachys* (DC.) Fisch. ex A. Berger, *Hylotelephium* H. Ohba, *Aizopsis* Grulich, *Phedimus* Raf., and *Dudleya* Britton et Rose, *Prometheum* (A. Berger) H. Ohba etc.]. In addition, various infrageneric classifications of the genus have been presented (e.g., Berger 1930, Jansson & Rechinger 1970, Chamberlain 1972, Akhiani 2000, Sarwar 2002). Lack of morphological characters, homoplasy of phenotypic features, crossing ability and several taxonomically complex species, led to more sophisticated species relationship. Based on Berger's classification (Berger 1930), sect. *Sedum* includes majority of the perennial white flowered species of *Sedum*, while sect. *Epeteium* contains annual species. This classification has been widely accepted by authors of Flora Iranica (Jansson & Rechinger 1970), and Flora of Turkey (Chamberlain 1972).

According to Flora Iranica (Jansson & Rechinger 1970), the genus *Sedum* consists of 16 species, arranged into two sections *Sedum* (= sect. *Seda jenuina* Koch.) with seven and *Epeteium* Boiss. with nine species. Later, Akhiani (2000) in Flora of Iran, listed 22 *Sedum* species mainly occur in semiarid and mountainous regions throughout the country. The systematic delimitation of Iranian species of *Sedum* shows many contradictions. According to phylogenetic and morphological evidences, two species of *S. pilosum* Fisch. ex M. Bieb. and *S. sempervivoides* Fisch. ex M. Bieb. were classified in the genus *Prometheum.* Three taxa of *S. stoloniferum* S.G. Gmel., *S. spurium* M. Bieb., and *S. obtusifolium* C.A.Mey. were placed in the genus *Phedimus* according to phylogenetic observations (Ohba 1978, 't Hart 1995, Amini Rad *et al*. 2017). Jansson & Rechinger (1970) and Akhiani (2000) regarded above-mentioned taxa as species of *Sedum.*

Previous studies revealed the taxonomic importance of palynological characters of the genus *Sedum* (Zheng 1997, Xie *et al*. 2014). However, this was primarily limited to the family *Crassulaceae* ('t Hart 1975, Calie 1981, Oybak *et al.* 1997, Karaer *et al.* 2010). Few palynological studies of the genus have been carried out (Sorsa 1968) including, 24 European species ('t Hart 1975), 20 Korean representatives of the genus belonging to three sections *Sedum*, *Aizoon* L., and *Telephium* S.F. Gray (Kim 1994), five species of *Sedum* from Pakistan and Kashmir (Qaiser *et al.* 2015) and 20 species of *Sedum s.l.* from Italy (Giuliani *et al*. 2017). The aims of the current survey is: 1. to evaluate the pollen morphological characters of Iranian species of *Sedum s.l*., and 2. to identify their valuable diagnostic traits for the generic delimitations and species relationships.

Materials and Methods

- Plant materials

Pollen grains of the 22 existing Iranian species of the genus *Sedum s.l.* (including 40 specimens) were investigated using light (LM) and scanning electron microscopy (SEM). The pollen grains were mainly collected from the field. However, for few species, we used dried herbarium samples of Guilan University Herbarium (GUH), Rasht; Herbarium of Guilan Research Center (GRC) Rasht, and Research Institute of Forests and Rangelands (TARI), Tehran, Iran. The voucher specimens are listed in table 1.

To ensure the constancy of pollen characters, measurements were taken from at least 30 grains in each sample, and two to three populations of the same species.

Table 1. Voucher specimens of the examined species in Iran

Taxa	Locality and herbarium No.
Phedimus stolonifer (S.G. Gmel.) 't Hart	Gilan prov.: 25 km of Masal South, Olesbelanga, 1500 m, Mohammadi & Saeidi 8299 (GUH); Mazandaran prov.: 40 km of Ramsar Southwest, Garasma Sar village, 1800 m, Hosaini 8300 (GUH); Golestan prov.: Azad-shahr, Farsian village, 500 m, Mohammadi 8301 (GUH)
Ph. spurius (M. Bieb.) 't Hart	Gilan prov.: Rezvan-Shahr, Paresar, Arde village, 1000 m, Mohammadi 8302 (GUH); Mazandaran prov.: Chalus, Kuhestan, Dalir village, 2100 m, Mohammadi & Alizadeh 8303 (GUH)
Ph. obtusifolius (C.A. Mey.) 't Hart	E Azerbayejan prov.: Salavat, Goli Daragh village, Goli Daragh mountain, 1500–1850 m, Mozaffarian & Nowrouzi 35065 (TARI)
Prometheum pilosum (M. Bieb.) H. Ohba	E. Azarbaijan prov.: Majarshin village, Uriyan mountain, sn 8296 (GUH)
P. sempervivoides (Fich. ex M. Bieb.) H. Ohba	E. Azarbaijan prov.: 17 km of Kaleybar, Pashtab village, 2000–2500 m, Ghahremani-Nejad 8297 (GUH)
Hylotelephium maximum subsp. *ruprechtii* (Jalas) Dostál	E. Azarbaijan prov.: 17 km of Kaleybar to Khoda-farin, 2000–2100 m, Mozaffarian & Mohammadi 37661 (TARI)
Petrosedum subulatum (C.A. Mey.) Afferni	Ardebil prov.: Khalkhal, Sardal West, 2300 m, Mohammadi & Nabizadeh 8307 (GUH)
Sedum album L.	Gilan prov.: Eshkevarat, Garmabdasht, 550 m, Naser 8304 (GUH); Gilan prov.: Amlash, Guraj village, 1600 m, Mohammadi 8305 (GUH); Mazandaran prov.: Chalus, Kelardasht, 1000 m, Soleymanpour 8306 (GUH)
S. tenellum M. Bieb.	Ardebil prov.: Sabalan mountain, 2500 m, sn 8298 (GUH)
S. gracile C.A. Mey.	Mazandaran prov.: Noor, Chamestan, Vaz village, 900–1000 m, Mohammadi 8308 (GUH)
S. lenkoranicum Grossh.	Gilan prov.: Asalem to Khalkhal, 1800 m, Mohammadi 8309 (GUH); Ardebil prov.: Heyran pass, 500 m, Mohammadi & Nabizadeh 8310 (GUH)
S. aetnense Tineo	Fars prov.: 15 km of Noorabad Southeast, Hararіz, 950–1000 m, Mohammadi & Nabizadeh 8311 (GUH); Fars prov.: 23 km of Lar, Grash, 800 m, Mohammadi & Nabizadeh 8312 (GUH); Kerman prov.: Sirjan to Bandar-Abbas, 70 m, Mohammadi & Jalali 8313 (GUH)
S. pallidum M. Bieb.	Gilan prov.: Fuman to Masuleh, 1000 m, Mohammadi & Saeidi 8314 (GUH); Gilan prov.: Talesh, Arasbaran, Moradi & Gholami 847 (GRC); Mazandaran prov.: Noor, Chamestan, Vaz, 970 m, Mohammadi 8315 (GUH)
S. hispanicum L.	Gilan prov.: South of Chaboksar, Sarvelat village, 1200–2100 m, Mohammadi & Fahmideh 8316 (GUH); Khorasan prov.: Amiri 8317 (GUH); Golestan prov.: Ziyarat, 1560 m, Mohammadi 8318 (GUH)
S. pentapetalum Boriss.	Gilan prov.: South of Chaboksar, Sarvelat village, 800 m, Mohammadi & Fahmideh 8319 (GUH); Mazandaran prov.: Noshahr, Sisangan park, 100 m, Mohammadi 8320 (GUH); Gilan prov.: Rudbar, Dogah-e, 1293 m, Moradi & Ladani 429 (GRC)
S. rubens L.	Hormozgan prov.: Bander-Abbas, Geno mountain, 1500 m, Mohammadi 8321 (GUH), Gilan: Talesh, Dokhalekuh, 1700 m, Mohammadi 8322 (GUH)
S. caespitosum (Cav.) DC.	Gilan prov.: Rudsar, Eshkevarat, 900 m, Shahi 8323 (GUH); Fars: 9 km of Shiraz to Noorabad, Mohammadi & Nabizadeh 8324 (GUH)
S. kotschyanum Boiss.	Lorestan prov.: Sefid-Kuh mountain, 2300 m, Roudi 8325 (GUH)
S. callichroum Boiss.	Fars prov.: Noorabad, Harayerz, 900–1000 m, Mohammadi 8331(GUH)
S. nanum Boiss.	Lorestan prov.: Sefid-Kuh mountain, 2000 m, Roudi 8327(GUH); Lorestan prov.: Makhmal-Kuh mountain, 500 m, Rudi 8328 (GUH)
S. annuum L.	Gilan prov.: Jirandeh, 1850 m, Mohammadi 8329 (GUH); Fars prov.: Rostam, Shahi 8330 (GUH)
S. elburzense Akhiani & Assadi	Zanjan prov.: Tarom, Sandestan, 1900 m, Mohammadi & Saeidi 8326 (GUH)

- LEM studies

For LM analysis, buds containing mature indehiscent anthers were selected and fixed in FAA (formaldehyde/acetic acid/ethanol). The pollen grains were stained with basic fuchsine, mounted in glycerin and observed with LM (Nikon microscope, model Optiphot-Z optic). The examined features consisted of: polar axis, equatorial diameter, colpus length, colpus width, mesocolporium, exine thickness and apocolporium index. Pollen heteromorphism was estimated based on Mignot *et al.* (1994), and Saeidi Mehrvarz *et al.* (2014). The number of apertures were firstly counted for 100 pollen grains, then the percentage of each aperture-class were calculated and percentage lower than 95 was considered as heteromorphic pollen.

- SEM studies

For SEM analysis, the collected pollen were soaked in 100% ethanol, air-dried at room temperature before mounting on metallic stubs (12.5 diameter), then coated in a sputter coater with 25 nm of gold-palladium. The quantitative data were provided as minimum (min), maximum (max) and mean (M) ± standard deviations (SD).

- Numerical analysis

Two numerical analysis methods cluster analysis (CA) and principal component analysis (PCA) were carried out using NTSYS-pc ver. 2 (Rohlf 1997). The un-weighted pair-group method with arithmetic averages (UPGMA) showed high cophenetic correlation and was chosen for cluster analysis. For the UPGMA, 15 pollen characters comprising four qualitative and 11 quantitative were scored (Table 2). The similarity matrix was calculated from the standardized data matrix, via cophenetic coefficient of resemblance for mixed data sets. The resulting pollen data for PCA consisted of 11 characters, i.e., one qualitative and 10 quantitative. We used multistate qualitative and quantitative features for this analysis and four characters that were mentioned in table 2 were deleted. The raw data were used to generate a correlation matrix, and two eigenvectors were extracted, providing two axes onto which the standardized data were projected to give two-dimensional plots of the taxa and characters. Pollen was described following the terminology of Punt (Punt *et al.* 2007). The pollen characters of 22 taxa are presented in figures 1–5 and table 1.

Results

- LEM and SEM studies

- General pollen grain features of *Sedum s.s.* and its allied genera

The pollen grains were monads, radially symmetrical, isopolar, mostly trizonocolporate and rarely tri-tetrazonocolporate (in *S. rubens* L.). The size of pollen varied from medium to small (Table 2). Three types of pollen shapes were identified including, prolate, prolate-spheroidal, and subprolate which the latter is the most dominant type. The outline of the pollen varied from circular to triangular in polar view and elliptical to almost circular in equatorial view. The mean values for the length of polar axis (P) varied from 16.7 to 28.9 µm. The length of equatorial diameter (E) ranged from 11.8 to 22.9 µm. The apertures were tri-tetra colporate. Colpi ranged from 11 to 25.4 µm in length and 1 to 2.6 µm in width, occurring on each angles. The thickest exine was measured in *S. rubens* (1.55 µm) and *S. aetnense* (1.5 µm), and the thinnest was recorded in *P. pilosum* (0.76 µm). The mesocolporium length (ML) varied between 3.8 to 12 µm and apocolporium index (Apo. In.) ranged from 1.9 to 4.1 µm (Table 2, Figs 1 & 2).

Two main types of exine sculpturing were identified, regulate-striate and regulate-striate-perforate. The lirae were randomly anastomosed and irregularly arranged in the exine surface of all the studied taxa. However, the length and diameter of lirae varied between species (Table 2, Figs 3–6).

Table 2. Pollen morphological characters of *Sedum s.l.* in Iran

Taxa	P (µm) Min. (M±SD) Max.	E (µm) Min. (M±SD) Max.	P/E (µm)	CL (µm) Min. (M±SD) Max.	CW (µm) Min. (M±SD) Max.	ML (µm) Min. (M±SD) Max.
Phedimus stolonifer	24.0 (25.9±0.6) 27.7	18.4 (19.1±0.5) 20.1	1.34	17.9 (21.0±1.2) 23.0	1.4 (2.2±0.6) 2.8	9.5 (10.7±0.50) 11.6
Ph. spurius	25.5 (27.4±0.8) 30.3	19.0 (21.2±0.7) 24.3	1.34	20.8 (23.9±1.1) 26.5	1.4 (1.8±0.6) 2.3	10.3 (12.0±0.7) 13.7
Ph. obtusifolius	25.8 (28.9±0.7) 30.5	18.5 (20.9±0.7) 23.3	1.35	18.0 (23.8±0.9) 26.1	1.0 (1.5±0.7) 2.2	11.2 (11.8±0.4)12.7
Prometheum pilosum	18.5 (20.9±0.8) 22.7	12.5 (15.9±0.9) 18.9	1.29	12.3 (16.3±0.6) 19.0	1.2 (1.4±0.4) 1.6	6.10 (7.50±0.80) 9.0
P. sempervivoides	19.0 (23.7±1.5) 28.5	14.0 (19.7±1.1) 25.0	1.22	15.1 (18.6±0.9) 20.3	1.2 (1.6±0.5) 2.0	11.1 (11.8±0.4) 12.6
Hylotelephium maximum subsp. *ruprechtii*	18.0 (20.4±0.9) 23.3	13.0 (14.0±0.4) 15.0	1.46	9.0 (11.09±0.8) 14.3	1.2 (1.1±0.7) 1.6	10.6 (11.6±0.5) 12.5
Petrosedum subulatum	22.6 (24.4±0.6) 25.7	19.1 (19.6±0.2) 20.0	1.24	17.4 (19.1±0.6) 20.0	1.4 (1.7±0.7) 2.0	10.5 (11.6±0.7) 12.8
Sedum album	20.0 (23.2±1.2) 26.5	17.0 (19.8±0.6) 23.0	1.13	16.9 (20.13±1.5) 22.8	1.5 (1.7±0.6) 2.1	10.0 (11.8±0.6) 13.6
S. tenellum	21.0 (24.2±0.9) 29.0	18.0 (18.7±0.7) 22.6	1.28	16.0 (21.8±1.0) 24.8	1.4 (1.7±0.5) 2.2	8.30 (9.6±0.50) 11.0
S. gracile	18.4 (22.8±0.8) 26.6	17.2 (21.7±0.9) 25.0	1.05	14.5 (18.1±0.8) 20.2	1.5 (1.8±0.7) 2.3	8.70 (12.5±0.9) 17.2
S. lenkoranicum	22.0 (24.4±0.7) 27.0	20.8 (22.9±0.6) 25.4	1.06	15.2 (18.2±0.8) 23.7	1.3 (1.8±0.9) 2.8	10.6 (11.6±0.7) 13.5
S. aetnense	21.7 (23.2±0.6) 24.7	16.7 (17.4±0.7) 19.5	1.32	12.7 (16.0±0.9) 18.4	1.0 (1.2±0.7) 1.5	6.50 (6.9±0.40) 7.30
S. pallidum	22.3 (24.0±0.8) 26.5	19.0 (21.2±1.1) 24.0	1.13	17.5 (21.0±0.7) 23.4	1.7 (1.9±0.6) 2.2	9.8 (10.2±0.20) 10.5
S. hispanicum	20.5 (22.9±0.9) 26.0	17.5 (20.5±0.9) 23.5	1.12	18 (21.7±0.8) 24.15	1.3 (1.9±0.8) 3.0	10.7 (11.1±0.3) 12.0
S. pentapetallum	20.2 (22.5±0.9) 25.8	17.0 (20.0±0.9) 24.7	1.12	17.8 (21.5±0.8) 24.0	1.3 (1.9±0.8) 3.1	10.7 (11.0±0.6) 12.0
S. rubens	23.0 (24.6±0.9) 27.0	17.0 (21.6±1.6) 26.0	1.13	20.0 (25.4±1.0) 31.3	2.0 (2.6±0.5) 3.3	8.10 (9.04±0.5) 10.0
S. cespitosum	21.0 (24.2±1.5) 29.9	15.0 (18.2 0.9) 20.8	1.32	18.3 (21.1±0.8) 25.9	1.5 (2.1±0.8)2.8	9.20 (10.1±0.5)11.0
S. kotschyanum	18.0 (19.2±0.7) 20.0	15.8 (17.1±0.6) 18.5	1.12	15.7 (15.8±0.1)17.0	1.4 (1.7±0.2) 2.0	4.20 (4.30±0.60) 6.0
S. callichrum	16.2 (16.7±0.5) 17.3	11.0 (11.8±0.6) 14.8	1.32	11.0 (11.7±0.3) 13.0	1.1 (1.3±0.09) 1.6	3.40 (3.8±0.50) 4.70
S. nanum	19.5 (20.9±0.7) 23.6	15.3 (16.4±0.4) 17.5	1.26	15.3 (16.5±0.6) 18.9	1.0 (1.1±0.8) 1.8	4.80 (6.3±0.80) 7.7
S. annuum	20.1 (21.1±0.6)22.0	15.0 (15.7±0.2) 16.5	1.32	16.2 (16.7±0.6) 18.0	1.1 (1.3±0.09) 1.6	6.0 (7.1±0.50)8.90
S. elburzense	19.5 (22.8±1.3) 26.0	16.0 (18±0.4) 19.27	1.26	18.0 (21.5±0.7) 25.4	1.6 (2.0±0.6) 2.39	10.6 (11±0.40) 11.5

Abbreviations: P: polar axis length (µm), E: equatorial diameter (µm), CL: colpus length (µm), CW: colpus width (µm), ML: mesocolporium length (µm), ET: exine thickness (µm), SD: Standard deviation.

Table 2. (contd)

Taxa	ET (µm)	Apo. In. (µm)	Shape	*Outline of pollen grain	*No. of apertures (3+4)	*Het (<95%)	*Exine sculpture	Length of lirae	Diameter of lirae
Phedimus stolonifer	1.0±0.8	2.47	Pr	Cir	(100+0)	No	RS	0.19 (0.38±0.06) 0.92	0.05 (0.08±0.02) 0.07
Ph. spurius	1.0±0.6	2.81	Pr	Cir	(100+0)	No	RS	0.14 (0.38±0.06) 0.99	0.07 (0.06±0.03) 0.11
Ph. obtusifolius	1.1±0.5	2.88	Pr	Cir	(100+0)	No	RS	0.16 (0.42±0.09) 1.09	0.07 (0.08±0.06) 0.10
Prometheum pilosum	0.7±0.6	2.3	Subpr	Cir	(100+0)	No	RS	0.28 (0.68±0.90) 1.50	0.60 (0.09±0.08) 0.12
P. sempervivoides	1.2±0.7	2.9	Subpr	Cir	(100+0)	No	RS	0.32 (0.77±0.80) 1.70	0.07 (0.10±0.06) 0.14
Hylotelephium maximum subsp. *ruprechtii*	0.9±1.2	1.9	pr	Cir	(100+0)	No	RSP	0.21 (0.67±0.80) 1.30	0.03 (0.06±0.03) 0.09
Petrosedum subulatum	1.1±0.7	3.29	Subpr	Tri	(100+0)	No	RSP	1.29 (1.48±0.80) 2.29	0.04 (0.08±0.07) 0.10
Sedum album	1.2±0.7	2.7	Prsph	Tri	(100+0)	No	RSP	0.20 (0.59±0.90) 1.40	0.04 (0.06±0.05) 0.09
S. tenellum	1.3±0.9	3.6	Subpr	Tri	(100+0)	No	RS	0.42 (1.15±0.80) 1.90	0.11 (0.14±0.09) 0.20
S. gracile	1.1±0.6	3.25	Prsph	Tri	(100+0)	No	RS	0.19 (0.49±0.50) 0.88	0.05 (0.06±0.06) 0.10
S. lenkoranicum	1.0±0.9	3.07	Prsph	Tri	(100+0)	No	RS	0.10 (0.50±0.70) 0.75	0.05(0.09±0.060)0.15
S. aetnense	1.5±0.8	2.55	Subpr	Cir	(100+0)	No	RSP	0.38 (1.08±1.00) 1.60	0.03 (0.04±0.40) 0.06
S. pallidum	1.2±0.4	3.9	Prsph	Cir	(98+2)	No	RS	0.21 (0.56±0.80)1.41	0.04 (0.05±0.02) 0.06
S. hispanicum	1.3±0.8	3.8	Prsph	Cir	(100+0)	No	RSP	0.22 (0.59±0.80) 1.02	0.06 (0.07±0.05) 0.09
S. pentapetallum	1.3±0.8	3.85	Prsph	Cir	(100+0)	No	RSPr	0.20 (0.59±0.70) 1.04	0.06 (0.07±0.01) 0.07
S. rubens	1.5±0.6	4.12	Prsph	Cir	(92+8)	Yes	RSP	0.20 (0.81±0.70) 1.89	0.04 (0.06±0.04) 0.07
S. cespitosum	1.2±0.9	3.3	Subpr	Cir	(100+0)	No	RS	0.23 (0.80±0.90) 1.36	0.05 (0.07±0.06)0.09
S. kotschyanum	1.0±1.5	3.7	Prsph	Cir	(100+0)	No	RS	0.25 (0.57±0.09.) 0.75	0.04 (0.06±0.09) 0.09
S. callichrum	1.0±0.3	2.8	Subpr	Cir	(100+0)	No	RS	0.32 (0.59±0.70) 1.13	0.04 (0.06±0.09) 0.08
S. nanum	1.1±0.3	2.5	Subpr	Cir	(100+0)	No	RSP	0.20 (0.58±0.90) 1.19	0.04 (0.05±0.01) 0.05
S. annuum	1.0±0.9	3.4	Subpr	Cir	(100+0)	No	RS	0.21 (0.61±1.00) 1.77	0.06 (0.07±0.06) 0.09
S. elburzense	1.3±0.6	3.8	Subpr	Cir	(100+0)	No	RS	0.31 (0.68±0.90) 1.11	0.06 (0.08±0.09) 0.11

Asterisks denote characters that were not used in the PCA.

Abbreviations: Apo. In.: Apocolporium index (µm), the ratio of the distance between the apices of two ectocolpi to the equatorial diameter (d/D), Pr: Prolate, Subpr: Subprolate, Prsph: Prolate-spheroidal, Cir: Circular; Tri: Triangular, Het: Heteromorphism, RS: Rugulate-striate, RSP: Rugulate-striate-perforate.

- Pollen grains features in the genus *Phedimus* (Table 2, Figs 1A-F, 2A-F)

Pollen grains with medium size and prolate shape are characteristic of representatives of this genus (*ph. stolonifer*, *Ph. spurius*, and *Ph. obtusifolius*). *Phedimus obtusifolius* has a large polar axis (28.9 μm). The pore shape varies from elliptical in *Ph. spurius* to circular in *Ph. stolonifer* and *Ph. obtusifolius* (Fig. 2F). The widest colpi (2.2 μm) and the shortest mesocolporium (10.7 μm) were measured in *Ph. stolonifer*.

- Pollen grains features in the genus *Prometheum* (Figs 1G-J, 2G & H)

The two studied species share many identical pollen morphological traits with each other and with the Iranian taxa of *Sedum s.s*. However, the pollen grains of *P. pilosum* are slightly smaller than *P. sempervivoides* (P = 20.9 μm). The thinnest exine was measured in the former species (0.73 μm).

- Pollen grains features in the genus *Hylotelephium* (Table 2, Figs 1K-L, 2I)

Hylotelephium maximum subsp. *ruprechtii* (Grossh.) H. Ohba is the only representatives of this genus examined in this study. The lowest value of apocolporium index (1.9 μm) and prolate pollen shape are two characteristics of *H. caucasicum*. The later character can separate *H. maximum* subsp. *ruprechtii* from all the studied taxa of *Sedum s.s.* and *Prometheum*. This species has a rugulate-striate-perforate exine ornamentation.

- Pollen grains features in the genus *Sedum* sect. *Sedum* (Table 2, Figs 1M-V, 2J-P).

This section contains the perennial taxa of the genus *Sedum s.s*. The triangular outline of pollen (in polar view) is the only feature constantly observed in this section (Fig. 1N, P, R, T, and V). The pollen shapes isprolate-spheroidal in *S. album*, *S. gracile*, *S. lenkoranicum*, and subprolate in *S. tenellum*, and *S. sabulatum*. The exine sculpture is also variable from regulate-striate in *S. tenellum*, *S. gracile*, and *S. lenkoranicum* to regulate-striate-perforate in *S. album*. Among all the studied taxa, the thickest and the longest lirae was recorded in *S. tenellum* (Fig. 2K-L) and *Pe. subulatum* (Fig. 2M), respectively.

- Pollen grains features in the genus *Sedum* sect. *Epetieum* (Table 2, Figs 1W-AT, 2P-T, 3A-P)

This section comprises all annual studied taxa of *Sedum s.s.*. Within *S. rubens* complex comprising *S. hispanicum*, *S. pallidum*, and *S. rubens*, the exine sculpture is regulate-striate in *S. pallidum* (Fig. 2R-S) and regulate-striate-perforate in *S. rubens* (Fig. 3D-E), and *S. hispanicum* (Figs 2T & 3A). Furthermore, *S. rubens* showed pollen heteromorphism including: 92% tri-aperturate (Fig. 1AF) and only 8% tetra-aperturate (Fig. 1AG) pollen grains. The shortest polar axis was recognized in *S. hispanicum.* In this section, mesocolporium varied from 11.13 μm (in *S. hispanicum*) to 3.83 μm (in *S. callichroum* Boiss.). The lirae interval were minimum (spacious, the most loosely arranged) in *S. aetnense* (Fig. 2Q) and maximum (very compact and the most densely arranged) in *S. annuum* (Fig. 3M-N). *Sedum rubens* had the largest apocolporium index (4.12 μm) among the studied taxa. *Sedum hispanicum* and *S. pentapetalum* shared similar pollen morphological characters.

- Numerical analysis

Among three methods of UPGMA, single linkage and complete, the UPGMA dendrogram (Fig. 4) was chosen for its high co-phenetic correlation coefficient (95%) between dendrogram and original matrix. The UPGMA dendrogram delimitated two major clusters (1 and 2) and showed palynological differentiation between *H. maximum* subsp. *ruprechtii* in one cluster (1) and the other examined taxa in another (2). The maximum Euclidean distance was observed for *H. maximum* subsp. *ruprechtii*. The studied taxa of *Phedimus* (*Ph. stolonifer*, *Ph. spurius*, and *Ph. obtusifolius*) formed a cluster (2A) separate from taxa of *Sedum s.s.* and *Prometheum*. The examined taxa of *Prometheum* are placed among taxa of *Sedum s.s.* in a similar cluster (2B). The cluster 2B formed two groups c and d, the first group with the highest number of taxa includes some species of *Sedum s.s.* and the second comprised *P. pilosum*, *P. sempervivoides*, and some other taxa of *Sedum s.s.* (Fig. 4).

Fig. 1. LM micrographs of pollen grains: A, B. *Ph. stolonifer*, C, D. *Ph. spurius*, E, F. *Ph. obtusifolius*, G, H. *P. pilosum*, I, J. *P. sempervivoides*, K, L. *H. maximum* subsp. *ruprechtii*, M, N. *S. album*, O, P. *S. tenellum*, Q, R. *Pe. subulatum*, S, T. *S. gracile*, U, V. *S. lenkoranicum*, W, X. *S. aetnense*, Y, Z. *S. pallidum*, AA, AB. *S. hispanicum*, AC, AD. *S. pentapetalum*, AE, AG. *S. rubens*, AH, AI. *S. cespitosum*, AJ, AK. *S. kotschyanum*, AL, AM. *S. callichroum*, AN, AO. *S. nanum*, AP, AQ. *S. annuum*, AR, AT. *S. elburzense* (Bar = 10 µm).

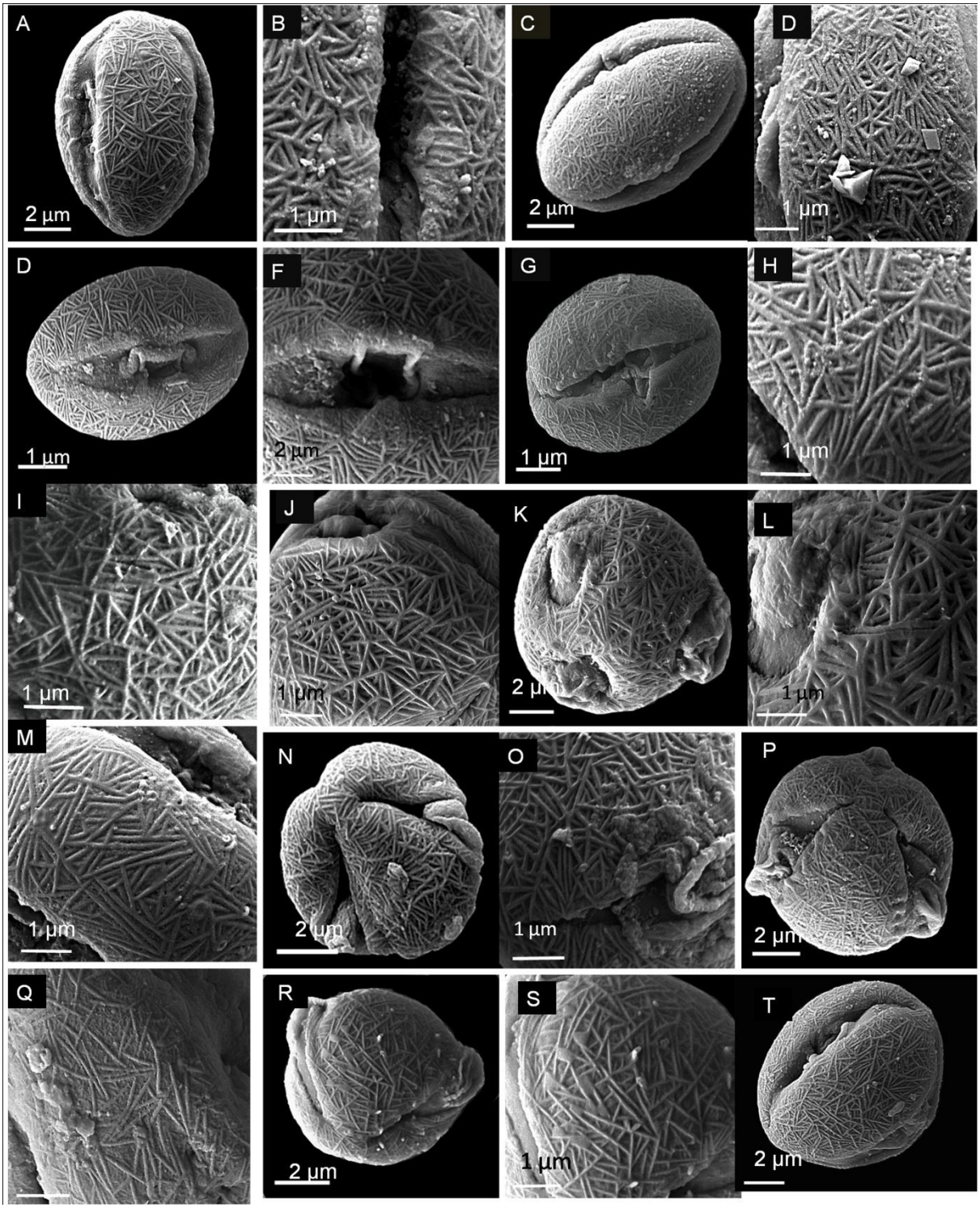

Fig. 2. SEM micrographs of pollen grains: A, B. *Ph. stoloniferus*, C, D. *Ph. spurius*, E, F. *Ph. obtusifolius*, G. *P. pilosum*, H. *P. sempervivoides*, I. *H. maximum* subsp. *ruprechtii*, J. *S. album*, K, L. *S. tenellum*, M. *Pe. subulatum*, N, O. *S. gracile*, P. *S. lenkoranicum*, Q. *S. aetnense*, R, S. *S. Pallidum*, T. *S. hispanicum.*

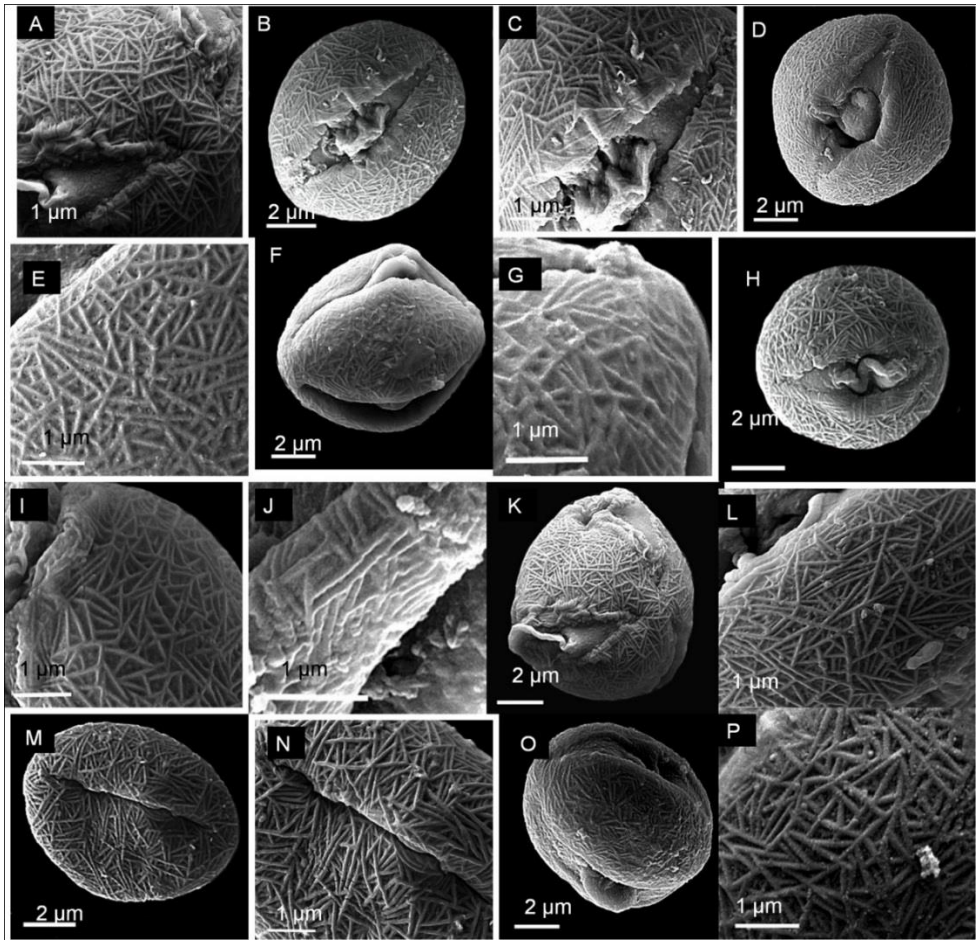

Fig. 3. SEM micrographs of pollen grains: A. *S. hispanicum*, B, C. *S. pentapetalum*, D, E. *S. rubens*, F, G. *S. cespitosum*, H, I. *S. kotschyanum*, J. *S. callichrum*, K, L. *S. nanum*, M, N. *S. annuum*, O, P. *S. elburzense*.

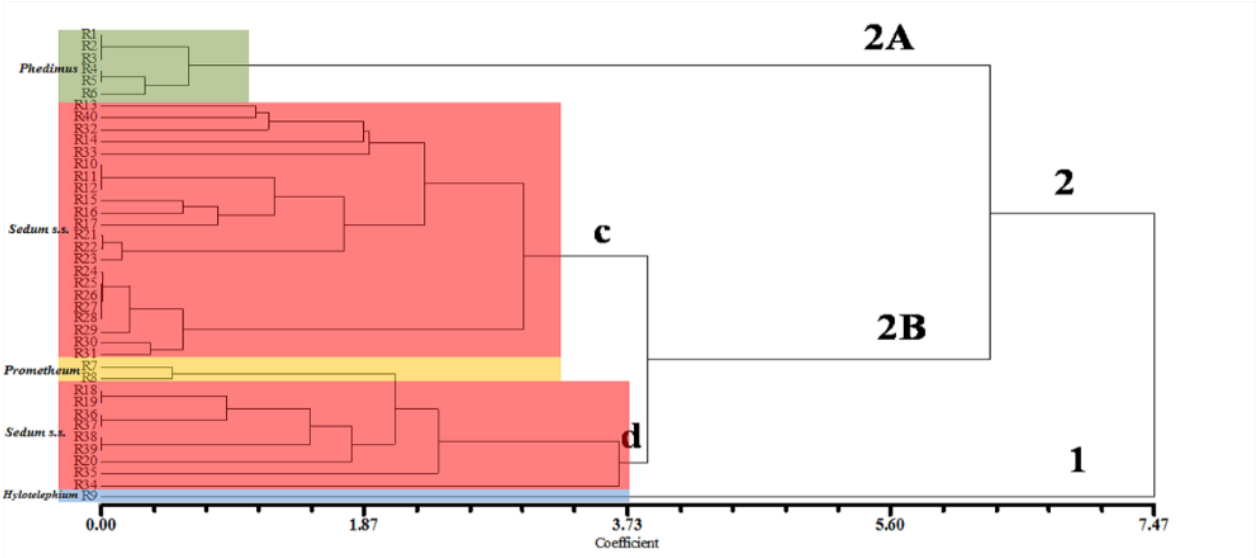

Fig. 4. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram. R: represents the examined individuals (R1-R3: *Ph. stolonifer*, R4-R5: *Ph. spurius*, R6: *Ph. obtusifolius*, R7: *P. pilosum*, R8: *P. sempervivoides*, R9: *H. maximum* subsp. *ruprechtii*, R10-R12: *S. album*, R13: *S. tenellum*, R14: *Pe. subulatum*, R15: *S. gracile*, R16-R17: *S. lenkoranicum*, R18-R20: *S. aetnense*, R21-R23: *S. pallidum*, R24-R26: *S. hispanicum*, R27-R29: *S. pentapetalum*, R30-R31: *S. rubens*, R32-R33: *S. cespitosum*, R34: *S. kotschyanum*, R35: *S. callichrum*, R36-R37: *S. nanum*, R38-R39: *S. annuum*, R40: *S. elburzense*).

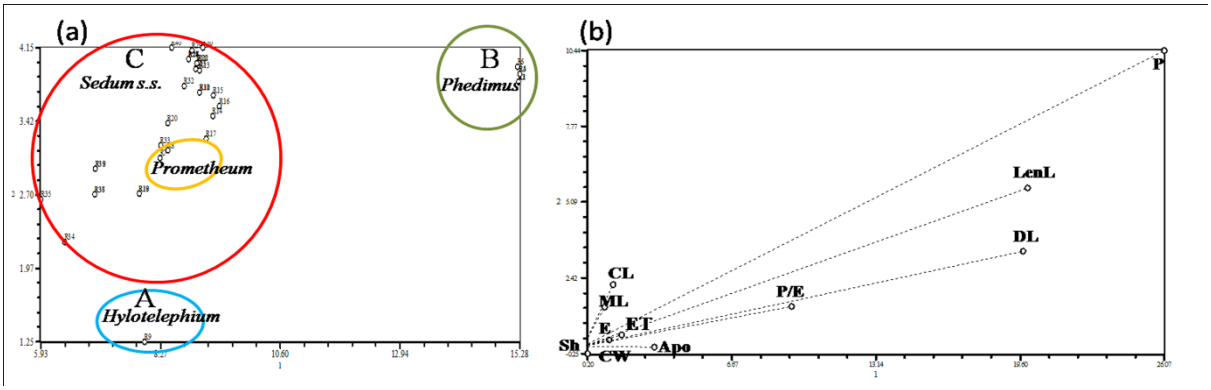

Fig. 5. Principal component analysis (PCA) of the pollen morphological features of 22 species of *Sedum s.l.* (a) Scatter plot of specimens for the two first PCA axes. (b) Scatter plot of characters for the two first PCA axes. Abbreviations: P: polar axis length, LenL: lengh of lirae, DL: diameter of lirae, P/E: ratio of polar axis length to equatorial axis length, CL: colpus length, Apo: apocolporium index, ML: mesocolporium length, ET: exine thickness, E: equatorial length, Cw: colpus width, Sh: shape.

The first and second components of the PCA explained 70.6% and 14.8% of the total variation among 22 studied species, respectively. The scatter plot of the two first PCA axes showed three groups of taxa (A, B and C) and confirmed the findings in the cluster analysis regarding separation of *H. maximum* subsp. *ruprechtii* (group A) and *Phedimus* taxa (group B) from *Sedum s.s.*, and *Prometheum* taxa (Group C) (Fig. 5a). The following characters had the greatest influence on division of groups in the PCA, respectively: polar axis length, length of lirae, diameteroflirae, ratio of polar axis length to equatorial diameter length, colpus length, apocolporium index, mesocolporium length, exine thickness, equatorial length, colporus width and pollen shape. Among the 11 characters used in PCA, only two colpus length and mesocolporium length, overlapped with PCA axis 2, and others overlapped with axis 1 (Fig. 5b).

Discussions

The present study, revealed the most outstanding palynological characters of the genus *Sedum s.s.* and related genera. According to current results, the pollen size and shape are valuable taxonomic traits that support the classification proposed by ’t Hart (1995) concerning the segregation of *Ph. spurius*, *Ph. stoloniferus*, and *Ph. obtusifolius* (prolate in shape and medium in size) from the representative of *Sedum s.s.*, and *Prometheum* (mainly subprolate to prolate-spheroidal in shape and small in size). However, these three species have been treated under *Sedum* in Flora Iranica (Jansson & Rechinger 1970), Flora of Iran (Akhiani 2000), and Flora of Turkey (Chamberlain 1972). According to phylogenetic results (’t Hart 1995, Van Ham &’t Hart 1998, Mayuzumi & Ohba 2004, Thiede & Eggli 2007), *Phedimus* and *Sedum* situated in two distinct tribes and phylogenetic clades (*Phedimus*: in the tribe *umbliceae*, clade *Telephium* and subclade *Phedimus*; *Sedum s.s.*: in the tribe *Sedae*, clades *Acre* and *Leucosedum*). Giuliani (2017) also isolated *Phedimus* [e.g., *Ph. stellatus* (L.) Raf.] from *Sedum s.l.* using pollen evidences (especially pollen shape), floral and micro morphological data. The genus *Phedimus* is recognized morphologically from *Sedum s.s.*, by its leaf blade and margins shape. *Phedimus* has laminar leaf with crenate margin while *Sedum s.s.* has terete leaf or sub terete with entire margin.

Prometheum sempervivoides and *P. pilosum* have been reported as taxonomically complex species. Several authors (e.g., Borissova 1939, Jansson & Rechinger 1970) treated these species as representatives of the genus *Rosularia*, while others (Berger 1930, Chamberlain 1972, Akhiani 2000) classified them in the genus *Sedum s.s.* However, later Ohba (1978) placed eight species of *Sedum* in a distinct genus i.e. *Prometheum*, including *P. pilosum* and *P. sempervivoides* (Sarwar & Qiser 2012). Independence of *Prometheum* was supported by phylogenetic, cytological and

morphological studies (’t Hart 2003, Amini Rad *et al*. 2017). Nevertheless, based on our observations and the results of the numerical taxonomy, these two species and *sedum s.s* taxa have mainly uniform pollen morphology and do not support the previous researches of Ohba (1978), ’t Hart (2003), and Amini Rad (2017) concerning isolation and taxonomic delimitation of the two genera.

Hylotelephium maximum subsp. *ruprechtii* that has been treated as *S. caucasicum* (Grossh.) Boriss. in the Flora of Iran by Akhiani (2000), is morphologically distinct from the genus *Sedum s.s.* by its laminar and broad leaves, free carpels and attenuate base of ovary (Ohba 1978, Giuliani *et al.* 2017). Some characters (i.e., the smallest polar area, prolate pollen shape and regulate-striate-perforate ornamentation) are taxonomically important and can be used for separating the later species from three genera *Sedum s.s.*, *Prometheum* and *Phedimus.* Our results of UPGMA dendrogram (Fig. 4) and PCA plots (Fig. 5) confirmed this segregation.

Several flora especially Flora Iranica (Jansson & Rechinger 1970) and Flora of Turkey (Chamberlain 1972) arranged *Sedum s.s.* taxa in the sect. *Sedum* and sect. *Epeteium.* However, in several recent studies these two sections have been unified (Ohba 1978). Our palynological findings support the earlier studies regarding the alliance of two sections. Despite the great diversity of pollen traits, they are not delimited within each section, and species of the two sections show identical pollen traits.

Based on Berger’s classification, the species of *S. rubens* complex (comprising *S. hispanicum*, *S. rubens*, and *S. pallidum*) have a systematically complicated position (’t Hart 1985). *Sedum pallidum* and *S. hispanicum* have many identical morphological characters such as terete leaves; 10 stamens; triangular sepals with equal size, white petals with a reddish median line. Froderstrom (1932), and Jansson & Rechinger (1970) considered *S. pallidum* as a synonym for *S. hispanicum*, while Borrisova (1939), Chamberlain (1972), and Akhiani (2000) treated *S. pallidum* as a distinct species. According to phylogenetic evidences, *S. pallidum*, *S. hispanicum*, and *S. rubens* are distinct species occurring in *Leucosedum* clade. According to present palynological data, these three closely related species can be easily separated by their exine ornamentation, polar axis length, and pollen heteromorphism. The observations of heteromorphy in the aperture number (3 and 4 colporate) of *S. rubens* is in agreement with the Giuliani’s findings (2017). Pollen heteromorphism is a direct result of polyploidy (Nadot *et al*. 2000), thus, the observed heteromorphism in *S. rubens* may be due to its ploidy level (octoploidy 2n = 80), previously reported by ’t Hart (1985).

Sedum hispanicum and *S. pentapetalum* were classified as two distinct species in both Flora Iranica (Jansson & Rechinger 1970) and Flora of Iran (Akhiani 2000). Nevertheless, there are some ambiguities regarding the separation of the species. Sarwar (2002) combined these two species together. These two taxa have several identical morphological and anatomical traits (Mohammadi Shahrestani 2021). The present palynological data also revealed no significant differences in pollen morphological evidences of the two species.

Acknowledgements

Authors would like to thank Dr. Moradi, the curator of Research Institute of Forest and Rangelands of Gilan province (N Iran) for allowing us to obtain pollen materials from herbarium specimens. Authors also honor Late Dr. Sh. Saeidi Mehrvarz, who unfortunately, did not have the opportunity to see this work completed.

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