Micro-morphological study of *Polygonaceae* tribes in Iran

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Abstract. As a family, *Polygonaceae* has a complex taxonomic historical position that experienced different treatment in the floras. In order to clear delimitation of its tribes in Iran, in this study we consider the macro and micro-morphological features of tepal epidermis, dorsal leaf epidermis, achene surface, and pollen grains in 10 populations from different habitats in Iran. The results of cluster analysis have revealed that *Rumiceae* has an interesting position in phenogram topology. Micro-morphological characters proved more efficient in the separation of *Persicarieae* elements in Iran than in two other tribes. The results of this project show distinctly *Polygonum* s. str. and *Persicaria* as separate genera in the flora of Iran.

Key words: Iran, *Persicarieae*, *Polygonaceae*, *Polygoneae*, *Rumiceae*

Introduction

Polygonaceae is composed of three tribes and more than 12 genera in Iran (Rechinger & Schiman-Czeika 1968). The family has a complex taxonomic history and different treatment in the various floras (Davis 1966; Rechinger & Schiman-Czeika 1968; Brandbyge 1993). Subdivisions of *Polygonum* L. s.l. have been always problematic (Ronse Decraene & Akeroyd 1988). In 1826, this large genus was divided into seven sectional levels by Meissner. He extended this subdivision to nine sections in 1857: *Bistorta* (Adans.) D. Don., *Aconogonon* Meisn., *Tiniaria* Meisn., *Avicularia* Meisn., *Persicaria* Meisn., *Fagopyrum* Meisn., *Amblygonon* Meisn., *Tephis* (Adans.) Meisn., and *Cephalophilon* Meisn. His sections with three other units were confirmed by Bentham & Hooker in 1880. Apparently, the first attempt for recognition of the various genera in *Polygonum* s.l. was made in 1913 by Gross (Ronse Decraene & Akeroyd 1988). Although Gross maintained Meissner's subdivisions, he recognized seven genera on the basis of pollen characters: *Polygonum* L., *Persicaria* L., *Bistorta* Mill., *Pleuropterus* Turcz., *Pleuropteropyrum* Gross., *Pteroxygonum* Dammer & Diels, and *Fagopyrum* Mill. Harldson (1978) distinguished seven other genera in *Polygonum* s.l. on the basis of anatomical features, and they were distributed between the different tribes (*Polygoneae* and *Persicarieae*) composed of *Polygonum*, *Persicaria*, *Bistorta*, *Fagopyrum*, *Aconogonon* Meisn., *Koenigia* L., *Fallopia* Adans., and *Reynoutria* Houtt.

Presently, a taxonomic survey without micro-morphology is considered incomplete. Rejdali (1991) mentioned leaf and hair micro-morphology in recognition of *Sideritis* L. (*Lamiaceae*) in Africa. Varieties of hair characteristics were also useful in the infrasectional division of this genus (Nunez & al. 1990). In *Bromus* L. (*Poaceae*), Acedo & Liamas (2001) identified six subgenera by the micro-morphology of lemma and palea. In *Polygonaceae*, the authors have studied tepal and fruit surface morphology (Hong & al. 1998; Ronse Decraene & al. 2000). Their observations have led to clear segregation of the genera in *Polygoneae* and *Persicarieae* tribes. In Iran, such researches for *Polygonaceae* have not been carried out so far. The only micro-morphological survey in Iran was the evaluation of some pollen grain characters in some species of *Polygonum* s.l. (Amiri & Sharifnia

2007). Studied were some genera of the *Persicaria*, *Tiniaria*, *Bistorta*, and *Pleuropterus* sections and three species of *Polygonum*. It has been found that pollen features are of taxonomic value in sectional division.

In this study, tepal epidermis, leaf dorsal epidermis, pollen grains and nut surfaces have been studied for the first time in Iran in some elements of three tribes of *Polygonaceae*.

Material and methods

In the present study, 10 populations (10 individuals per each accession) from three tribes of *Polygoneae*, *Persicarieae* and *Rumiceae* were collected from different parts of Iran and studied out. For each sample, proper replications were made. Species were confirmed by different floras (Hooker 1885; Rechinger & Schiman-Czeika 1968; Webb & Chater 1964). Fresh materials of *Polygonum*, *Persicaria* and *Pteropyrum* species and herbarium specimen of *Rumex acetosa* were used in the study (Table 1). Voucher specimens were deposited in the Herbarium of Alzahra University (AUH). For epidermis surveys, the tissue removal method was used. According to this method, the plant segments were immersed in 10% of hydrogen peroxide for 20 minutes. This caused lysis of the additional tissues and required lubrication of the scalpel. Then the destroyed tissues were removed by the scalpel at an angle of approximately 45°. After removal, the epidermis was washed and stained out with Methyl green. The Stomatal Index was calculated by means of the formula

$$\frac{S}{E+S} \times 100$$

where **S** stands for the number of stomata per unit area and **E** stands for the number of epidermal cells in the same area (Metcalfe & Chalk 1979). Photographs were taken by light microscope equipped with a digital camera Olympus DP12.

For SEM studies, the pollen grains suspended in a drop of water were directly transferred by a fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Sputter Coater BAL-TEC, SCDOOS). Nuts were studied by SEM without any treatment. Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 A (Hacking & al. 2007). The SEM examination was carried out on a Philips microscope XL30. The measurements were based on 10–20 readings for each specimen.

The terminology of Buchner & Weber (2000) was followed for pollen and nut sculptures and of Metcalfe & Chalk (1979) and Hong & al. (1998) for epidermal cell walls patterns.

In order to detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. To reveal the species relationships, we have used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). For multivariate analysis, the mean of the quantitative characters was used, while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of morphological data. In order to determine the most variable micro-morphological characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 9 (1998) software was used for statistical analysis.

Table 1. Collection data for populations used in this study

Collector	Origin	Voucher No	Species
Mosaferi	Iran, Mazandaran province, Kelardasht, Gavitar village	500	*Persicaria hydropiper* L.
Keshavarzi	Iran, Mazandaran province, Zirab, Kechid village	502	*P. maculosa* Gray.
Mosaferi	Iran, Hamadan province, Heydareh village	504	*P. lapathifolia* subsp. *nodosa* Danser.
Gholami	Iran, Kermanshah province, Kermanshah, Gharesoo river	506	*P. lapathifolia* subsp. *lapathifolia* L.
Amini	Iran, Mazandaran province, Noushahr	513	*P. lapathifolia* subsp. *brittingeri* (Opiz) Rechenb.
Mosaferi	Iran, Mazandaran province, Abbas Abad, Abbas Abad forest	535	*P. mitis* Schrank.
Mosaferi	Iran, Isfahan province, Golpaygan, Saravar village	514	*P. minor* Hudson.
Gholami	Iran, Tehran province, Karaj-Challus road, Shahrestanak village	303	*Polygonum alpestre* C. A. Mey
Gholami	Iran, Kermanshah province, Kermanshah	302	*P. aviculare* L.
Mosaferi	Iran, Tehran province, Karaj, Baghestan	205	*Pteropyrum aucheri* Jaub. & Spach
Mosaferi	Iran,15 km Tehran-Saveh road- Ma'mooniyeh town	202	*P. olivieri* Jaub. & Spach
Ahmadi	Iran, Golestan province, Kaboodval forest	412	*Rumex acetosa* L.

Results

Tepal epidermis

Persicarieae

In *Persicaria* species, tepal epidermis consisted of rectangular to elongated cells with different outline. For instance, in *P. mitis* cell walls were sinuate, while in *P. hydropiper* cells had an undulating outline. *P. maculosa* was with short rectangular cells, with sinuate anticlinal walls. In some taxa, a few stomata could be observed in the tepal epidermis. Among the three subspecies of *P. lapathifolia*, subsp. *nodosa* showed rectangular cell shape, with mostly straight anticlinal walls, while subsp. *brittingeri* showed sharply sinuate cells (Figs 1-7).

Polygoneae

Polygonum. The tepal epidermis in *Polygonum* had sinuate cell walls. *P. aviculare* showed strongly sinuate outline, while *P. alpestre* had a long rectangular cell shape, with sinuate anticlinal walls (Figs 8-9).

Pteropyrum. *P. aucheri* had irregular to polygonal cells, with mostly sinuate anticlinal walls, while *P. olivieri* had smaller polygonal cells with a similar outline (Figs 10-11).

Rumiceae

In *Rumex acetosa,* the tepal epidermis consists consisted of irregular to rectangular cells, with sinuate outline (Fig. 12).

Leaf epidermis

Persicarieae

In most studied taxa of this tribe, the leaf epidermis consisted of irregular and short cells, with undulated cell walls. The length and width of stomata, subsidiary cells and Stomata Index differed between the taxa (Table 2). The stomata type was paracytic, except for in *P. lapathifolia*. In the leaf epidermis of this species, rectangular to polygonal cells with smooth cell walls and anisocytic stomata type have been observed (Figs 13-19).

Polygoneae

In this tribe, the leaf epidermis had irregular, rectangular to polygonal cells, with anisocytic stomata. Occasionally, the cell shape was fairly long, with undulated cell walls, (*Polygonum alpestre*), or with smooth cell walls (*P. aviculare*), while in the *Pteropyrum* species small polygonal epidermis cells have been observed (Figs 20-23).

Table 2. Micro-morphological characters of tribes *Polygoneae*, *Persicarieae* & *Rumiceae*

Taxon	Tribe	Leaf epidermis characters						Tepal epidermis characters		
		Length of stomata	Width of stomata	Length of subsidiary cells	Width of subsidiary cells	Index of stomata	Type of stomata	Length of epidermis	Width of epidermis	Distance between waves
Persicaria hydropiper	*Persicarieae*	14.98	13.42	20.31	5.08	% 27.45	paracytic	57.79	11.64	12.36
P. maculosa		41.92	29.31	52.84	17.76	% 25	paracytic	73.39	24.44	17.24
P. lapathifolia subsp. *nodosa*		23.27	16.99	34.14	9.91	% 15.71	anisocytic	45.15	13	0
P. lapathifolia subsp. *lapathifolia*		24	16	19.19	9.86	% 29.58	anisocytic	55.52	11.63	11.59
P. lapathifolia subsp. *Brittingeri*		15.54	12.35	28.57	13.2	% 24.35	anisocytic	34.11	22.38	11.10
P. mitis		24.73	15.67	40.09	10.76	% 53	paracytic	71.45	18.34	9.28
P. minor		24.11	16.73	33.73	9.37	% 28.26	paracytic, anisocytic	47.15	16.98	14.07
Polygonum alpestre	*Polygoneae*	38.5	30.52	92.54	29.42	% 34	anisocytic	107.51	20.75	25.01
P. aviculare		17.13	15.61	74.89	34.29	% 61	anisocytic	53.97	14.14	8.82
Pteropyrum aucheri		25.27	23.79	32.09	14.16	% 68	anisocytic	61.76	22.28	19.94
P. olivieri		23.18	17.28	32	7.61	% 70	anisocytic	48.62	19.76	26.54
Rumex acetosa	*Rumiceae*	21.71	15.24	39.69	11.58	% 24	anisocytic	29.99	15.12	17

Table 2. Continuation.

Taxon	Pollen grain characters								Nut characters		
	Length of equatorial axis	Length of polar axis	Number of aperture	Space between sculpture	Equatorial shape	Polar shape	Pollen type	Sculpture type	Nut length	Nut width	Nut sculpture
Persicaria hydropiper	29.63	28.5	5	0.85	circular	spheroidal	porate	reticulate heterobrochate	2.56	1.64	sinuate & striate
P. maculosa	32.61	27.97	5	0.19	circular	spheroidal	porate	reticulate	2.28	1.6	fossulate
P. lapathifolia subsp. *lapathifolia*	26.32	22.52	5	0.31	circular	spheroidal	porate	reticulate	2.49	1.84	smooth with scattered dots
P. mitis	32.5	29.41	5	0.38	circular	spheroidal	porate	reticulate microgranulate	2.26	1.44	punctuate
P. minor	31.71	26.6	5	0.3	circular	spheroidal	porate	reticulate clavate	2.37	1.77	smooth with rounded ledges
Polygonum alpestre	11.57	20.52	3	0.24	elliptic	prolate	colpate	granulate	2.32	0.87	shallowly striate
P. aviculare	28.09	12.55	3	0.66	elliptic	subprolate	colpate	granulate	2.64	1.26	smooth with tubercle
Pteropyrum aucheri	15.62	23.96	3	0.12	lobate	prolate	colporate	perforate granulate	4.80	5.5	wrinkled
P. olivieri	14.44	19.29	3	0.41	lobate	prolate	colporate	perforate	4.20	3.37	deeply striate in a wrinkled substrate
Rumex acetosa	14.52	11.64	3	0.29	cup shape	spheroid	colporate	densely granulate	2.20	1.8	reticulate

Rumiceae

In *Rumex acetosa*, the leaf epidermal cells were fairly irregular and small, with undulating outline (Fig. 24).

Pollen grains

Persicarieae

In this tribe, the pollen grains were pantoporate, with spheroidal form and circular shape in polar and equatorial view, respectively. The most variable feature between species was the exine sculpture patterns (Figs 25-34).

Polygoneae

The pollen grains in *Polygoneae* were smaller than in *Persicarieae*, with 3-colpate or 3-colporate pollen grains (Table 2). In polar view they were prolate to subprolate (Figs 35-42).

Rumiceae

In *Rumex acetosa*, the pollen grains were distinct 3-colporate, with granulate sculpture. In polar and equatorial view, this species was the same as *Persicaria* (Figs 43-44).

Nut macro- and micro-morphology

Persicarieae

In the *Persicaria* species, the nuts were biconvex, biconcave or trigonous. Nut surface sculpture showed variations between species (Table 2). In *P. hydropiper,* a sinuate and striate pattern was observed, while the nuts in *P. lapathifolia* were smooth, with scattered dots. Furthermore, the length and width of nuts differed between the taxa (Figs 45-54).

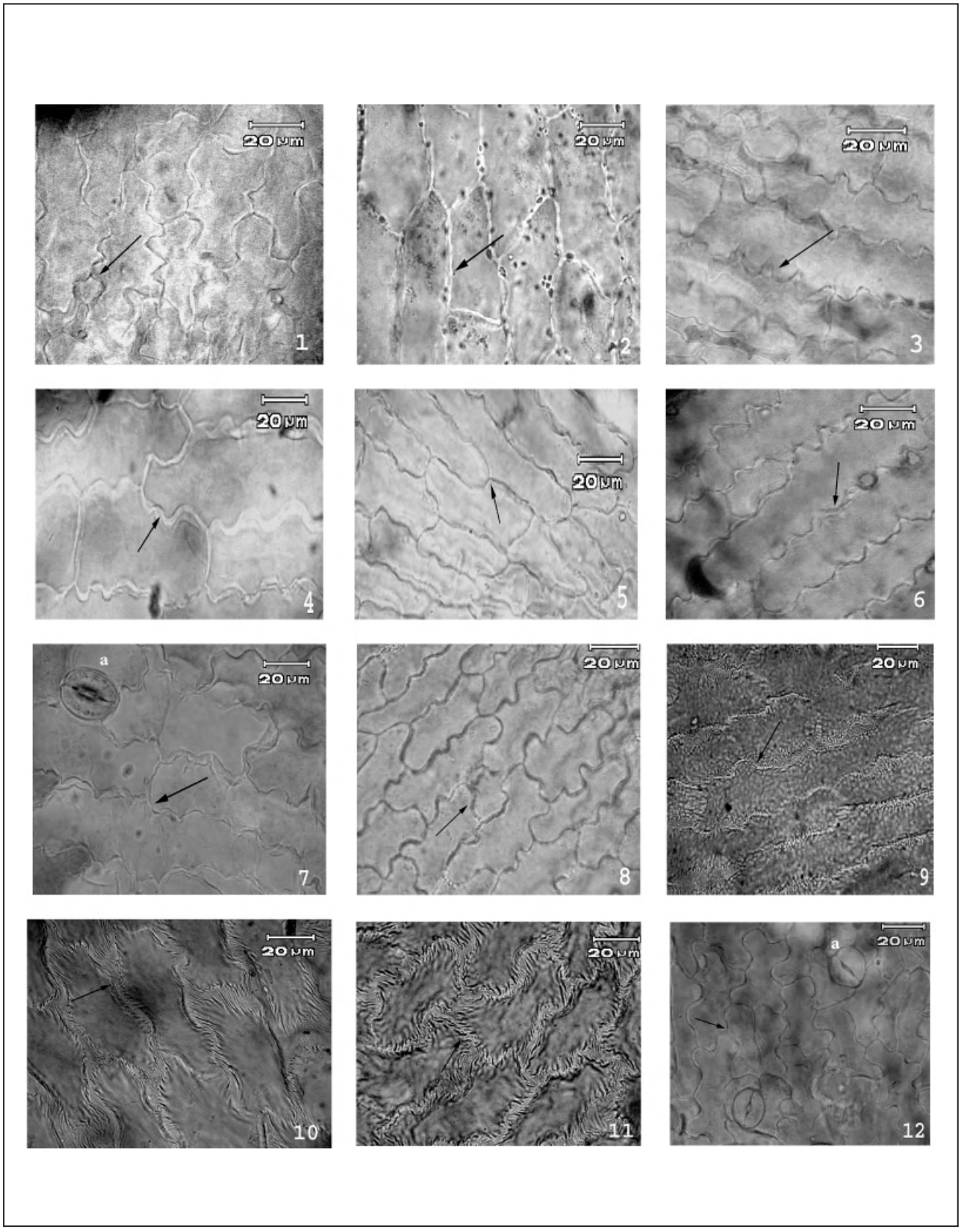
Polygoneae

Polygonum. In *Polygonum,* the fruit shape was triangular. In *P. alpestre,* the fruit had a shallowly striate surface pattern, while in *P. aviculare* the nut had tubercle (Figs 45-54).

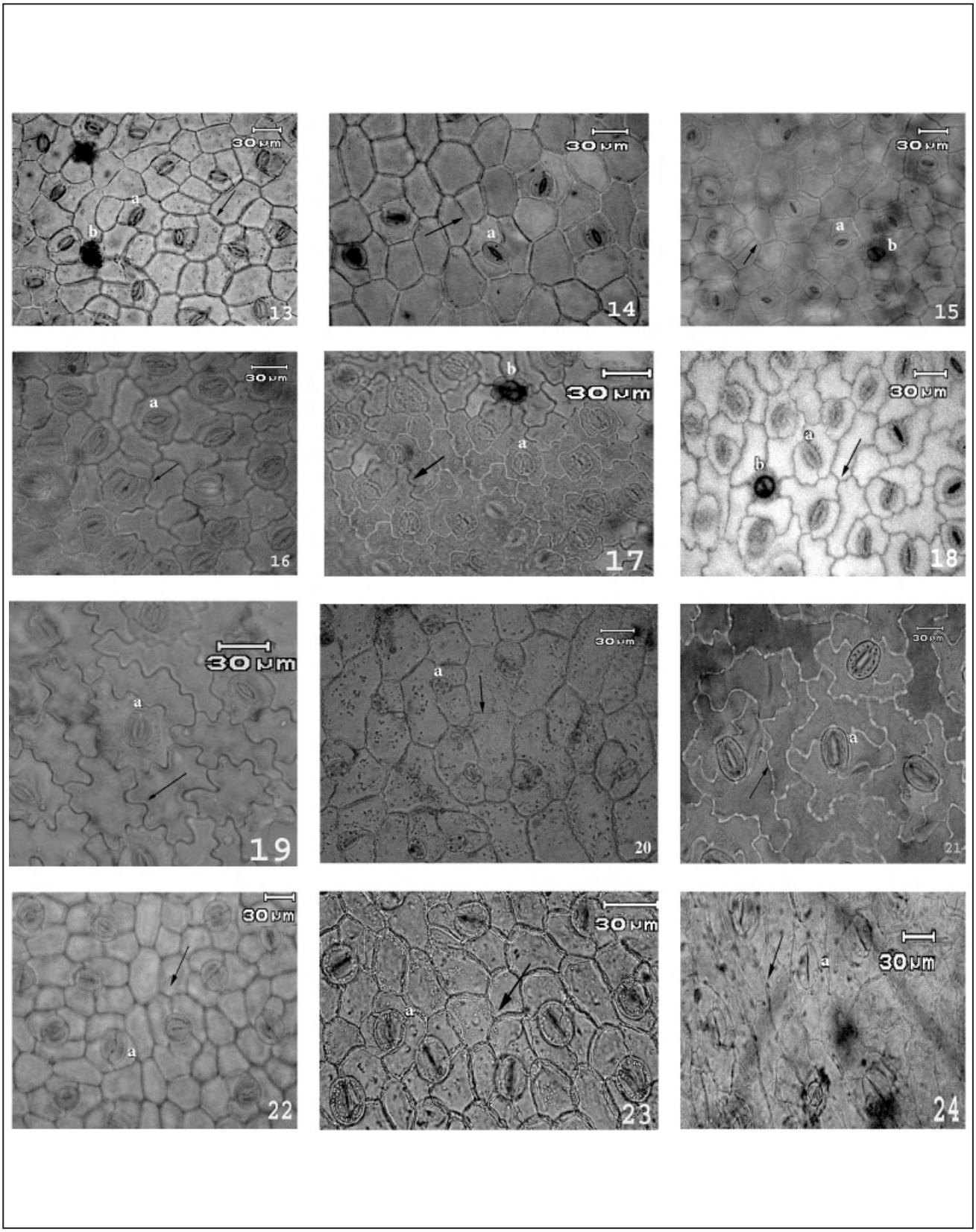
Pteropyrum. In this taxon, the achenes were surrounded by three wings. The fruit surface in this genus has shown wrinkled and striate patterns (Figs 55-62).

Rumiceae

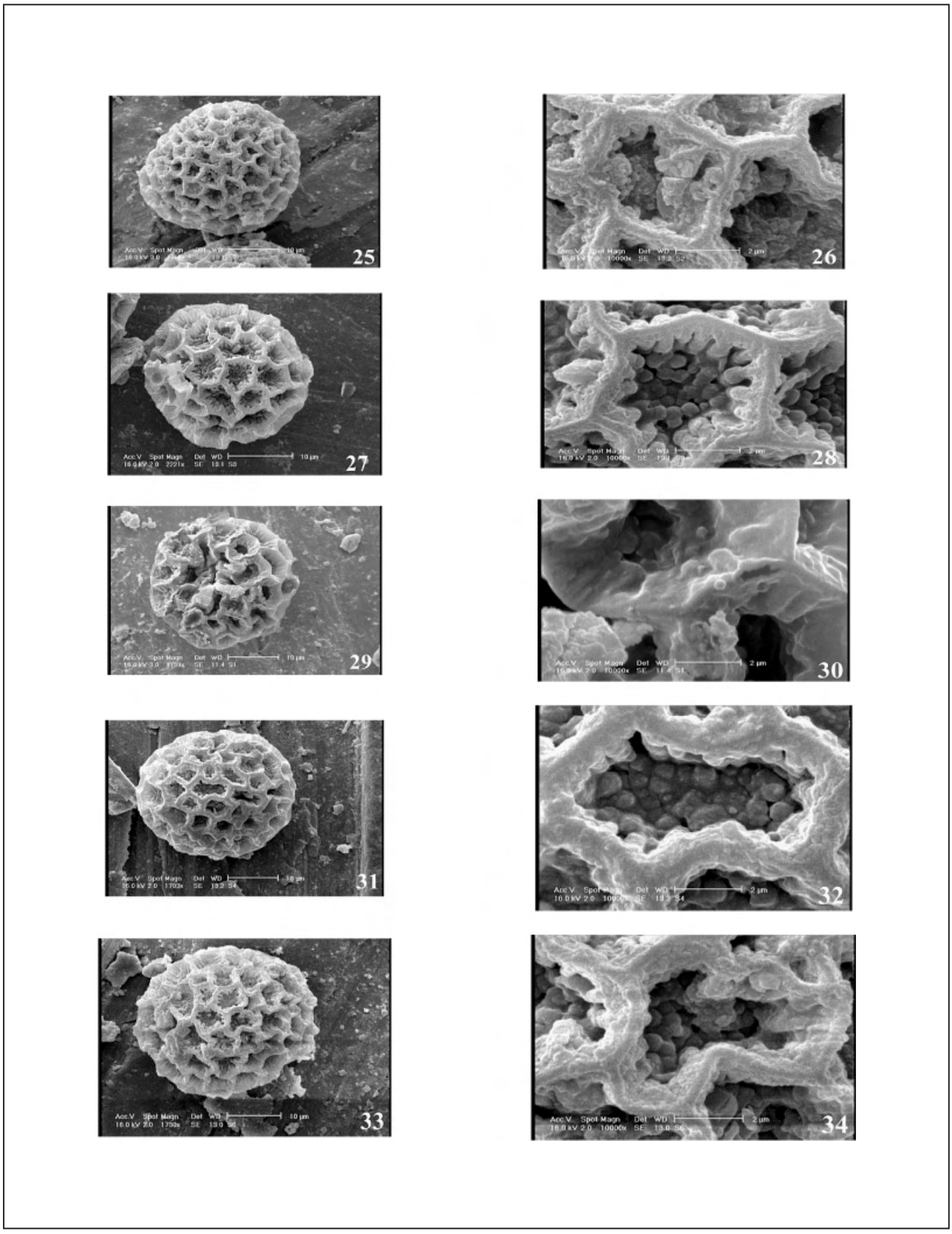
In *Rumex acetosa,* the nuts were small, with reticulate surface.



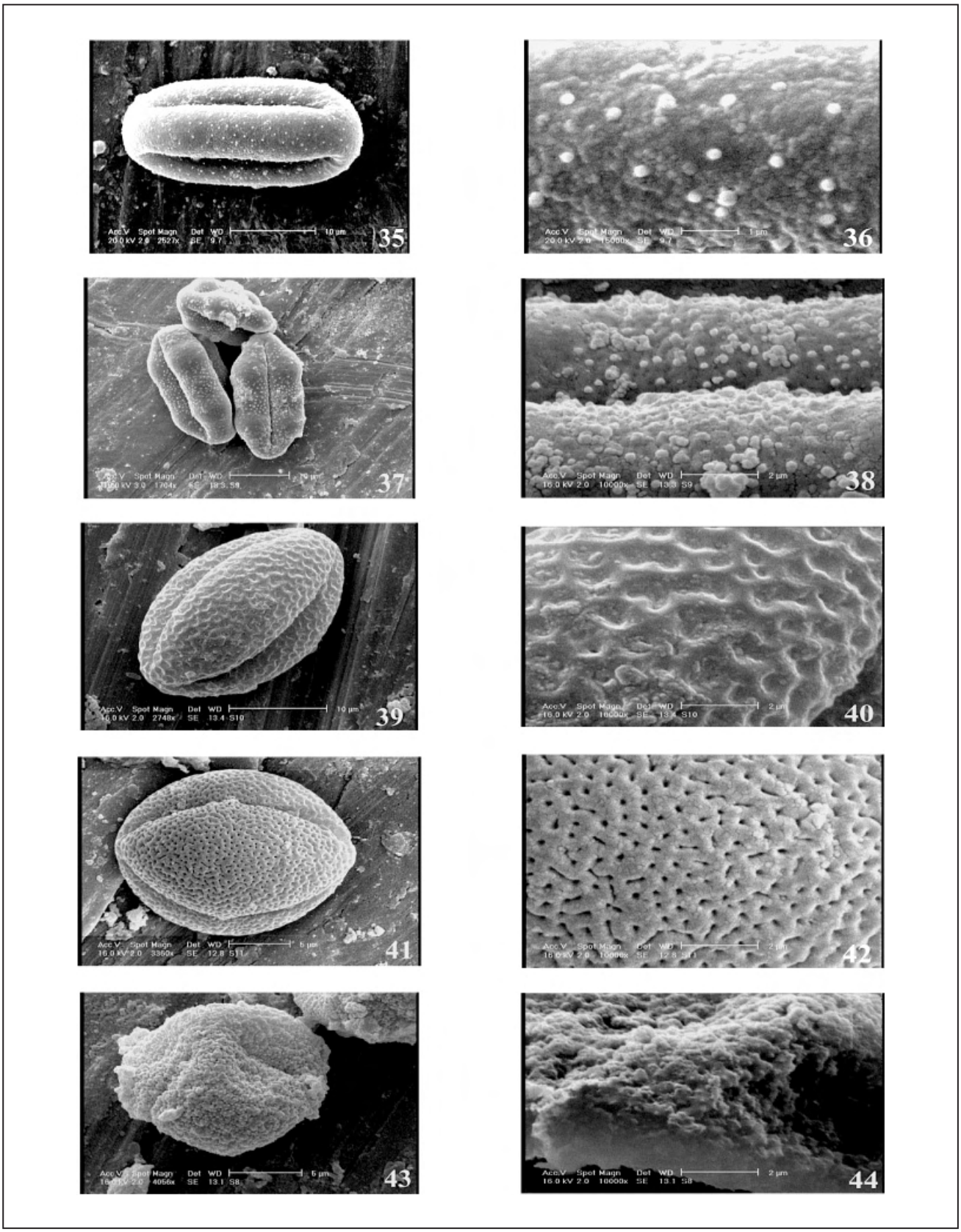
Figs 1-7. Tepal epidermis in *Persicarieae*. **1,** *Persicarieae lapathifolia* subsp. *brittingeri*, **2,** *P. lapathifolia* subsp. *nodosa*, **3,** *P. lapathifolia* subsp. *lapathifolia*, **4,** *P. maculosa*, **5,** *P. hydropiper*, **6,** *P. minor*, **7,** *P. mitis*. **Figs 8-12.** Tepal epidermis in *Polygoneae*. **8,** *Polygonum aviculare*, **9,** *P. alpestre*, **10,** *Pteropyrum aucheri*, **11,** *P. olivieri*. **Fig. 12.** *Rumex acetosa*. (**a** = stomata, arrows show the cell walls,.



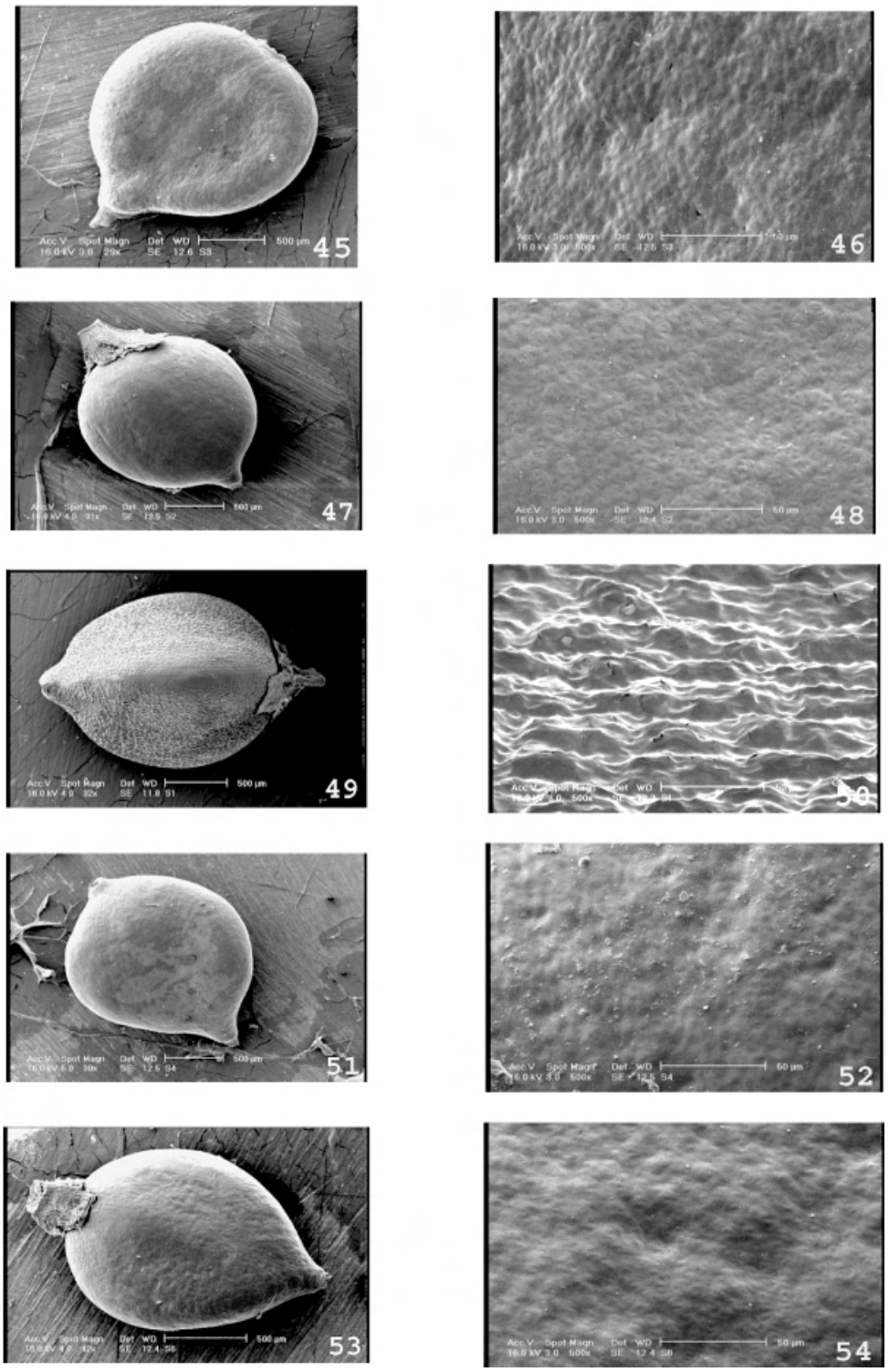
Figs 13-19. Leaf epidermis in *Persicarieae*. **13,** *Persicarieae lapathifolia* subsp. *brittingeri*, **14,** *P. lapathifolia* subsp. *nodosa*, **15,** *P. lapathifolia* subsp. *lapathifolia*, **16,** *P. maculosa*, **17,** *P. hydropiper*, **18,** *P. minor*, **19,** *P. mitis*. **Figs 20-23.** Leaf epidermis in *Polygoneae*. **20,** *Polygonum aviculare*, **21,** *P. alpestre*, **22,** *Pteropyrum aucheri*, **23,** *P. olivieri*. **Fig. 24.** *Rumex acetosa*. (**a** = stomata, **b** = hair cross-section, arrows show cell walls).



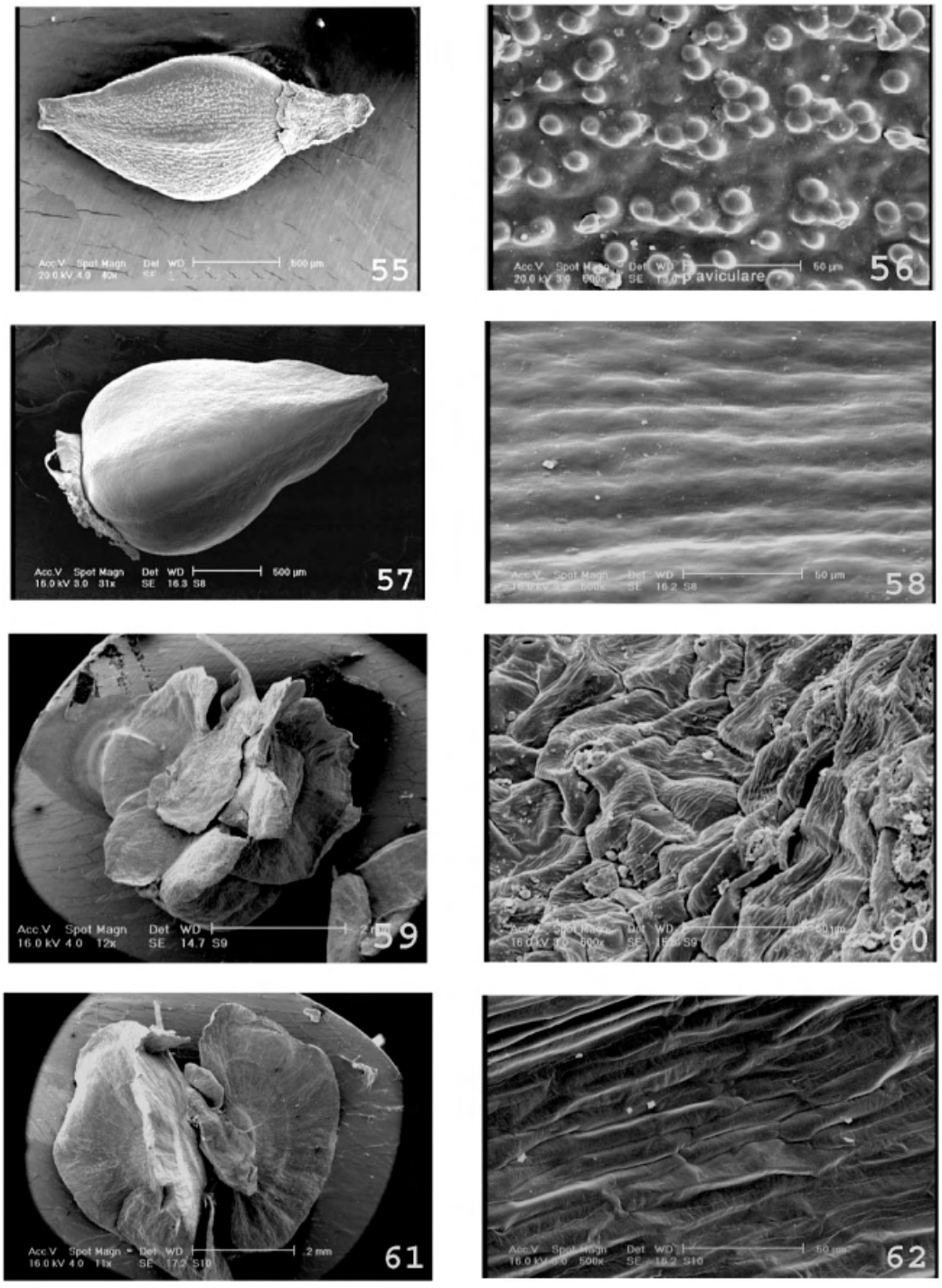
Figs 25-34. Pollen grains in *Persicarieae*. **25-26,** *P. lapathifolia*, **27-28,** *P. maculosa*, **29-30,** *P. hydropiper*, **31-32,** *P. minor*, **33-34,** *P. mitis*. (Left column scale bar equal to 10 μm shows pollen grains and right column scale bar equal to 2 μm shows pollen sculpture).



Figs 35-42. Pollen grains in *Polygoneae*. **35-36,** *Polygonum aviculare*, **37-38,** *P. alpestre*, **39-40,** *Pteropyrum aucheri*, **41-42,** *P. olivieri*.
Figs 43-44. Pollen grains in *Rumex acetosa* (Left column with scale bar equal 10 µm shows pollen grains and right column with scale bar equal 2 µm shows pollen sculpture).



Figs 45-54. Achenes in *Persicarieae*. **45-46,** *Persicarieae lapathifolia*, **47-48,** *P. maculosa*, **49-50,** *P. hydropiper*, **51-52,** *P. minor*, **53-54,** *P. mitis*. (Left column scale bar equal to 500 μm shows achenes and right column scale bar equal to 50 μm shows achenes sculpture).



Figs 55-62. Achenes in *Polygoneae*. **55-56,** *Polygoneae aviculare*, **57-58,** *P. alpestre*, **59-60,** *Pteropyrum aucheri*, **61-62,** *P. olivieri*. (In the left column, the scale bar equals 500 µm, except for Figs 59 & 61 where it equals 2 mm, and in the right column it equals 50 µm).

Discussion

A phenogram by the Ward's method has revealed two main clusters (Fig. 63). The first main cluster consists of elements of the Persicarieae tribe, while the second cluster consists of *Polygoneae* and *Rumiceae* elements. In the first cluster, *Persicaria minor* and *P. mitis* show some similarities in their morphological features, which correspond to the macro-morphological data (Mosaferi 2010). Three other species showed separate positions in the phenogram topology. They are morphologically separate too. In the second cluster there are two subsets, one of which consists of two *Pteropyrum* species and the other contains *Polygonum* species (*P. alpestre* and *P. aviculare*). These clusters show the close relationship of these taxa. The *Rumiceae* tribe has an interesting position in the phenogram topology. *Rumex acetosa* is nested between the *Pteropyrum* clusters. Thus, it could be concluded that these characters are more efficient in the differentiation of *Persicarieae* elements in Iran than in the two other tribes.

The factor analysis has shown that the three first factors are responsible for more than 76% of the observed variation. In the first factor, such features as the number of subsidiaries, length of equatorial and polar axis of the pollen grains, and the number of apertures are important (Table 3). In the second factor, the width and length of stomata and the length of tepal epidermal cell show high correlation (Table 3).

The results in tepal observations correspond to those of Ronse Decraene and Akeroyd (1988) and Hong & al. (1998). They recognize three main patterns of sculpturing and cell shape in their studies. The first pattern has rectangular to elongated cells, with straight or undulating anticlinal walls. This pattern was found in *Persicaria*. The second pattern, with irregular to elongated cells and mostly sinuate outline, was found in the genera *Polygonum* and *Pteropyrum*. The above-mentioned tepal cell types were found in the present study too.

With the help of nut studies by Ronse Decraene & al. (2000), the anatomical and morphological features of *Persicarieae* and *Polygoneae* tribes were discovered. They believe that the exocarp anatomical features are of taxonomic importance for delimitation of tribes and genera in *Polygonaceae*. Our results of the achene surface confirm this differentiation too. The observed variations in the leaf epidermal cells corresponded to the findings of Yasmin & al. (2010). They have found that variation in size and shape of the epidermal cells, stomata, glandular and non glandular trichomes are of taxonomic importance for the species delimitation in *Persicaria*. Owing to the various studied species, two different stomatal patterns are reported here for *Persicaria*.

Table 3. Factor analysis.

Feature	Component	
	1	2
Length of stomata	–	0.935
Width of stomata	–	0.893
Number of subsidiary cells	0.721	–
Type of stomata	0.721	–
Length of tepal epidermis	–	0.846
Length of equatorial axis	0.827	–
Length of polar axis	0.702	–
Number of aperture	0.958	–
Equatorial view	0.754	–
Polar view	0.773	–
Aperture type	0.899	–
Type of pollen sculpture	0.816	–
Type of nut sculpture	0.829	–

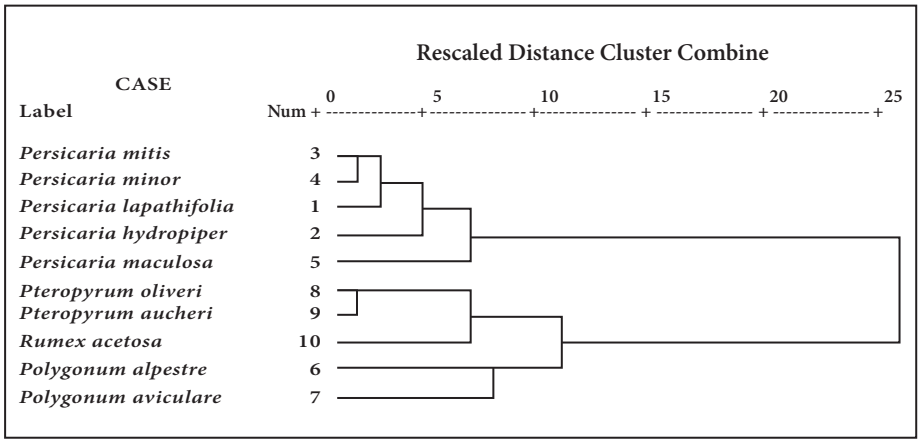


Fig. 63. Phenogram by the Ward's Method based on the micro-morphological characters in three tribes of *Polygonaceae*.

Pollen grain features are of taxonomic importance in *Polygonaceae* elements. Amiri & Sharifnia (2007) have found that the pollen grain features could provide valuable diagnostic traits for distinguishing *Polygonum* and *Persicaria* in Iran. Our results correspond to their findings. The results of this project are in support of distinguishing *Polygonum* s. str. and *Persicaria* as separate genera. The *Rumiceae* tribe is not clearly differentiated in the present study, so molecular methods are proposed in further studies for distinguishing this tribe from other tribes of the *Polygonaceae* family.

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