

A DETAILED STUDY OF LEAF AND POLLEN MICROMORPHOLOGY OF SOME SPECIES OF *PARIETARIA* AND ITS SYSTEMATIC IMPLICATION (URTICACEAE)

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Parietaria L. is a genus comprised of 20 species distributed throughout temperate and subtropical areas of the world. The leaf and pollen characters of four species of *Parietaria* from different locations in Iran were investigated with light and scanning electron microscopy, in order to assess their diagnostic significance to provide additional evidence on species delimitation. All pollen grains were small in size and found to be porate. Most of the pollen characters, such as shape and sculpturing of pollen grains, exine diameter, were overlapped between species and indicated limited taxonomic value. The pore and annulus diameter could be provided additional taxonomic characters that can be used for species delimitation in the genus. Moreover, the leaf epidermis provided new qualitative data (laminar hydathodes, bladder-based trichomes) that could delimit the species. The characters like twin stomata and laminar hydathode observed in *P. officinalis* were reported for the first time in this genus.

Key words: laminar hydathodes, species delimitation, stomata, trichome

INTRODUCTION

Parietaria Linnaeus (1753: 1052) comprises of 20 species distributed throughout temperate and subtropical areas of the world (Boufford 1992, Friis 1993). This genus belongs to the Urticaceae family, with 53 genera and 2,625 species worldwide (Christenhusz and Byng 2016). Four species in Iran represent *Parietaria*, none of which are endemic (Janighorban 2001). The genus is characterised by hermaphrodite flowers, leaves with entire margins, and an apparent modification of the flower after anthesis (Weddell 1856).

Based on recent molecular data (Wu *et al.* 2013), the genus is currently placed in the tribe Parietarieae in the subfamily Boehmerioideae alongside *Gesnouinia* and *Solierola*.

Leaf anatomical features have provided valuable taxonomic information, including the shape of epidermal cells, stomata feature, as well as the size,

type, distribution, and number of cells in trichome at the different ranks of plants (Shokefun *et al.* 2014, Stace 1984, Stuessy 2009).

Also, pollen features have been utilised in many fields, including geology, criminology, medicines, palaeobotany, and allergy prohibitions (Huang 1972). Moreover, it has significant uses in resolving phylogenetic relationship problems (Huang 1972, Judd and Olmstead 2004, Perveen *et al.* 2004). Different palynologists worked on the significance of pollen morphology in plant systematics and found it a beneficial character for identifying plant species (Choi *et al.* 2022, Erdtman 1952, Kadluczka *et al.* 2022, Mete *et al.* 2021, Ullah *et al.* 2022, Wodehouse 1935).

The research studies on the micromorphology (pollen and foliar epidermis) of *Parietaria* genus are few, despite that we can refer to Punt and Malotau (1984), Sorsa and Huttunen (1975), Kadiri *et al.* (2011), Sultani and Al-shemarri (2016), Burn and Mayle (2008), Selim (2020). From most of the previous literature, it was noticed that the structure, size of the pores, and exine sculpture of the pollen of *Parietaria* have the most diagnostic characteristics. Moreover, the variations in the length and trichome type of the epidermal surface of this genus were indicated to have reliable systematic value (Selim 2020). Therefore, the present study aimed to investigate the leaf epidermis and pollen morphology of the four *Parietaria* species distributed in various Iran regions. Given the importance of the Urticaceae and *Parietaria* genus in palaeobotanical studies and allergic works, the results can be helpful in advancing scientific purposes and increasing the encyclopaedia of this genus. The taxonomic importance of epidermal features was discussed.

MATERIAL AND METHODS

Pollen study

Pollen grains of 16 populations were studied by light microscopy (LM) and scanning electron microscope (SEM) in the following *Parietaria* species: *P. alsinifolia* Delile, *P. lusitanica* L., *P. judaica* L., *P. officinalis* L. The populations belong to four geographical regions in 11 provinces of Iran (Fig. 1, Table 1). The taxonomic identification of *Parietaria* species was accomplished based on Flora of Iran (Janighorban 2001). The pollen samples were obtained from herbarium materials. Voucher specimens have been deposited in Shahid Beheshti University Herbarium (HSBU) and the herbarium of the Museum of Natural History in Vienna, Austria (W) (Table 1).

The pollen grains of five randomly collected specimens were mixed and prepared for LM and SEM investigations. The LM study was conducted using the standard methods described by Erdtman (1952) and photographed using an Olympus BX51 microscope with an automatic camera, under (E40, 0.65) oil

Table 1
The locality properties and details of examined characters of *Parietaria* specimens in this study.

Taxa	Code: locality	Pp	Ee	Exine	P/E	Pore diameter (Pd)	Annulus diameter (Ad)	Pollen shape classes	Voucher number
Alsini	1: Hormozgan, Hajjabad	20.03±1.95	17.78±1.78	1.11±0.16	1.13	0.97±0.17	2.7±0.36	prolate-spheroidal	W1970-3335
Alsini	2: Khozestan, Behbahan	13.92±0.9	13.3±0.96	0.85±0.08	1.05	0.98±0.21	2.36±0.32	prolate-spheroidal	HSBU2018646
Juda	3: Khorasan, Mashhad, Karmabad	13.2±1.14	13.14±1	0.73±0.19	1	1.15±0.28	2.66±0.23	spheroidal	HSBU2018645
Juda	4: Tehran, Darakeh	12.53±1.5	13.27±0.93	0.93±0.26	0.94	1.3±0.31	3.29±0.4	oblate-spheroidal	HSBU2018641
Juda	5: Semnan, Sorkheh	20.24±1.28	17.96±1.22	1.03±0.2	1.13	1.3±0.28	3.37±0.32	prolate-spheroidal	W1979-12310
Juda	6: Kermanshah, Rijab	13.98±0.71	13.79±0.91	0.91±0.23	1.01	1.06±0.1	2.62±0.36	prolate-spheroidal	W1972-09786
Juda	7: Kerman, Khebr	13.55±1.71	13.81±0.56	0.95±0.16	0.98	0.99±0.28	2.97±0.37	oblate-spheroidal	HSBU2018643
Juda	8: Baluchistan, Taftan	14.36±0.77	13.97±1.02	1.04±0.28	1.03	0.91±0.18	2.91±0.41	prolate-spheroidal	W1982-07329
Juda	9: Azarbayjan, Khoy	13.51±0.89	13.73±0.68	0.94±0.12	0.98	1.14±0.24	3.1±0.42	oblate-spheroidal	W1972-00364
Lusi	10: Khozestan, Ramhormoz	18±1.64	17.81±1.17	0.89±0.16	1.01	1.04±0.24	3.24±0.37	prolate-spheroidal	W 1972-15031
Lusi	11: Khozestan, Izeh	11.59±0.72	11.44±0.96	0.87±0.13	1.01	0.9±0.18	2.43±0.25	prolate-spheroidal	W1972-13530
Lusi	12: Khozastan, Behbahan	12.33±0.37	12.4±0.61	0.89±0.17	0.99	0.97±0.15	2.62±0.58	oblate-spheroidal	HSBU2018648*
Lusi	13: Fars, Takht-e Jamshid	18.13±1.54	18.1±1.43	1.17±0.32	1	1.26±0.26	3.4±0.56	spheroidal	W1970-20626
Offi	14: Mazandaran, Chalus	13.66±0.96	13.35±1.08	0.84±0.12	1.02	0.79±0.17	2.45±0.29	prolate-spheroidal	HSBU2018640
Offi	15: Mazandaran, Qaemshahr	12.99±0.81	13.13±0.62	0.88±0.22	0.99	0.97±0.2	2.65±0.37	oblate-spheroidal	HSBU2018636*
Offi	16: Mazandaran, Amol	12.01±0.66	12.11±0.9	0.88±0.11	0.99	0.84±0.15	2.42±0.32	oblate-spheroidal	HSBU2018637

Alsini = *P. alsinifolia*, Juda = *P. judaica*, Lusi = *P. lusitanica*, Offi = *P. officinalis*, * = the specimens used in epidermis study.

immersion (E100, 1.25). The photographs were measured with the software Image Tools Version 3.0. For SEM studies, small pollen grains were attached to double-sided cello tape mounted on the aluminium stub and coated with the gold. The specimens were examined with a Phillips $\times$ L20 SEM. UTHSCSA Image Tool Version 3.0 was used to carry out the required measurements. Ten to 30 fully developed pollen grains were randomly selected for analysis. Principal Components Analysis (PCA) was performed by PAST software to identify useful palynological features for separating the specimens (Hammer *et al.* 2001). Six quantitative palynological features were used for multivariate analysis, including Polar axis size (P), equatorial axis size (E), P/E, exine thickness, pore diameter, and annulus diameter. Moreover, the qualitative features like exine ornamentation, pollen shapes, and pores were compared and discussed.

Leaf epidermis study

Leaf epidermal materials were prepared from mature leaves. For LM studies, laminas were boiled in water for 30 s, and then macerated overnight in a 1:1 (by volume) hydrogen dioxide solution and glacial acetic acid at 60 °C. Pieces of leaf epidermis were stained with Safranin–alcohol (50%) prior

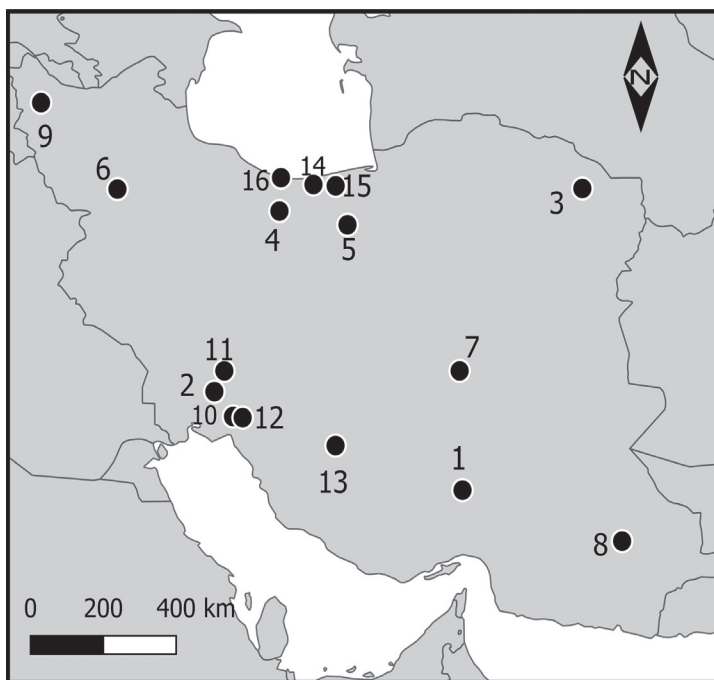


Fig. 1. Distribution map of the studied *Parietaria* L. specimens in Iran. The numbers are in accordance with Table 1. 1–2 = *P. alsinifolia*, 3–9 = *P. judaica*, 10–13 = *P. lusitanica*, 14–16 = *P. officinalis*

Table 2
Leaf epidermal characteristics of *Parietaria* species (from SEM and LM)

Taxa	Adaxial epidermis				Abaxial epidermis											
	Trichome		Wax orna- men- tation	Cys- tolithe	Cuticular membrane		Shape of the guard cell	Sto- mata eleva- tion	Anti- clinal cell walls	Anti- clinal wall eleva- tion	Periclinal wall		Trichome			
	size	Bb		pres- ence	stria- tion	ridged					eleva- tion	sur- face	size	den- sity		
<i>P. alsinifolia</i>	SH & LO	+	(HD)	LG	+	RG	HR	E	SU	U&W	SU	RA	SM	SH & LO	HD	+
<i>P. judaica</i>	SH	-		HG	+(LD)	RG	SR	E	SU	SI	SU	RA	SM	SH	LD	-
<i>P. lusitanica</i>	SH & LO	+		HG	+(HD)	LS	NR	NE	AL	SI & U	RA	SU	SM	SH	HD	-
<i>P. officinalis</i>	SH	+(LD)		LG	+	LS	NR	WE	AL	SI & U	RA	SU	SM	SH	LD	-
Abbreviations: Bb = Bladder base; SH = short, SH & LO = short and long, LD = low density, HD = high density, HG = high granular wax, LG = low granular wax, RG = radiating from the guard cell, LS = low striation, SR = slightly ridged, NR = normal ridged, HR = heavy ridged, E = elliptical, NE = narrow elliptical, WE = wide elliptical, SU = sunken, AL = at the same level of other epidermal cells, SI = sinuate, U & W = undulate and wavy, SI & U = sinuate & undulate, RA = raised, SM = smooth																

to mounting in glycerine gel. Prepared cuticles were observed using an Olympus BX51 microscope with an automatic camera. For SEM study, a small segment from the central part of the leaf abaxial surface was placed on aluminium stubs with double-sided cellophane tape and coated with a gold. The specimens were examined with a Phillips × L20 SEM. UTHSCSA Image Tools, version 3.0 was used to measure the following features.

Qualitative features include the trichome size and situation, wax ornamentation, cystolithe presence, cuticular membrane feature, the shape of guard cell, the shape of anticlinal cell walls, anticlinal and periclinal wall elevation were compared and discussed (Figs 5–6; Table 2).

The terminology used for describing pollen grains and epidermis micromorphology followed Erdtman (1952), Punt and Malotau (1984), Punt *et al.* (2007), Hesse *et al.* (2009), and Klimko and Truchan (2006), Selim (2020).

RESULTS

Pollen morphology

Table 1 summarises the quantitative parameters measured for all pollen specimens. SEM and LM images of the studied pollen grains are presented in Figures 2–3. All grains are 3–4 zonoporate with the annulus surrounding their circular pores (annulus sensu Punt *et al.* 2007).

The specimens investigated in this genus have 3-porate or rarely 3-porate along with 4-porate grains (observed in *P. judaica* and *P. lusitanica*).

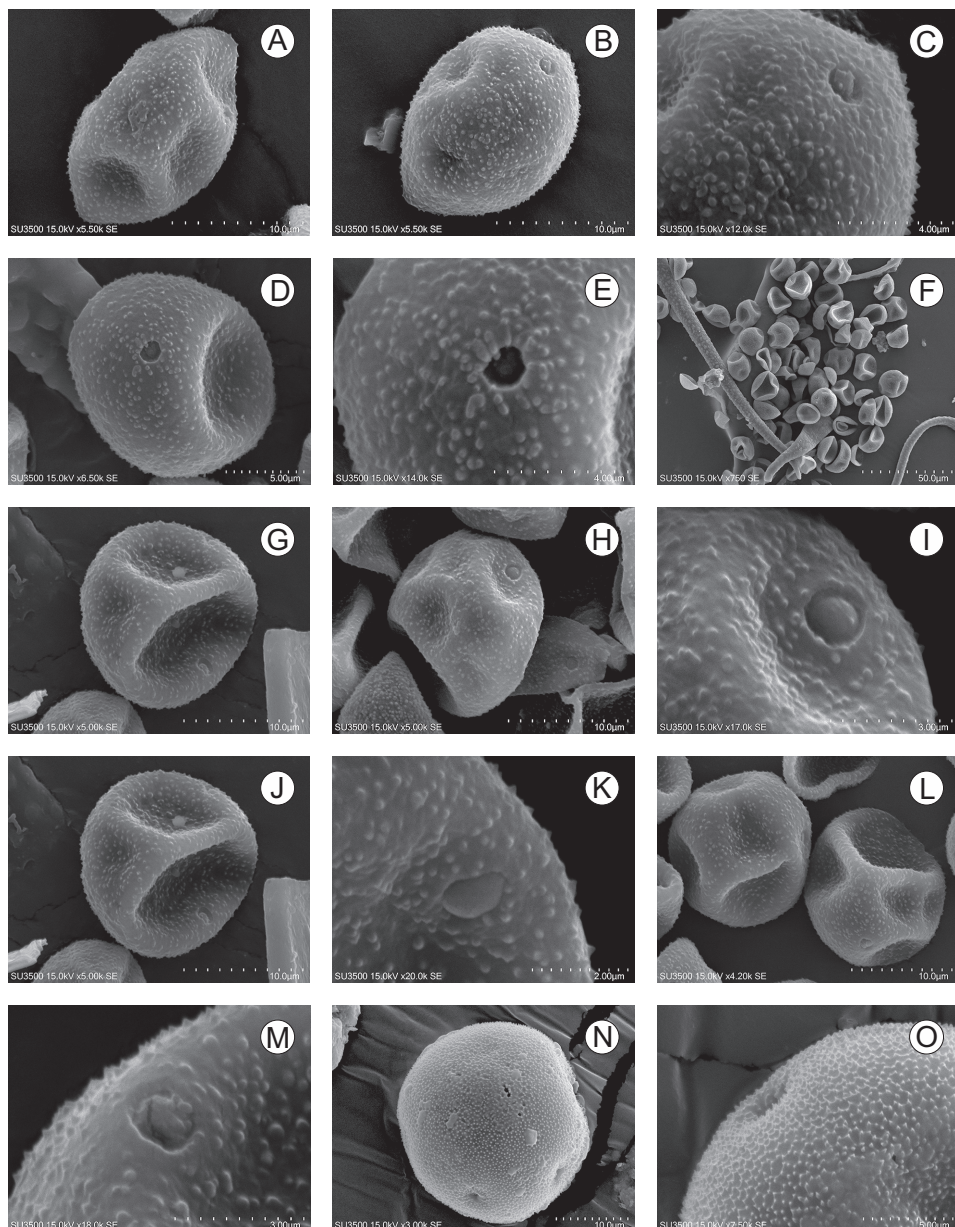


Fig 2a. SEM micrographs of pollen grains in *Parietaria* genus. A = *P. alsinifolia*, B–M = *P. judaica*, N–O = *P. lusitanica*

The shape class (P/E) ranged from oblate-spheroidal in *P. judaica*, *P. lusitanica*, and *P. officinalis*, spheroidal in *P. judaica* and *P. lusitanica* to prolate-spheroidal in some specimens of all four species (Figs 2a, b).

In general, all pollen grains belong to different specimens and are small, as the polar axis never exceed 25 μm , with the mean length of the polar axis (P) varying between $11.59 \pm 0.72 \mu\text{m}$ for *P. lusitanica* (code 11), 20.24 ± 1.28 and $20.03 \pm 1.95 \mu\text{m}$ for *P. judaica* (code 5), and *P. alsinifolia* (code 1). The mean equatorial diameter (E) varying between $11.44 \pm 0.96 \mu\text{m}$ for *P. lusitanica* (code 11), 18.1 ± 1.54 and $17.96 \pm 1.22 \mu\text{m}$ for *P. lusitanica* (code 13) and *P. judaica* (code 5) (Table 1, Figs 2–3).

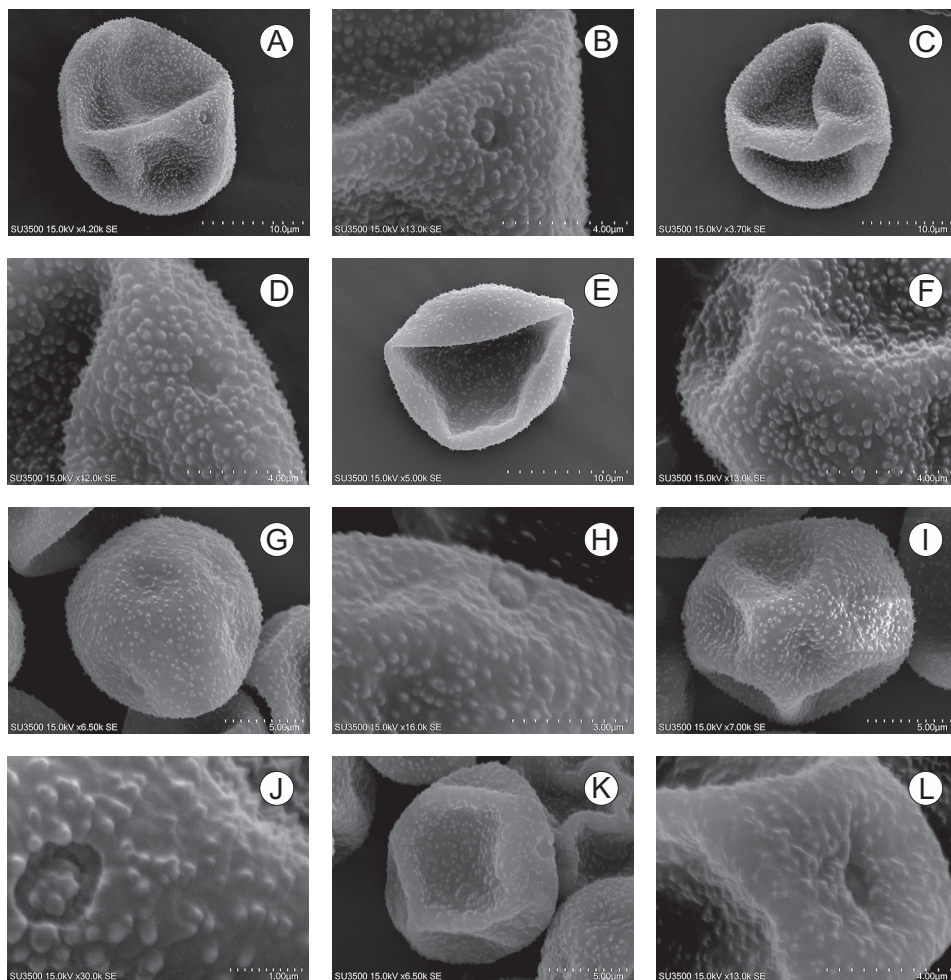


Fig. 2b. SEM micrographs of pollen grains in *Parietaria* genus. A–F = *P. lusitanica*, G–L = *P. officinalis*

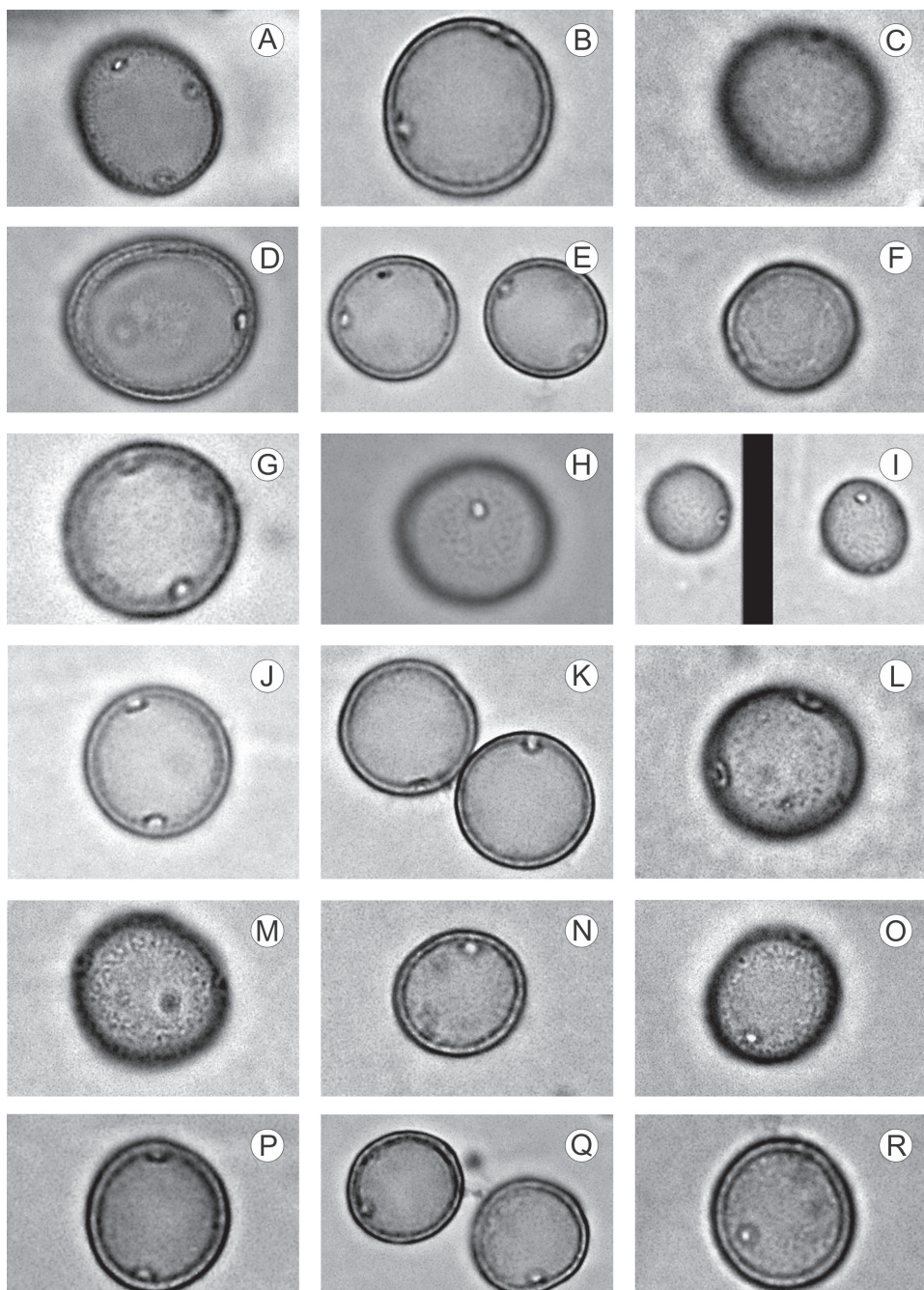


Fig. 3. Photomicrographs of pollen morphology of the studied species (LM). A–C = *P. alsinifolia*, D–L = *P. judaica*, M–O = *P. lusitanica*, P–R = *P. officinalis*

P. judaica (code 4, 5) has the most prominent pores with a mean diameter of $1.3\ \mu\text{m}$, and *P. officinalis* (code 14) exhibits the smallest mean pore diameter of $0.79\ \mu\text{m}$. The mean exine thickness varies between $0.73\ \mu\text{m}$ (*P. judaica*, code 3) and $1.17\ \mu\text{m}$ (*P. lusitanica*, code 13).

A similar shape and surface ornamentation were observed in almost all studied specimens except *P. alsinifolia* whose sculpturing needed more investigation on different specimens.

The surface sculpturing of the studied specimens is spinules. Except *P. lusitanica* (code 12), the sculpturing of all studied specimens exhibited mostly broad, cone-shaped spinules, occasionally together with smaller granules. The spinules of *P. lusitanica* (code 12) had a similar shape to others, but most spinules indicated broad bases and sharply tapering points. Moreover, the density of spinules on the surface of *P. officinalis* and *P. judaica* specimens was lower than *P. lusitanica* and *P. alsinifolia*.

As seen in the PCA plot (Fig. 4), just specimens belonging to *P. officinalis* nested close to each other could be separated from other specimens. Given to the quantitative features, the *P. judaica* species indicated the most intraspecific variation. PCA analysis of morphological traits revealed that the first two PCA components comprised about 90.03% of the total variation. In the first PCA axis, with about 71.47% of the total variation, morphological traits like pollen size (P, E) and pore diameter possessed the highest correlation. Thus, these features were the most variable morphological characters among the

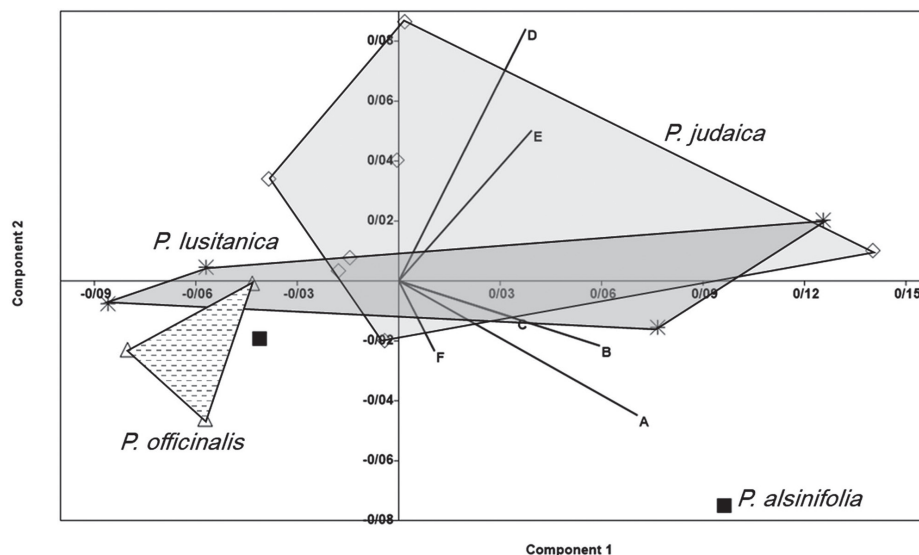


Fig. 4. PCA Biplot of morphological traits in individuals of the studied specimens based on pollen study. A = polar axis (P), B = equatorial axis (E), C = exine thickness, D = pore diameter, E = annulus diameter, F = P/E

studied specimens. PCA-biplot of morphological characters (Fig. 4) separated the specimens of *P. officinalis* and *P. alsinifolia* from members of *P. judaica* species. However, the high variation was in members of all species except for *P. officinalis*.

Micromorphology of leaf epidermis

The characteristics of the leaf epidermis of the studied species are listed in Table 2. The leaf was hypostomatic, in which stomata occurred on the lower surface. The anomocytic type of stomata was the dominant type in the studied specimens, but the paracytic type was seen in *P. lusitanica* beside the anomocytic one (Fig. 6). The epidermal cells in all studied species were regularly or irregularly polygonal with sinuous to undulate and wavy anticlinal walls (Fig. 6, Table 2). Trichomes were found on both adaxial and abaxial surfaces in all studied species. Given to the base of trichomes, two trichome types were

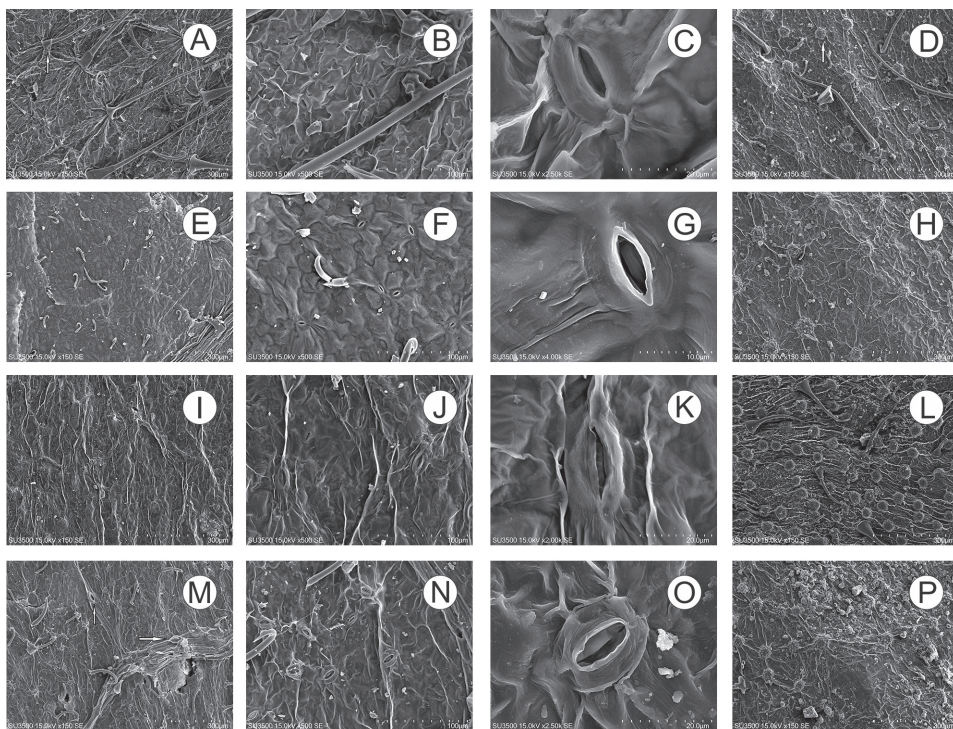


Fig. 5. Major aspects of lamina epidermal characters of the studied species (SEM). A–C = abaxial and D = the adaxial surface of *P. alsinifolia*, the arrow in “a” indicate bladder-based trichomes and the arrow in “d” indicates cystoliths; E–G = abaxial and H = the adaxial surface of *P. judaica*; I–K = abaxial and L = the adaxial surface of *P. lusitanica*; M–O = abaxial and P = the adaxial surface of *P. officinalis*. The arrows show laminar hydathodes

commonly found. (1) short unicellular non-glandular trichomes with a single-celled base, present in all studied species with various density, (2) filiform and long trichomes with a bladder-like base that observed in the abaxial surface of *P. alsinifolia* and the adaxial surface of *P. alsinifolia* (with significantly high density), as well as *P. lusitanica* (Figs 5–6; Table 2). Moreover, this type of trichome was seen in *P. officinalis* and *P. judaica* with very low density. In all two types, the apex of the trichome was curved. The sculpture of cuticular membranes was ridged on all species but to different degrees. Given the abaxial and adaxial surface of all species except *P. alsinifolia*, the higher degree of ridging on the adaxial surface was obvious. In *P. alsinifolia*, the degree of ridging on the abaxial surface was greater than the adaxial surface. Three statuses of striation were found in the studied species, including low striation (*P. lusitanica*, *P. officinalis*, Fig. 5) and radiating from the guard cell (*P. alsinifolia*, *P. judaica*, Fig. 5).

The anticlinal walls were undulate-wavy in *P. alsinifolia* and sinuous to undulated in three others. *P. alsinifolia* and *P. lusitanica* had deeper necks than

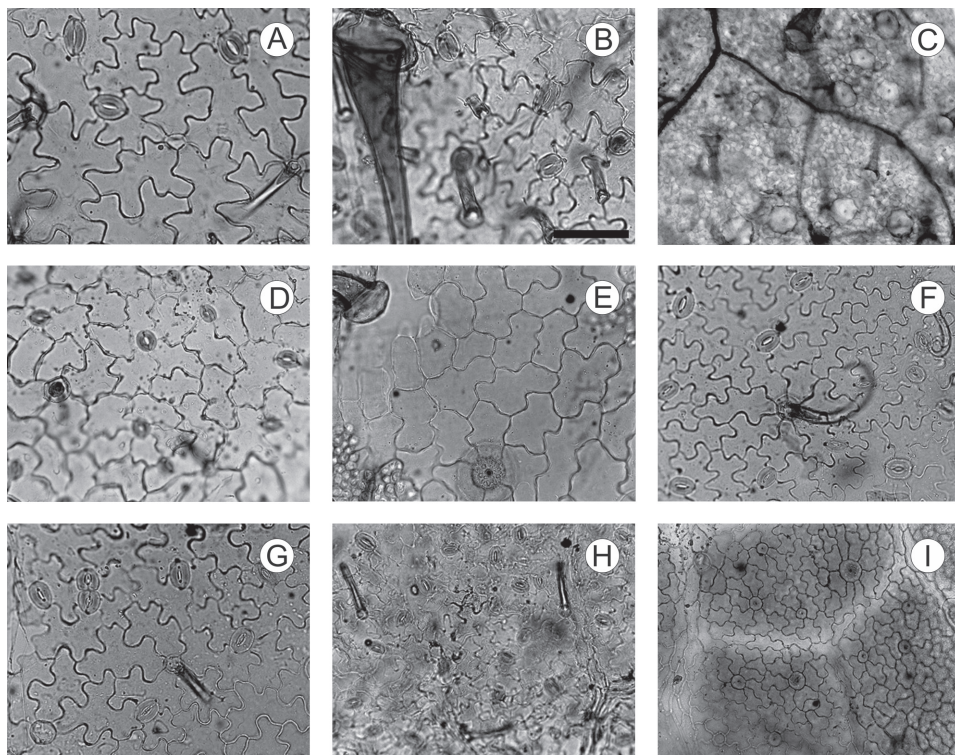


Fig. 6. Major aspects of lamina epidermal characters of the studied species (LM). A–B = abaxial and C = the adaxial surface of *P. alsinifolia*; D = abaxial and E = the adaxial surface of *P. judaica*; F–G = the abaxial surface of *P. lusitanica*; H–I = the abaxial surface of *P. officinalis*.

The twin stomata type is shown in *P. lusitanica* (G)

P. officinalis and *P. judaica* (Fig. 6). The two recent species indicated the lower sinuate status in the anticlinal walls. This wall was sunken in *P. judaica* and *P. alsinifolia* and raised in others. The periclinal wall surface was smooth in all studied species (Fig. 5). Stomata were located at the same level of epidermal cells in *P. lusitanica* and *P. officinalis* sunken in the two other species. The shape of the guard cell was elliptical (*P. judaica*, *P. alsinifolia*), narrow elliptical (*P. lusitanica*), and wide elliptical (*P. officinalis*). The cuticular was covered with granular epicuticular wax in different degrees (Table 2).

The cystoliths was another important epidermal structure found in the adaxial surface of all studied species with different degrees of density (Fig. 5). The adaxial surface of *P. lusitanica* indicated a much higher density than other species. The long trichomes (type II, see above) could be another type of cystoliths distributed with different densities in the epidermis of *Parietaria* species.

Some unusual points have been observed in this genus. For example, there were twin stomata in the abaxial surface of *P. lusitanica*. Also, the existence of laminar hydathode was confirmed in this genus. The laminar hydathode was observed in one of the studied species, the abaxial surface of *P. officinalis* (Fig. 5). Also, in *P. officinalis*, the secretory gland and papilla-like lithocyst were observed on the abaxial surface (Fig. 5).

DISCUSSION

The four Iranian species of *Parietaria* studied are found in various habitats throughout different geographical vegetation of Iran. They could be divided into annual herbs (*P. alsinifolia* and *P. lusitanica*) and perennial with woody basal stem (*P. judaica* and *P. officinalis*). While *P. judaica* colonises on the cliffs of the mountain throughout all floristic regions of Iran, the *P. officinalis* confines as an element belonging to Hyrcanian region and some Irano-Turanian of North of Iran. Moreover, *P. lusitanica* grows in Irano-Turanian and Sahara-Syndian. These two regions and Zagrosian areas are the habitat regions of *P. alsinifolia* (Janighorban 2001).

Palynological evidence from this study indicates similarity and homogeneity in the pollen characters of the specimens studied. The pollen grains were considered small, as the maximum size of the polar axis was *ca* 20 μm , spinulate ornamentation of exine, infolding, and asymmetric in dry pollens. The results indicated three pollen shapes in the studied specimens: spheroidal, oblate-spheroidal, and prolate-spheroidal. The prolate-spheroidal shape of pollen in *P. alsinifolia* and *P. judaica* was reported by (Selim 2020). Also, the homogeneity of the P/E ratio observed in the studied specimens confirmed previous *Parietaria* and *Urtica* genera studies (Selim 2020). According to our results, based on seven specimens of *P. judaica* belonging to various geographical regions of Iran (range of North, west, east, central, and south) indicated infraspecific variation in the pollen shape

(all three pollen shapes were observed). Therefore, it seems that the shape of the pollen could not be a reliable differentiation between the studied species.

The less useful taxonomic importance of pollen shape was emphasised by (Davis and Heywood 1963) and (Moore *et al.* 1991) because it can vary considerably within one species due to extracting methods and embedding media used.

Based on our results, the 3–4 porate apertures were observed in the studied specimens, while Selim (2020) reported the panto-colporoidate apertures in *P. alsinifolia* and *P. judaica* that could not be confirmed in our work. However, in accordance with our work, Punt and Malotau (1984) indicated the 4-porate and 3-porate pollen for *P. judaica* and *P. officinalis*, respectively. Also, in this genus, porate pollen was recorded for *P. alsinifolia*, *P. lusitanica*, *P. judaica* by Sultani and Al-shemarri (2016). Comparing pollen size indicated no strong differentiation between specimens of the studied species. As seen in Table 1, different specimens of *P. judaica* showed range of small to large pollen. Similar results were observed in *P. lusitanica* and *P. alsinifolia*. Only in *P. officinalis* specimens, the close range of pollen size was observed that be approximately the smallest among all species specimens. Thus, there were no obvious barriers between these four species based on their pollen size, shape, and pore number. This may be due to the influence of climatic and growing conditions of the plant on the pollen size, which must be taken into account (Rodriguez-Damian *et al.* 2006).

Perveen *et al.* (2004) reported the taxonomic importance of exine ornamentation patterns in systematic studies for delimitation at subspecies, varieties, and cultivars ranks.

However, the uniformity of spinules observed in all studied specimens indicated limited taxonomic value in this genus. Just *P. lusitanica* indicated different spinules in more tapering apex. There is a higher density of the spinule in SEM micrographs of *P. alsinifolia* and *P. lusitanica* than in two other species.

In a similar study, Sultani and Al-shemarri (2016) showed the low taxonomic importance of the shape of the pollen grain, number and dimension of pores, and sculpturing of pollen grains in the differentiation of *Parietaria* (*P. alsinifolia*, *P. lusitanica*, *P. judaica*) and *Urtica* (*U. urens*, *U. dioica*, and *U. pilulifera*) genera in Iraq.

Despite all points mentioned above, the PCA analysis based on pollen quantitative features indicated clear differentiation of *P. officinalis* from *P. judaica* and *P. alsinifolia*.

As obvious in the PCA plot, the specimen of *P. alsinifolia* related to Hormozgan area is located far from the other specimens, including the other specimen of its own species.

As our observations are based on several specimens of each species, especially *P. judaica*, its obvious distinction from *P. officinalis* indicated that the use of pore and annulus diameter beside could help discriminate the species even within the genera.

Sultani and Al-Shemarri (2016) indicated that most features of pollen were overlapped between species of *Urtica* and *Parietaria* genera. While Dopazo *et al.* (2000) could not discriminate some species of *Urtica* genus and *Parietaria judaica* using biometrical studies. Rodriguez-Damian *et al.* (2006) used an automatic algorithm to distinguish the species of the family Urticaceae combining shape and texture analysis.

Literature sources on the epidermal morphology of *Parietaria* genus are relatively scarce. The epidermal leaf traits of *Parietaria* revealed several important micromorphological characters that did not report previously in this genus.

The conducted LM and SEM observations show that on the epidermal surface of their leaves, there are e-glandular unicellular trichomes with bladder base, granular wax ornamentation, epidermal hydathodes, and cystoliths.

Some of the epidermal traits observed here can be used to delimit species. The micromorphological results of this work largely agree with those in Assaf *et al.* (2016), Jafari and Dehghan (2012), Kadiri *et al.* (2011), Metcalfe and Chalk (1950), Murugesan and Dan (2019), Selim (2020). Differences in some traits were observed and will be mentioned below.

On the abaxial surface of all species, epidermal cells are mostly polygonal to irregular with sinuous to undulate anticlinal walls except *P. alsinifolia* that indicated undulate and wavy ones.

Epidermal anticlinal walls can be regarded as a taxonomically stable character in some families like Myrtaceae, Sapindaceae, etc. (Carr *et al.* 1971, Pole 2010).

The anticlinal walls were considered sinuate in *P. alsinifolia* and *P. judaica* distributed in Egypt (Selim 2020), while the difference between the walls of these two species was obvious in our works. This could be related to the influence of different habitat conditions. As a consequence of environmental adaptation, the mesophytic species usually have sinuous anticlinal epidermal walls while xerophytic have straight walls (Gifford and Foster 1989). None of the studied species is known as xerophytic species, so the straight walls were not seen. Moreover, the anticlinal wall to epidermal cells indicated sunken in both *P. alsinifolia* and *P. judaica*, while considering Selim (2020), the sunken situation was reported for *P. alsinifolia*, and the raised situation was observed only in *P. judaica*.

Hypostomatic leaves and anomocytic stomata type have been reported for the family in previous studies (Assaf *et al.* 2016, Gangadhara and Inamdar 1977, Selim 2020), but Kadiri *et al.* (2011) showed the paracytic and anisocytic types as diagnostic traits for some species of *Laportea* genus and *P. laxiflora*. In accordance with this, the paracytic type was observed in *P. lusitanica* as well as anomocytic one.

Also, the present study is the first report on the occurrence of twin stomata in the genus *Parietaria* that is seen in *P. lusitanica*. Before, this kind of stomata had been recorded in *Pellionia* genus of this family (Murugesan and Dan 2019). Twin stomata, as the abnormal type, were formed from two adjacent

meristemoid cells by degeneration of one or two stomatal cells and abnormal stomatal development (Patel and Inamdar 1971).

Among the anatomical account of Urticaceae, Metcalfe and Chalk (1979) recorded the occurrence of hypostomata, presence of hydathodes, and cystoliths of different forms as well as some other traits like glandular, e-glandular, and stinging hair types.

Hydathodes have been reported as functional structures mediated guttation in thirty-four families of dicotyledons (Metcalfe and Chalk 1950). As usual form of them, Marginal hydathodes could be observed in all the families, while laminar hydathodes mainly occurred in some species of three families, including Crassulaceae (Fahn 1988, Rost 1969), Moraceae, and Urticaceae. (Lersten and Curtis 1991). This functional structure in various shapes normally occurred in different genera of Urticaceae like *Pellionia*, *Pilea*, and *Urtica* genera (Kohl 1889, Lersten and Curtis 1991). The occurrence of this guttation-mediated was not reported in the *Parietaria* genus. Based on our work, *P. officinalis* as species distributed widely in the wet areas of Hyrcanian regions, obviously indicated laminar hydathodes in the surface of leaf epidermis. At the same time, this interesting structure was not observed in the other three species. However, we do not deny the existence possibility of this structure on their epidermis surface.

Cystoliths have been reported as silicified bodies with cellulose skeletons or occasionally not encrusted (Metcalfe and Chalk 1950). Also, the cystoliths constructed from calcium carbonate are usually located in the lithocysts (Ummu-Hani and Noraini 2013). They are generally found only in a few families, including Acanthaceae, Cucurbitaceae, Moraceae, Ulmaceae, and Urticaceae (Ajello 1941, Arnott 1980, Fernández Honaine *et al.* 2018, Metcalfe and Chalk 1950, Patil and Patil 2011, Scott 1946, Setoguchi *et al.* 1989). Various types of cystoliths such as arc, cigar, elliptical, fusiform, linear, round, spindle, papillate, hair-like occur primarily in the epidermal layer of the leaves and are reported in different genera of Urticaceae (Kadiri *et al.* 2011, Murugesan and Dan 2019, Patil and Patil 2011, Ummu-Hani and Noraini 2013). Here we observed round cystolith type on the adaxial surface of all four species with different degrees of density. Their density was high in annual species (*P. lusitanica* and *P. alsinifolia*) and low in perennial species (*P. judaica* and *P. officinalis*). Based on Murugesan and Dan (2019), cystoliths were present conspicuously on both surfaces in *Pellionia heyneana* (Urticaceae). However, we do not observe them on the abaxial surface of the *Parietaria* genus.

The cystoliths usages in classification at the family level are uncommon. However, in some families such as Moraceae, Acanthaceae, Urticaceae, and Boraginaceae, various forms of cystoliths can be good criteria for genus and species identification (Metcalfe 1960, Solereder 1908). Also, the papillae type was seen on the surface of *P. officinalis* epidermis. This evidence could represent the taxonomic importance of cystoliths in *Parietaria* genus.

The result of the present investigation showed that leaf epidermal characters could be differentiated *P. alsinifolia*, *P. officinalis* and *P. lusitanica* because of specialised characters including paracytic types of stomata and twin stomata in *P. lusitanica*, luminaire hydathode and papilla-like type in *P. officinalis*, and very high density of bladder-based trichomes in *P. alsinifolia*.

*

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