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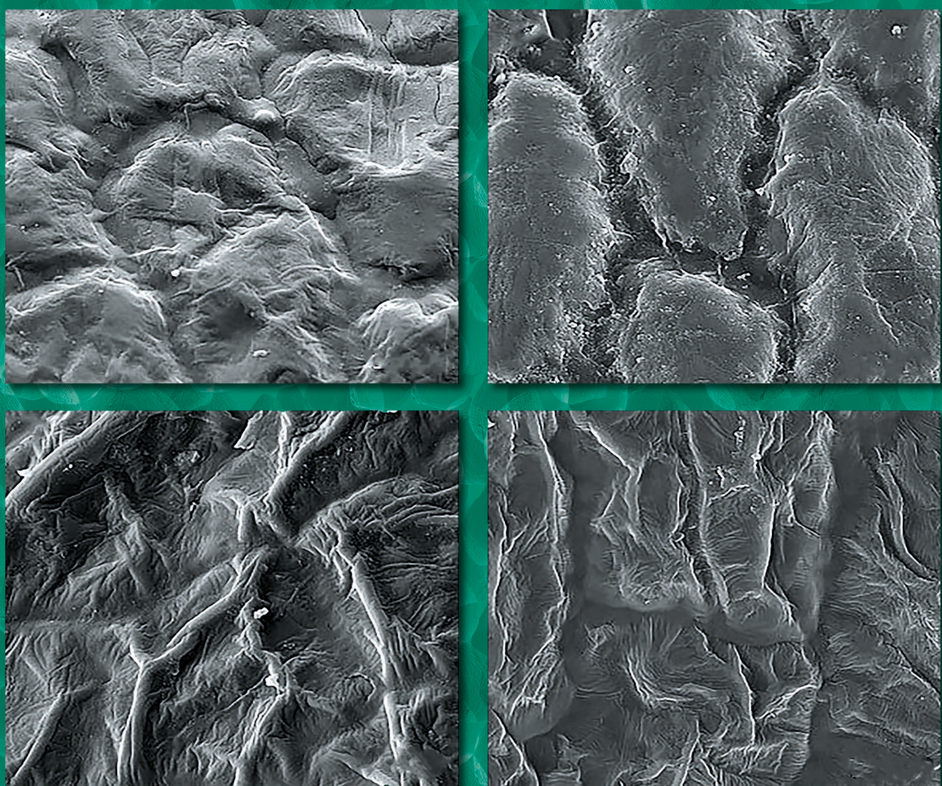

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Achene micromorphology of the genus *Alchemilla* L. (Rosaceae) in Iran

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ABSTRACT
In the current research, we examined achene micromorphological characteristics of 21 species of *Alchemilla* L. (Rosaceae) from Iran using a scanning electron microscope. Our findings revealed diversity in achene micromorphological features. According to the results, four main types of achene surface pattern (irregular reticulate elevations, ruminate lineate elevations, ruminate elevations and depressions, uneven and irregular fine elevations), three main types (reticulate, ruminate and colliculate) and nine subtypes (reticulate-falsifoveate, reticulate-foveate, reticulate-alveolate, ruminate reticulate-foveate-tuberculate, ruminate foaveat-tuberculate, ruminate reticulate-tuberculate-lineolate, colliculate with rugose, with fine folding and with papillae) of achene sculpturing were identified. We used multivariate analysis to determine species relationships. The results displayed that, achene width, base type, surface pattern, sculpturing, anticlinal cell wall, and outer periclinal layer types, epidermal cell wall type and shape, and hilum position are taxonomically informative and providing useful tools for species identification. The results of cluster and principal component analysis explained a close affinity among the studied species. However, this was not consistent with the current classification, except for four species (*Alchemilla sericata* Rchb., *A. rigida* Buser, *A. caucasica* Buser and *A. erythropoda* Juz.). An identification key based on diagnostic micromorphological characters of achene is presented. The obtained results from the present study slightly support the previous classifications.

KEY WORDS
Rosaceae
Alchemilla,
achene,
Scanning electron microscopy,
Principal component analysis,
identification key.

RÉSUMÉ

Micromorphologie des akènes du genre Alchemilla *L. (Rosaceae) en Iran.*

Dans cette recherche, nous examinons les caractéristiques micromorphologiques des akènes de 21 espèces d'*Alchemilla* L. (Rosaceae) d'Iran, en utilisant un microscope électronique à balayage. Nos résultats révèlent la diversité des caractéristiques micromorphologiques de l'akène. Selon les résultats, quatre types principaux de motifs de surface de l'akène (élévations réticulées irrégulières, élévations linéaires ruminées, élévations et dépressions ruminées, élévations fines inégales et irrégulières), trois types principaux (réticulé, ruminant et colliculé) et neuf sous-types (réticulé-falsifové, réticulé-falsifové, réticulé-alvéolé, réticulé-fové-tuberculé ruminé, foaveat-tuberculé ruminant, réticulé-tuberculé-linéolé, colliculé avec rugosité, avec plissement fin et avec papilles) de la sculpture des akènes ont été identifiés. Une analyse multivariée a été utilisée pour déterminer les relations entre les espèces. Les résultats montrent que la largeur de l'akène, le type de base, le motif de surface, la sculpture, les types de paroi cellulaire anticline et de couche péricline externe, le type et la forme de la paroi cellulaire épidermique et la position du hile sont des informations taxonomiques qui fournissent des outils utiles pour l'identification des espèces. Les résultats de l'analyse en clusters et de l'analyse en composantes principales expliquent une affinité étroite entre les espèces étudiées. Cependant, cela ne correspond pas à la classification actuelle, sauf pour quatre espèces (*Alchemilla sericata* Rchb., *A. rigida* Buser, *A. caucasica* Buser et *A. erythropoda* Juz.). Une clé d'identification basée sur les caractères diagnostiques micromorphologiques de l'akène est présentée. Les résultats obtenus par la présente étude confirment faiblement les classifications précédentes.

MOTS CLÉS
Rosaceae
Alchemilla,
akène,
microscopie électronique à balayage,
analyse en composantes principales,
clé d'identification.

INTRODUCTION

The genus *Alchemilla* L. (1753) comprises perennial herbaceous plants, with small apetalous flowers, round to heart-shaped leaves, including *c.* 1000 species (Fröhner 1995) and one of the most species-rich genera of the family Rosaceae. Also, with its frequent apomixis, polyploidization, and hybridization (Asker & Jerling 1992; Gehrke *et al.* 2008), *Alchemilla* represents a taxonomically difficult genus. Therefore, its circumscription has been a matter of controversy and demonstrating several changes (Linnaeus 1753; Juzepczuk 1941; Rothmaler 1944; Notova & Kusnetzova 2004). Based on Fröhner's taxonomic treatment (Fröhner 1986), Eurasian *Alchemilla* classify into four sections: *Alpinae* Buser ex Camus, *Pentaphylleae* Buser ex Camus, *Erectae* Fröhner, *Ultravulgares* Fröhner, and several intermediate species. *Alchemilla* is thought to be related to the subtribe *Sanguisorbinae* (Hutchinson 1964) because of its inconspicuous flowers. However, its relation to the tribe *Potentilleae* (Schulze-Menz 1964) was confirmed by DNA sequence data (Eriksson *et al.* 1998, 2003). This was supported by further morphological and molecular studies (Notova & Kusnetzova 2004; Gehrke *et al.* 2008; Soják 2008). Gehrke *et al.* (2008) molecular analysis revealed that *Alchemilla* s.l. is monophyletic and comprises four Eualchemilla-, Aphanes-, Lachemilla- and Afromilla-clades. The genus has 31 species in the area covered by Flora Iranica (Fröhner1969), 24 representatives (14 endemics to Iran) in the flora of Iran (Khatamsaz 1993), and to these Naqinezhad *et al.* (2017) added a new species (*Alchemilla mazandarana*) from the north. The most outstanding studies of the genus were based on morphological characters such as macro-micro morphological (Bradshaw 1963; Fröhner 1990; Kalheber 1994; Sepp & Paal 1998; Faghir *et al.* 2014a, b, 2017), palynological (Faghir *et al.* 2015) and anatomical evidence (Faghir *et al.* 2016).

The term achene was defined by Wagenitz (1976) as a one-seeded, dry, indehiscent fruit derived from an inferior ovary. Achenes contain a single seed that nearly fills the pericarp, but does not adhere to it. In *Alchemilla* the "seed" is an achene, a fruit containing the seed. The seed-like appearance is owed to the hardening of the fruit wall (pericarp), which encloses the solitary seed so closely as to seem like a seed coat. In immature achenes the fruit wall (pericarp) is soft, but it becomes hard during fruit maturation. This hardness in some families is due to the presence of a hard, black, resistant layer called Phytomelanin, which is found in the pericarp of plants. The identification of immature achenes is difficult. Usually achenes are ridged or wrinkled when mature and smooth when young. On the other hand, in young achene style may still persist (Pandey & Dehakal 2001; Kolodziejek 2010). Achene features have been used for traditional divisions of the family Rosaceae into subfamilies, however, these do not always correspond to the latest taxonomic descriptions of this family (Wagenitz 1976; Potter *et al.* 2007).

Many studies have shown that fruit or seed can very well serve in the identification and classification of species at various taxonomic levels (Cervantes & Martin-Gomez 2019). However, achene morphological data of *Alchemilla* are too scarce and poorly understood. The main aims of the present survey are to study detailed achene morphology of the Iranian species of *Alchemilla* using the scanning electron microscope for the first time, to identify diagnostic achene characters, to determine how helpful these characters are in explaining species relationships and the systematics of the genus *Alchemilla*.

TABLE 1. — Species of *Alchemilla* L. used in the current analysis.

Species	Location, Collector, Date	Accession Number
1. *A. pectiniloba* S.E.Fröhner	Iran, Guilan province, Deylama, Larikhani, 1530 m; *Saeidi*; 20.V.1993.	18837 (TUH)
2. *A. erythropoda* Juz.	Iran, Guilan province, Damash, Rudbar East, 1900 m; *Wendelbo* & *Annala*; 5.VI.1993	18232 (TARI)
3. *A. hessii* Rothm.	Iran, Mazandaran province, Kandovan, Ghahreman; *Augustine* & *Sheikholeslami*; 19.VI.1974	19418 (TUH)
4. *A. gigantodus* S.E.Fröhner	Iran, Mazandaran province, Kojur, Keikuh Mountain, 2000-2300 m; *Khatamsaz* & *Gholoizadeh*; 22.VI.1998.	57149 (TARI)
5. *A. caucasica* Buser	Iran, Guilan province, Asalem to Khalkhal road, 1900 m; *Faghir* & *Shokatyari*; 29.VI.2015	8344 (GUH)
6. *A. persica* Rothm.	Iran, Kurdistan province,Sanandaj, Narran village,1700 m; *Maroofi* & *Mansoori*; 13.VII.2016	8707 (GUH)
7. *A. rechingeri* Rothm.	Iran, Mazandaran province, Kojur, Firozabad village, 1700 m; *Ghahreman* & *Attar*; 19.VI.1997	20601 (TUH)
8. *A. sedelmeyeriana* Juz.	Iran, Mazandaran province, Firozabad Mountain, 2200 m; *Khatamsaz* & *Gholizade*; 22.VI.1998	57165 (TARI)
9. *A.melancholica* S.E.Fröhner	Iran, Guilan province, Espili, Larikhani, 1530 m; *Saeidi*; 3.V.1993	18841 (TUH)
10. *A. microscopica* S.E.Fröhner	Iran, Guilan province, Almas pass; *Aghai*, *Ahmadi*, *Faghir* & *Shahi*; 12.VII.2014	5275 (GUH)
11. *A. valdehirsuta* Buser	Iran, Guilan province, Almas pass; *Aghai*, *Ahmadi* & *Faghir*; 15.VII.2014	5359 (GUH)
12. *A. hyrcana* (Buser) Juz.	Iran, Guilan province, Asalem to Khalkhal road, 1800 m; *Faghir* & *Shokatyari*; 29.VI.2015	8347 (GUH)
13. *A. pseudocartalinica* Juz.	Iran, Mazandaran province, Kojur; Firozabad village; 1700 m; *Ghahreman* & *Attar*; 19.VI. 1997	20602 (TUH)
14. *A. citrina* S.E.Fröhner	Iran, Guilan province, Asalem to Khalkhal road, 2200 m; *Faghir* & *Shokatyari*; 29.VI.2015	8345 (GUH)
15. *A. kurdica* Rothm. ex Bornm.	Iran, Guilan province, Masal; Khashkhami; Chaichi, *Faghir* & *Shahi*; 10.VI.2012	4875 (GUH)
16. *A. farinosa* S.E.Fröhner	Iran, Guilan province, Khalkhal to Almas , 2400 m; *Faghir* & *Shokatyari*; 29.VI.2015	8349 (GUH)
17. *A. condensa* S.E.Fröhner	Iran, Guilan province, Asalem to Khalkhal road, 1800 m; *Faghir* & *Shokatyari*; 29.VI.2015	8346 (GUH)
18. *A. rigida* Buser	Iran, Guilan province, Asalem to Khalkhal road, 2200 m; *Faghir* & *Shokatyari*; 29.VI.2015	8351 (GUH)
19. *A. sericata* Rchb.	Iran, Guilan province, Asalem to Khalkhal road, 2400 m, *Faghir* & *Shokatyari*, 29.VI.2015	8348 (GUH)
20. *A. fluminea* S.E.Fröhner	Iran, Guilan province, Asalem to Khalkhal road, 2200 m, *Faghir* & *Shokatyari*; 29.VI.2015	8350 (GHE)
21. *A. plicatissima* S.E.Fröhner	Iran, Guilan province, Almas pass; Chaichi, *Faghir* & *Shahi*; 7.VIII.2012	4869 (GUH)

MATERIAL AND METHODS

PLANT MATERIAL

In the current study, achene micromorphological traits of 21 species of *Alchemilla* from Iran were studied by SEM. We used the healthy mature achenes of both freshly collected (from natural habitat during 2014-2016) and dried specimens. Species sampled are presented in Table 1. Achenes were washed, dried, and mounted on the stubs with double-sided cellophane tape and then coated in a sputter by 25 nm of gold-palladium at an accelerating voltage of 10-15 kV. The micrographs were prepared by scanning electron microscope Vega Tescan Razi instrument. The measurement provides were taken from 10-25 achenes for each species, using Photoshop (version 21.2.0.225) software. The achene morphological characters are presented in Table 2. The terminology used here follows Barthlott (1981), Bojnanský & Fargašová (2007), and Svetlana *et al.* (2009) with some modifications.

STATISTICAL DATA ANALYSIS

In this paper, two numerical analyses including cluster analysis and principal components analysis were carried out. Fruit character states used in the numerical analysis presented in Table 2. R software version 4.1.1 were employed for all analysis.

ABBREVIATIONS

Institutions

TUH Tehran university herbarium;
TARI Research Institute of Forests and Rangelands;
GUH Guilan university herbarium

Achene surface type (AST)

Un-Irr-Fi-El uneven and irregular fine elevation;
Irr. Ret. El irregular reticulate elevation;
Rum El. Dep ruminate elevation and depression;
Rum. Lin E ruminate lineate elevation.

Achene shape (AS)

O ovate;
OG ovate-globose;
TO tear-ovate;
BO broadly ovate;
LO long ovate.

Hilum position (HP)

SB subbasal;
B basal;
SBL subbasal-lateral.

Achene base type (ABT)

OV ovoid;
OB obtuse.

Apex type (AT)

AC acute;
ACC acute curved;
BA blunt accuminate.

Achene sculpturing type (AScut)

Alv alveolate;
Coll colliculate;
Fov foveate;
Fas falsifoveate;
Lin lineolate;
Pap papillate;
Rug rugose;
Rum ruminate;
Ret reticulate;
Tub tuberculate;
Rum Ret ruminate reticulate.

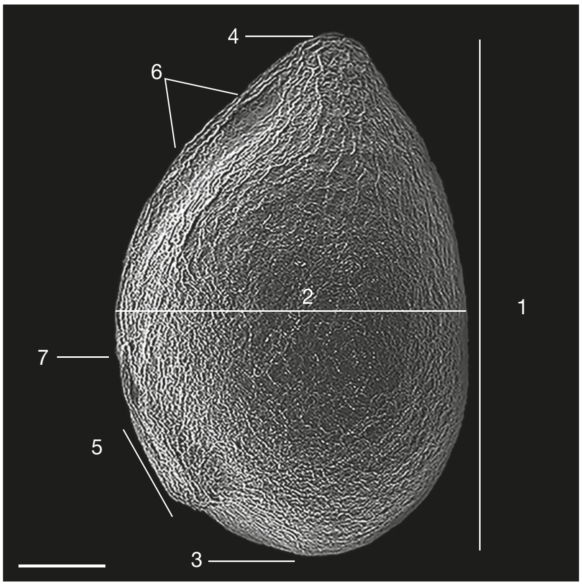

Fig. 1. — Achene of *Alchemilla* L.: *A. sericata* Rchb.: **1**, length; **2**, width; **3**, achene base; **4**, achene apex; **5**, hilum length; **6**, suture; **7**, style scare. Scale bar: 500 µm.

Folding (fold)
X Fold X-shape folding;
tri Fold triangular folding;
sli slightly.

Epidermal cell shape (ECS)
Po polygonal;
L-Po long polygonal;
Irr irregular;
R-Po rectangular-polygonal;
T-Po triangular-polygonal;
T-RH triangular-rhomboidal.

Anticlinal cell wall (ACW)
OB oblate;
Dep depressed;
Rai raised;
OPL outer periclinal layer.

Epidermal cell wall type (ECWT)
ST straight;
Un undulate;
HL hilum length;
AL achene length;
AW achene width.

Other abbreviations
SEM scanning electron microscope;
PCA principal components analyses;
CA cluster analysis;
UPGMA unweight pair-group method with arithmetic mean;
PC principal components;
Min minimum;
Max maximum;
SD standard deviation.

RESULTS

Achene micromorphological data of the studied species and their micrographs are presented in Table 2 and Figures 1-6. Achene in *Alchemilla*, is a bilateral, single-seeded dry indehiscent thin-walled fruit in which the seed coat is not part of the fruit coat, displaying a suture on the ventral side, hilum (its point of attachment to the funicle) (Bojnanský & Fargašová 2007), and style scar (Fig. 1).

Micromorphological traits

Achene shape

The shape of the achenes changed from ovate-globose (in *A. erythropoda, A. rigida,* and *A. farinosa*) to ovate (in *A. pectiniloba*, *A. hessii*, *A. caucasica, A. rechingeri, A. sedelmeyeriana, A. hyrcana, A. pseudocartalinica, A. citrina, A. sericata, A. fluminea, A.melancholica, A. plicatissima,* and *A. valdehirsuta*), broadly ovate (in *A. persica,* and *A. condensa*), long ovate (in *A. kurdica* and *A. microscopica*), and ovate-tear shape in *A. gigantodus*.

Achene size

The length of achene varied from a minimum of 1.53 mm (*A. rigida*) to a maximum of 2.5 mm (*A. kurdica*), and the width of achene changed from a minimum of 1.25 mm (*A. microscopica*) to a maximum of 1.71 mm (*A. erythropoda*).

Achene surface

Achene in all studied species demonstrated four types of ornamentation as followings:

Type I. Irregular reticulate elevations in *A. plicatissima* (Fig. 2A, B), *A. pseudocartalinica* (Fig. 2C, D), *A. pectiniloba* (Fig. 2E, F), *A. gigantodus* (Fig. 2G, H), *A. rechingeri* (Fig. 2I, J), *A. melancholica* (Fig. 2K, L), *A. farinosa* (Fig. 2M, N), and *A. hessii* (Fig. 2O, P).

Type II. Ruminate lineate elevations in *A. kurdica* (Fig. 2Q, R).

Type III. Ruminate elevations-depressions in *A. valdehirsuta* and *A. citrina* (Fig. 2S, T).

Type IV. Uneven and irregular fine elevations in *A. caucasica* (Fig. 3A, B), *A. persica* (Fig. 3C, D), *A. microscopica* (Fig. 3E, F), *A. fluminea* (Fig. 3G, H), *A. erythropoda* (Fig. 3I, J), *A. rigida* (Fig. 3K, L), *A. condensa* (Fig. 3M, N), *A. sedelmeyeriana* and *A. hyrcana* (Fig. 3O, P), and *A. sericata* (Fig. 6F).

Position and length hilum

The hilum position changed from basal in *A. persica* (Fig. 3C) to subbasal in *A. plicatissima, A. pectiniloba, A. gigantodus, A. rechingeri, A. farinosa, A. hessii, A. valdehirsuta,, A. caucasica, A. erythropoda, A. condenca, A. sedelmeyeriana* and *A. citrina* (Figs 2A, E, G, I, M, O; 3A, I, M, O) and subbasal-lateral in *A. sericata, A. pseudocartalinica, A. melancholica, A. kurdica, A. microscopica, A. fluminea, A. rigida,* and *A. hyrcana* (Figs 1B, C, K; 3E, G, K). The length of hilum varied from a minimum of 0.11mm in *A. persica* to a maximum of 0.24 mm in *A. pectiniloba*.

TABLE 2. — Achene morphological characters of the Iranian species of *Alchemilla* L. Abbreviations: see Material and méthods.

Species	AS	HP	ABT	AT	AST	AScuT	ECS
1. *A. pectiniloba*	O	SB	OV	BA	Irr. Ret. El	Ret-Fov	R-Po
2. *A. erythropoda*	OG	SB	OB	BA	Un Irr. Fi. El	Colli+ fine triFold	Po
3. *A. hessii*	O	SB	OV	BA	Irr. Ret. El	Colli+ fine triFold	Po
4. *A. gigantodus*	TO	SB	OB	AC	Irr. Ret. El	Ret-Fov	Po
5. *A. caucasica*	O	SB	OV	BA	Un Irr. Fi. El	Colli+ fine tri Fold	Po-Irr
6. *A. persica*	BO	B	OV	AC	Un Irr. Fi. El	Colli fine Fold	L-Po-Irr
7. *A. rechingeri*	O	SB	OV	BA	Irr. Ret. El	Ret-Alv	R-Po
8. *A. sedelmeyeriana*	O	SB	OB	AC	Un Irr. Fi. El	Colli+ Rug	L-Po-Irr
9. *A. melancholica*	O	SBL	OV	ACC	Irr. Ret. El	Rum Ret-Fov-sli Tub	Po-Irr
10. *A. microscopica*	LO	SBL	OV	AC	Un Irr. Fi. El	Colli+X Fold	T-Rh-Irr
11. *A. valdehirsuta*	O	SB	OB	ACC	Rum El. Dep	Rum -Fov- sliTub	R-Po-Irr
12. *A. hyrcana*	O	SBL	OV	ACC	Un Irr. Fi. El	Colli+ Rug	L-po-Irr
13. *A. pseudocartalinica*	O	SBL	OV	AC	Irr. Ret. El	Ret-Fov	R-Po
14. *A. citrina*	O	SB	OV	BA	Rum El. Dep	Rum -Fov-sliTub	po
15. *A. kurdica*	LO	SBL	OV	AC	Rum. Lin EL	Rum Ret –sliTub-Lin	Po
16. *A. farinosa*	OG	SB	OB	ACC	Irr. Ret. El	Ret-Fas	Po
17. *A. condensa*	BO	SB	OB	AC	Un Irr. Fi. El	Colli+ Rug	Po-Irr
18. *A. rigida*	OG	SBL	OV	BA	Un Irr. Fi. El	Colli fine Fold	Po-Irr
19. *A. sericata*	O	SBL	OV	BA	Un Irr. Fi. El	Colli+Pap+ Fold	Po-Irr
20. *A. fluminea*	O	SBL	OV	BA	Un Irr. Fi. El	Colli+ triFold	Po
21. *A. plicatissima*	O	SB	OB	ACC	Irr. Ret. El	Rum Ret-Fov-Tub	R-Po

Species	ACW	OPL	ECWT	HL Min-Max (mm±SD)	AL (mm) Min-Max (mm±SD)	AW (mm) Min-Max (mm±SD)
1. *A. pectiniloba*	Rai	Dep-Rai	ST	0.21-0.25 (0.24 ± 24.53)	1.8-2.2 (1.96 ± 0.208)	1.4-1.6 (1.4200 ± 0.085)
2. *A. erythropoda*	Dep	Rai	ST	0.15-0.17 (0.16 ± 9.75)	2.1-2.4 (2.23 ± 0.152)	1.6-1.8 (1.7120 ± 0.025)
3. *A. hessii*	Dep	Rai	ST	0.13-0.15 (0.14 ± 10.45)	2.2-2.5 (2.36 ± 0.152)	1.6-1.7 (1.6370 ± .0251)
4. *A. gigantodus*	Rai	Dep	ST	0.17-0.18 (0.18 ± 10.1)	2-2.2 (2 ± 0.2)	1.5-1.6 (1.5000 ± 0.081)
5. *A. caucasica*	Dep	Rai	UN	0.13-0.18 (0.16 ± 26.77)	2-2.3 (2.16 ± 0.153)	1.6-1.75 (1.56 ± 0.0057)
6. *A. persica*	Dep	Rai	ST-sliUN	0.09-0.12 (0.11 ± 19.91)	1.9-2.2 (2.03 ± 0.142)	1.6- 1.7 (1.60 ± 0.043)
7. *A. rechingeri*	Rai	Dep	ST	0.09-0.18 (0.12 ± 54.96)	2.2-2.5 (2.33 ± 0.125)	1.5-1.7 (1.600 ± 0.081)
8. *A. sedelmeyeriana*	Dep	Rai	UN	0.15-0.19 (0.17 ± 19.9)	1.9-2 (1.96 ± 0.057)	1.5-1.6 (1.500 ± 0.081)
9. *A. melancholica*	Rai-Ob	Dep-Rai	ST	0.17-0.18 (0.17 ± 17.5)	1.9-2 (2.025 ± 0.1)	1.4-1.5 (1.4250 ± 0.050)
10. *A. microscopica*	Dep	Rai	ST	0.18-0.2 (0.19 ± 14.95)	1.8-2 (1.93 ± 0.115)	1.2-1.4 (1.25 ± 0.0577)
11. *A. valdehirsuta*	Rai-Ob	Dep	ST	0.09-0.12 (0.11 ± 20.38)	2.7-2.9 (2.76 ± 0.305)	1.4-1.5 (1.4500 ± 0.057)
12. *A. hyrcana*	Dep	Rai	ST	0.17-0.18 (0.16 ± 13.1)	1.8-2.4 (2.13 ± 0.156)	1.5-1.7 (1.6100 ± 0.040)
13. *A. pseudocartalinica*	Rai	Dep	ST	0.15-0.17 (0.16 ± 9.75)	1.9-2.2 (2.03 ± 0.1731)	1.6-1.7 (1.600 ± 0.0408)
14. *A. citrina*	Rai-Ob	Dep-Rai	ST	0.09-0.17 (0.14 ± 41.03)	2-2.4 (2.2 ± 0.2)	1.5-1.7 (1.500 ± 0.0547)
15. *A. kurdica*	Rai-Ob	Dep-Rai	ST	0.17-0.2 (0.19 ± 55.18)	2.3-2.7 (2. 5 ± 0.290)	1.5-1.6 (1.45 ± 0.07)
16. *A. farinosa*	Rai	Dep	ST	0.18-0.21 (0.19 ± 17.95)	2.1-2.4 (2.23 ± 0.1)	1.6-1.7 (1.700 ± 0.0100)
17. *A. condensa*	Dep	Rai	UN	0.15-0.17 (0.16 ± 9.75)	1.8-2.2 (1.96 ± 0.15)	1.3-1.5 (1.4700 ± 0.069)
18. *A. rigida*	Dep	Rai	UN	0.15-0.18 (0.17 ± 17.25)	1.4-1.7 (1.53 ± 0.145)	1.1-1.3 (1.2700 ± 0.057)
19. *A. sericata*	Dep	Rai	UN	0.15-0.17 (0.16 ± 9.76)	1.8-2.2 (2.1 ± .0570)	1.5-1.7 (1.600 ± 0.0400)
20. *A. fluminea*	Dep	Rai	ST	0.12-0.15 (0.14 ± 14.89)	1.7-2.2 (1.93 ± .250)	1.4-1.5 (1.4200 ± 0.057)
21. *A. plicatissima*	Rai-Ob	Dep-Rai	ST	0.15-0.17 (0.16 ± 9.75)	2.1-2.4 (2.26 ± 0.081)	1.5-1.7 (1.580 ± 0.008)

Achene base

Two types of achene bases were identified, including:

Type I: Achene with ovoid base, observed in *A. sericata, A. pseudocartalinica, A. pectiniloba, A. rechingeri, A. melancholica, A. hessii, A. kurdica, A. caucasica, A. persica, A. microscopica, A. fluminea, A. rigida, A. hyrcana* and *A. citrina* (Figs 1C, E, I, K, O, Q; 3A, C, E, G, K).

Type II: Achene with obtuse base, recognized in *A. plicatissima, A. gigantodus, A. farinosa, A. valdehirsuta*, and *A. erythropoda. A. condensa,* and *A. sedelmeyeriana* (Figs 2A, G, M; 3I, M, O).

Achene apex

The apex of achene showed variation among the studied species. Three types recorded:

Type I: Achene with acute apex type identified in *A. pseudocartalinica*, *A. gigantodus*, *A. kurdica*, *A. persica, A. microscopica, A. condensa,* and *A. sedelmeyeriana* (Figs 2C, G, Q; 3C, E, M, O).

Type II: Achene with acute and curved apex recorded in *A. plicatissima, A. melancholica, A. farinosa, A. valdehirsuta,* and *A. hyrcana* (Fig. 2A, K, M, S).

Type III: Achene with blunt acuminate apex identified in *A.sericata*, *A. pectiniloba, A. rechingeri*, *A. hessii, A. caucasica, A. fluminea, A. erythropoda, A. rigida,* and *A. citrina* (Figs 1B, E, I, O; 3A, G, I, K).

The epidermal cell shape

The epidermal cell shape of the studied taxa varied from polygonal (in seven species: *A. erythropoda, A. hessii, A. gigantodus, A. citrina, A. kurdica, A. farinosa,* and *A. fluminea*) to polygonal-irregular (in six species: *A. caucasica, A. melancholica, A. condensa, A. rigida, A. sericata*), long

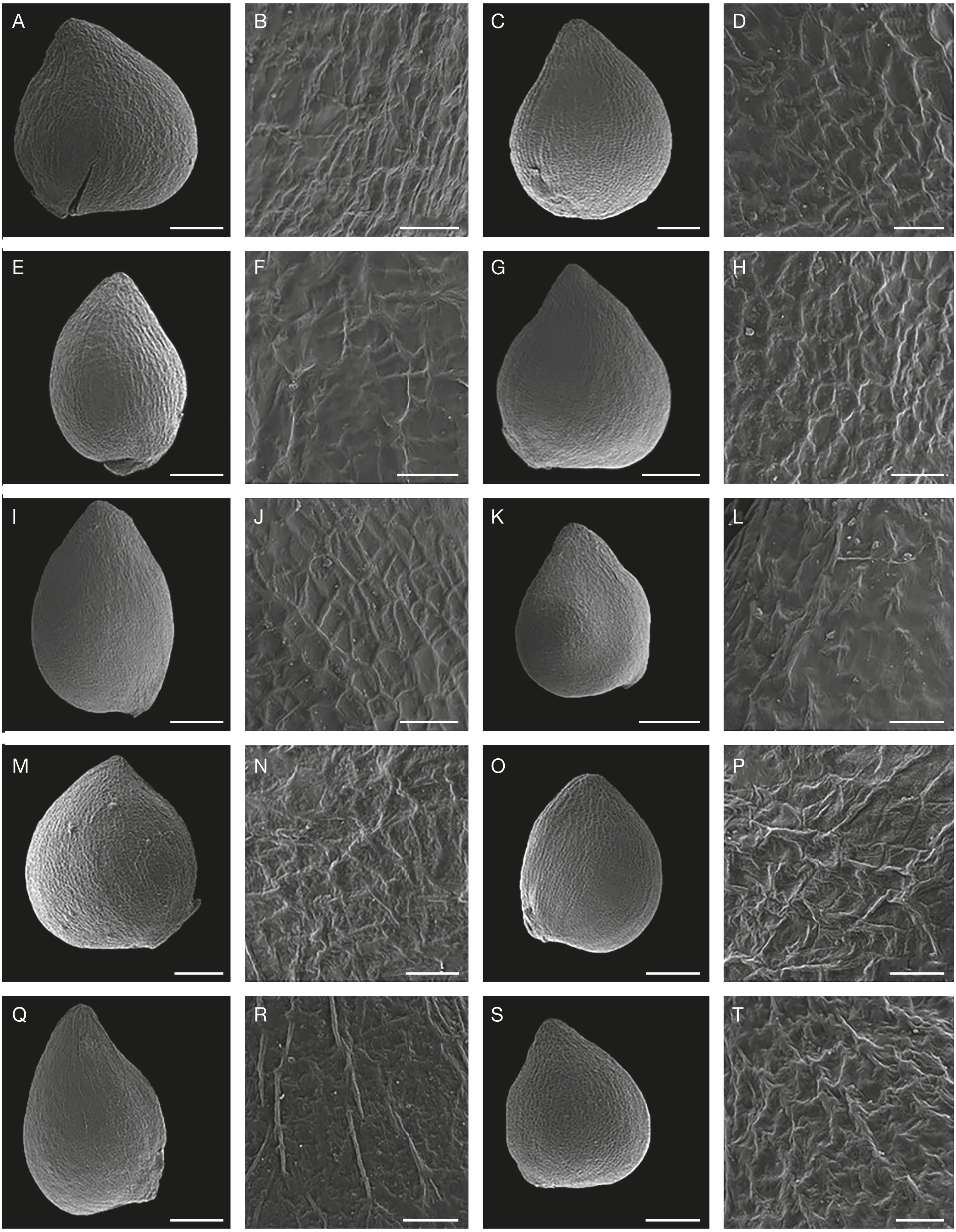

Fig. 2. — SEM micrographs showing the achene surface types of the *Alchemilla* L. species. Irregular Reticulate Elevation: **A**, **B**, *A. plicatissima* S.E.Fröhner; **C**, **D**, *A. pseudocartalinica* Juz.; **E**, **F**, *A. pectiniloba* S.E.Fröhner; **G**, **H**, *A. gigantodus* S.E.Fröhner; **I**, **J**, *A. rechingeri* Rothm.; **K**, **L**, *A. melancholica* S.E.Fröhner; **M**, **N**, *A. farinosa* S.E.Fröhner; **O**, **P**, *A. hessii* Rothm. **Q**, **R**, Ruminate Lineate Elevation, *A. kurdica* Rothm. ex Bornm.; **S**, **T**, Ruminate Elevation and Depression, *A. valdehirsuta* Buser. Scale bars: A, C, E, G, I, K, M, O, Q, S, 500 µm; B, D, F, H, J, L, N, P, R, S, 50 µm.

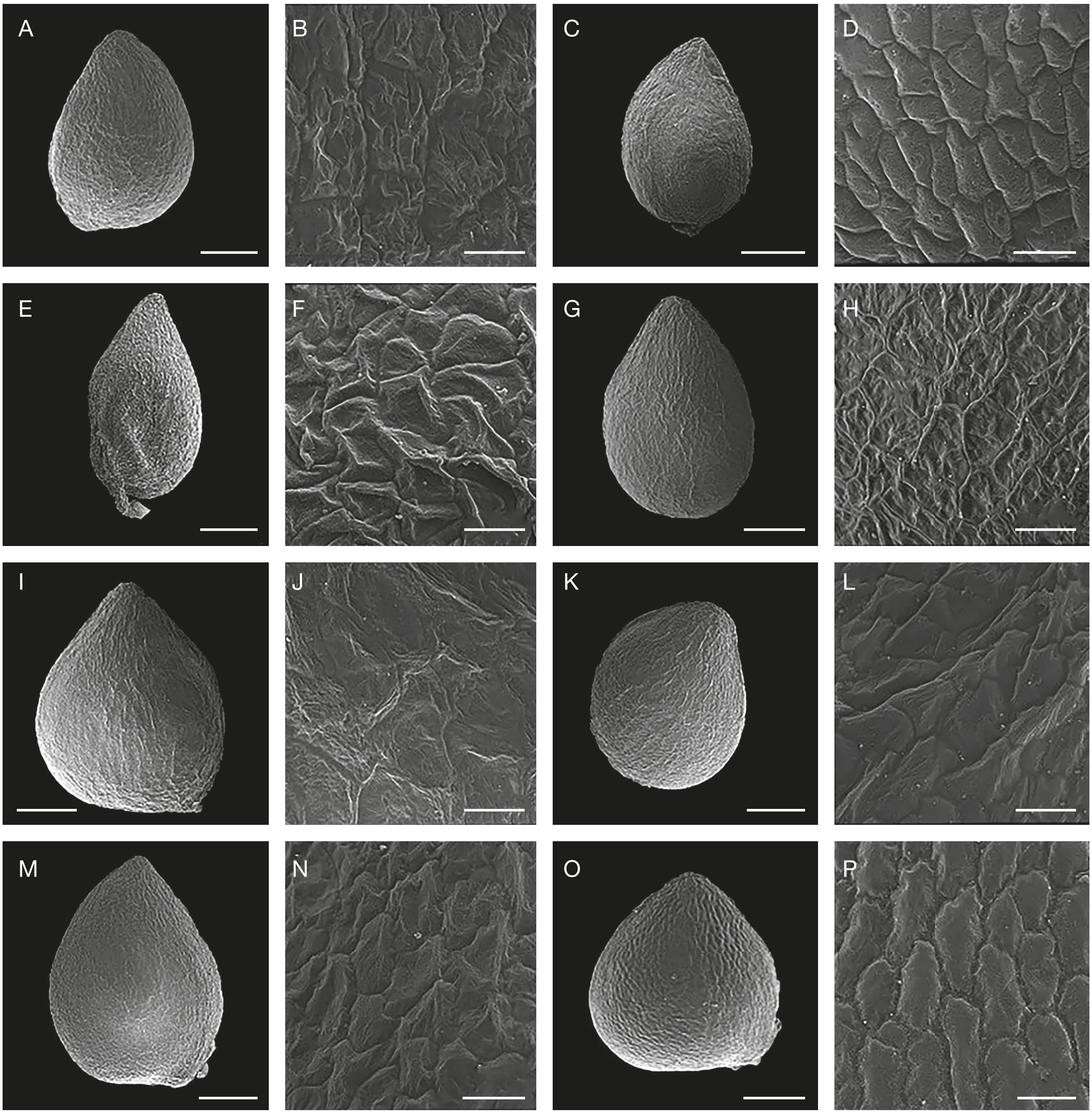

Fig. 3. — SEM micrographs showing the achene surface types of the *Alchemilla* L. species. Uneven and Irregular Fine Elevations: **A**, **B**, *A. caucasica* Buser; **C**, **D**, *A. persica* Rothm.; **E**, **F**, *A. microscopica* S.E.Fröhner; **G**, **H**, *A. fluminea* S.E.Fröhner; **I**, **J**, *A. erythropoda* Juz.; **K**, **L**, *A. rigida* Buser; **M**, **N**, *A. condenca* S.E.Fröhner; **O**, **P**, *A. sedelmeyeriana* Juz. Scale bars: A, C, E, G, I, K, M, O, 500 µm; B, D, F, H, J, L, N, P, 50 µm.

polygonal-irregular (in three species: *A. persica, A. sedelmeyeriana,* and *A. hyrcana*), rectangular-polygonal (in 4 species: *A. pectiniloba, A. rechingeri, A. pseudocartalinica,* and *A. plicatissima*), rectangular-polygonal-irregular in *A. valdehirsuta* and triangular-rhomboidal-irregular in *A. microscopica.*

Anticlinal cell walls

Three types of anticlinal cell boundaries were identified:

Raised (Type I) in *A. pectiniloba, A. gigantodus, A. rechingeri, A. pseudocartalinica,* and *A. farinosa*;

Raised-oblate (Type II) in *A. melancholica, A. valdehirsuta, A. citrina, A. kurdica,* and *A. plicatissima*;

and Depressed (Type III) in twelve representatives, including *A. erythropoda, A. hessii, A. caucasica, A. persica, A. sedelmeyeriana, A. microscopica, A. hyrcana, A. condensa A. rigida, A. sericata, and A. fluminea.* Also, we found straight anticlinal cell walls in *A. pectiniloba, A. gigantodus, A.rechingeri, A. pseudocartalinica, A. farinosa, A. erythropoda, A. hessii, A. microscopica, A. hyrcana, A. melancholica, A. valdehirsuta, A. citrina, A. kurdica,* and *A. plicatissima*; undulate in *A. caucasica, A. sedelmeyeriana, A. condensa A. rigida,* and *A. sericata,* and straight-undulate in *A. persica.*

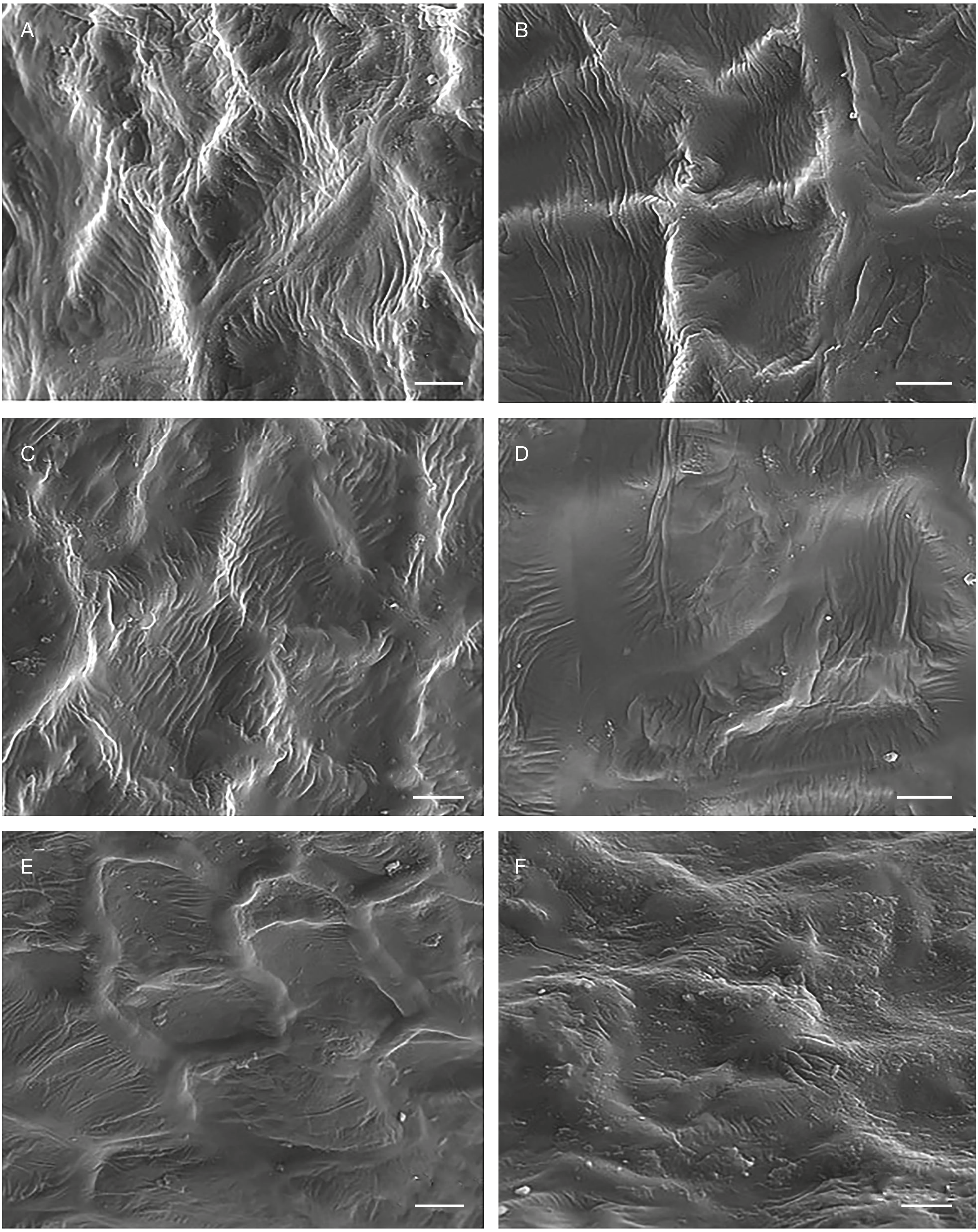

Fig. 4. — SEM Micrographs showing the achene sculpturing types of the *Alchemilla* L. species: Reticulate-Falsi Foveate: **A**, *A. farinosa* S.E.Fröhner. Reticulate Foveate: **B**, *A. pectiniloba* S.E.Fröhner; **C**, *A. gigantodus* S.E.Fröhner; **D**, *A. pseudocartalinica* Juz.. Reticulate Alveolate: **E**, *A. rechingeri* Rothm.. Ruminate Reticulate- Foveate-Tuberculate: **F**, *A. melancholica* S.E.Fröhner. Scale bars: A, B, F, 10 µm; C, D, E, 20 µm.

Periclinal cell wall

Three types of periclinal layers were recorded in the studied species. They were raised (Type I) in twelve species (*A. erythropoda, A. hessii, A. caucasica, A. persica, A. sedelmeyeriana, A. microscopica, A. hyrcana, A. condensa A. rigida, A. sericata,* and *A. fluminea*), depressed-raised (Type II) in five species

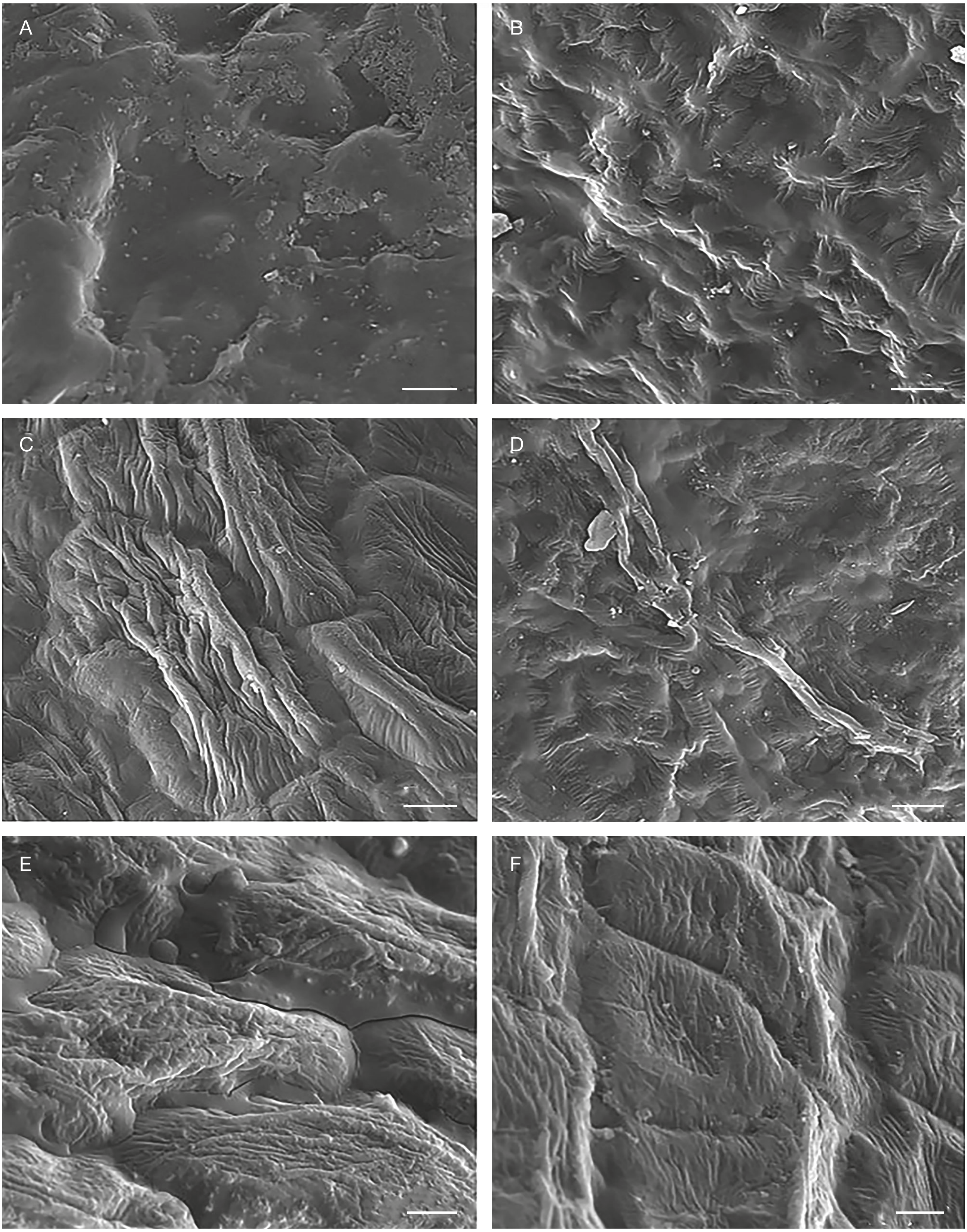

FIG. 5. — SEM Micrographs showing the achene sculpturing types of the *Alchemilla* L. species. Reticulate- Foveate-Tuberculate: **A**, *A. plicatissima* S.E.Fröhner. Ruminate-Foveate-Tuberculate: **B**, *A. citrina* S.E.Fröhner; **C**, *A. valdehirsuta* Buser. Ruminate Reticulate-Tuberculate: **D**, *A. kurdica* Rothm. ex Bornm. Colliculate without folding: **E**, *A. rigida* Buser; **F**, *A. condensa* S.E.Fröhner. Scale bars: 10 µm.

Table 3. — Eigenvalues, variance percent, and cumulative percentage of variation explained by the first three principal components on 13 morphological traits in *Alchemilla* L. species (characters with high coefficients (**bold fonts**) in the PC axes should be considered more important).

	PC1	**PC2**	**PC3**
Eigen values	4.77	1.9	1.82
Variance percent	36.73	14.67	14.07
Cumulative variance	36.73	51.41	65.48
Traits		Eigen vectors	
ABT	–0.16	0.48	**0.71**
AS	–0.25	0.27	0.53
ASUT	**0.9**	–0.129	0.04
ECWT	**0.68**	0.3	0.2
ACW	**0.91**	–0.16	0.16
OPL	**–0.832**	0.14	–0.348
ECS	**0.744**	0.351	–0.3
AST	**0.9**	–0.01	0.08
AL	–0.44	**–0.63**	0.08
AW	0.13	**–0.7**	0.22
HL	–0.5	0.37	–0.07
HP	0.29	–0.07	**–0.74**
AT	0.06	0.38	–0.3

(*A. pectiniloba, A. melancholica*, *A. citrina*, *A. kurdica,* and *A. plicatissima*) and depressed (Type III) in *A. gigantodus, A. rechingeri, A. valdehirsuta, A. pseudocartalinica,* and *A. farinosa*.

Achene sculpture

The achene sculpture included three main types and nine subtypes as followings:

Type I. Reticulate. Having a raised network of narrow and sharply angled lines frequently presenting a geometric appearance, each area or depression outlined by the reticulum being an interspace. This type includes three subtypes:

Type I subtype I: Reticulate-falsifoveate, it is an intermediate between reticulate and having pits that do not have the same depth throughout, as a little depression made laterally, in *A. farinosa* (Fig. 4A).

Type I subtype II: Reticulate-foveate, an intermediate between reticulate and pitted, in *A. pectiniloba* (Fig. 4B) and *A. gigantodus* (Fig. 4C), and *A. pseudocartalinica* (Fig. 4D).

Type I subtype III: Reticulate-alveolate, it is reticulate with depressions (lumina) and walls (muri) giving the appearance of a honey-comb or windows; lumina and muri composed of transparent parenchymous tissue; honey-combed, the elevation not rounded off, the depression or area outlined by the elevation in *A. rechingeri* (Fig. 4E).

Type II. Ruminate. This type is very uneven and looking as if chewed. This type includes three subtypes:

Type II subtype I Ruminate reticulate-foveate-tuberculate, an intermediate between Ruminate reticulate and foveate with tubercles, in *A. melancholica* (Fig. 4F), *A. plicatissima* (Fig. 5A).

Type II subtype II Ruminate foaveat-tuberculate, an intermediate between Ruminate foveate and tuberculate, in *A. citrina* and *A. valdehirsuta* (Fig. 5B, C).

Type II subtype III Ruminate reticulate-tuberculate-lineolate, an intermediate type between Ruminate reticulate tuberculate and marked with fine broken lines in *A. kurdica* (Fig. 5D).

Type III. Colliculate. It comprises rounded broad elevations closely spaced covering the seed coat. This type includes three subtypes:

Type III subtype I Colliculate without folding in *A. persica* and *A. rigida* (Fig. 5E), *A. condensa* (Fig. 5F), and *A. hyrcana* (Fig. 6F), *A. sedelmeyeriana* (Fig. 6B).

Type III subtype II Colliculate with folding in *A. erythropoda, A. hessii, A. fluminea* (Fig. 6C), *A. caucasica* (Fig. 6D) and "X" form folding in *A. microscopica* (Fig. 6E).

Type III subtype III Colliculate having folding and papillae in *A. sericata* (Fig. 6F).

Cluster analysis (CA)

On the UPGMA dendrogram, two main clusters (A and B) were recognized by a Euclidean distance ranged between 0 and 5.38 (Fig. 7). Cluster A comprises two subclusters A1 and A2. Subcluster A1 contained eight representatives and divided into two further small subclusters, A1a contained *A. fluminea*, *A. rigida, A. sericata,* and A1b included *A. sedelmeyeriana, A. condensa, A. hyrcana, A. microscopica and A. persica.* In the former subcluster (A1a), *A. fluminea* derived from *A. rigida* and *A. sericata* with a distance coefficient of 1.73. While, in the later subcluster (A1b), *A. hyrcana* separated from *A. microscopica* and *A. persica* at a distance coefficient of 2. In subcluster A1b smallest distance was found between *A. sedelmeyeriana,* and *A. condensa* with an Euclidean distance (ED) = zero. Subcluster A2 composed of two further small subclusters (A2a and A2b). *A. citrina* and *A. valdehirsuta* formed a small cluster A2a. In subcluster A2b, *A. hessii* derived from the other two species, with a distance coefficient of 2.64.

Cluster B composed of two subclusters (B1 and B2). Subcluster B1 contained three representatives (*A. kurdica, A. melancholica,* and *A. pseudocartalinica*). The first two species formed a small cluster and the third species separated from them at a distance coefficient of 2.4. Subcluster B2 included five species divided into two further subclusters B2a (*A. gigantodus* with *A. farinosa*), and B2b composed three representatives *(A. rechingeri, A. pectiniloba,* and *A. plicatissima*). In this cluster, *A. plicatissima* derived from others with a distance coefficient of 2.2. The results showed that the distance between *A. sedelmeyeriana* and *A. condensa* was the smallest (ED= 0), whereas the distance between *A. hyrcana* and *A. pectiniloba* was the largest (ED= 5.38).

Principal components analyses (PCA)

The result of PCA explained 65.48% of the total variation among the 21 *Alchemilla* species (Table 3). The first principal component displayed 36.73% of the variance, positively associated with achene sculpturing type, epidermal cell wall type, anticlinal cell wall, and epidermal cell shape as well as achene surface type. Whereas, outer periclinal layer was negatively associated with principal component (PC) 1. The second principal component exhibited 14.67% of the observed

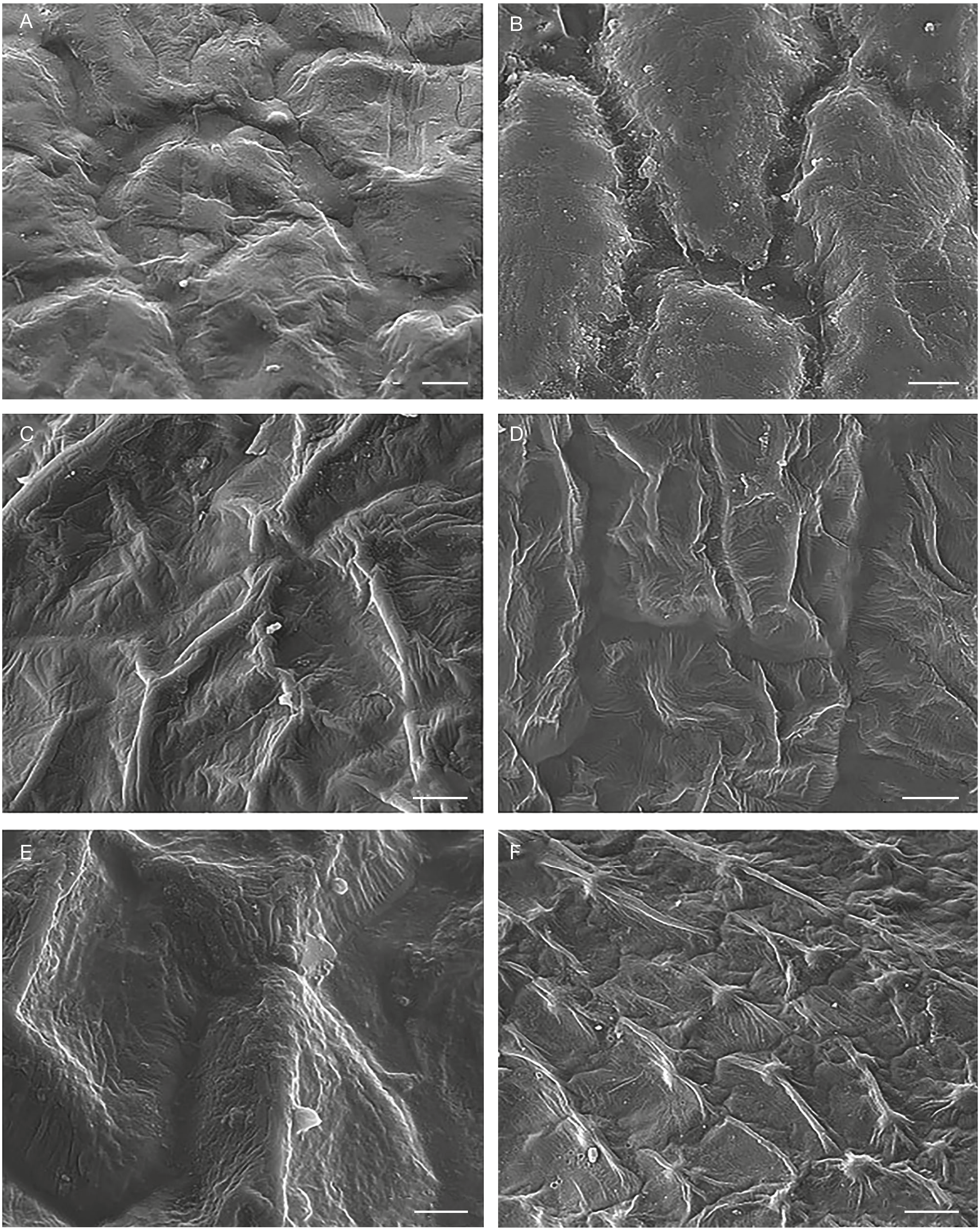

Fig. 6. — SEM Micrographs showing the achene sculpturing types of the *Alchemilla* L. species. Colliculate without folding: **A**, *A. hyrcana* (Buser) Juz.; **B**, *A. sedelmeyeriana* Juz.. Colliculate with folding: **C**, *A. fluminea* S.E.Fröhner; **D**, *A. caucasica* Buser; **E**, *A. microscopica* (with X folding). Colliculate with folding and Papillae: **F**, *A. sericata* Rchb.. Scale bars: A, B, C, D, 20 µm; E, 10 µm; F, 50 µm.

Identification key of the species of *Alchemilla* L. based on the achene micromorphological characters

1. Achene sculpturing colliculate or ruminate foveate-tuberculate (with rugose, folding or papillae); anticlinal wall depressed and outer periclinal layer raised 2
— Achene sculpturing, reticulate falsifoveate with papillae, reticulate-foveate, reticulate-alveolate, ruminate reticulate-foveate-tuberculate, ruminate foveate-tuberculate, ruminate reticulate-tuberculate-lineolate; anticlinal wall raised-oblate and raised and outer periclinal layer depressed 14

2. Hilum position subbasal 3
— Hilum position basal and subbasal-lateral 9

3. Apex acute and acute curved 4
— Apex blunt acuminate 6

4. Apex acute 5
— Apex acute curved .... *A. valdehirsuta* Buser

5. Achene shape ovoid, epidermal cell shape long polygonal irregular .... *A. sedelmeyeriana* Juz.
— Achene shape broadly ovate, epidermal cell shape polygonal irregular .... *A. condensa* S.E.Fröhner

6. Achene sculpturing ruminate foaveat-tuberculate .... *A. citrina* S.E.Fröhner
— Achene sculpturing colliculate 7

7. Achene ovoid globose in shape, obtuse at the base .... *A. erythropoda* Juz.
— Achene ovoid in shape, ovoid at the base 8

8. Epidermal cell shape polygonal irregular, epidermal cell wall undulate .... *A. caucasica* Buser
— Epidermal cell shape polygonal, epidermal cell wall straight .... *A. hessii* Rothm.

9. Achene with blunt acuminate apex 10
— Achene with acute or acute curved apex 12

10. Epidermal cell shape polygonal irregular, epidermal cell wall undulate 11
— Epidermal cell shape polygonal, epidermal cell wall straight .... *A. fluminea* S.E.Fröhner

11. Achene ovate-globose in shape and sculpturing colliculate with fine folding .... *A. rigida* Buser
— Achene ovate in shape and sculpturing colliculate with folding and papillae .... *A. sericata* Rchb.

12. Achene with acute apex 13
— Achene with acute curved apex .... *A. hyrcana* (Buser) Juz.

13. Hilum position basal, achene broadly ovate in shape, sculpturing colliculate with folding, epidermal cell shape long polygonal irregular, epidermal cell wall straight-slightly undulate .... *A. persica* Rothm.
— Hilum position subbasal-lateral, achene long ovate, sculpturing colliculate, with X-formed folding, epidermal cell shape triangular-rhomboidal irregular, epidermal cell wall straight .... *A. microscopica* S.E.Fröhner

14. Hilum position subbasal-lateral, apex acute or acute curved, ovoid or obtuse at the base 15
— Hilum position subbasal, apex blunt acuminate, ovoid at the base 17

15. Achene ovate and long ovate in shape, apex acute or acute curved, sculpturing ruminate reticulate-foveate-tuberculate or ruminate reticulate-tuberculate 16
— Achene ovate in shape, apex acute, sculpturing reticulate-foveate .... *A. pseudocartalinica* Juz.

16. Achene ovate in shape, with irregular reticulate elevation on the surface, acute curved apex, epidermal cell shape polygonal irregular .... *A. melancholica* S.E.Fröhner
— Achene long ovate in shape, with ruminate lineate elevation on the surface, acute apex, epidermal cell shape polygonal .... *A. kurdica* Rothm. ex Bornm.

17. Achene obtuse at the base, epidermal cell shape polygonal 18
— Achene ovate or obtuse at base, epidermal cell shape rectangular polygonal 19

18. Achene tear-ovate in shape, apex acute, sculpturing reticulate-foveate .... *A. gigantodus* S.E.Fröhner
— Achene ovate-globose in shape, apex acute curved, sculpturing reticulate falsifoveate .... *A. farinosa* S.E.Fröhner

19. Achene ovoid at the base, apex blunt acuminate 20
— Achene obtuse at the base, apex acute curved .... *A. plicatissima* S.E.Fröhner

20. Achene sculpturing reticulate-foveate, outer periclinal layer raised-depressed, hilum 0.24 mm long .... *A. pectiniloba* S.E.Fröhner
— Reticulate-alveolate, outer periclinal layer depressed, hilum length shorter than 0.2 mm .... *A. rechingeri* Rothm.

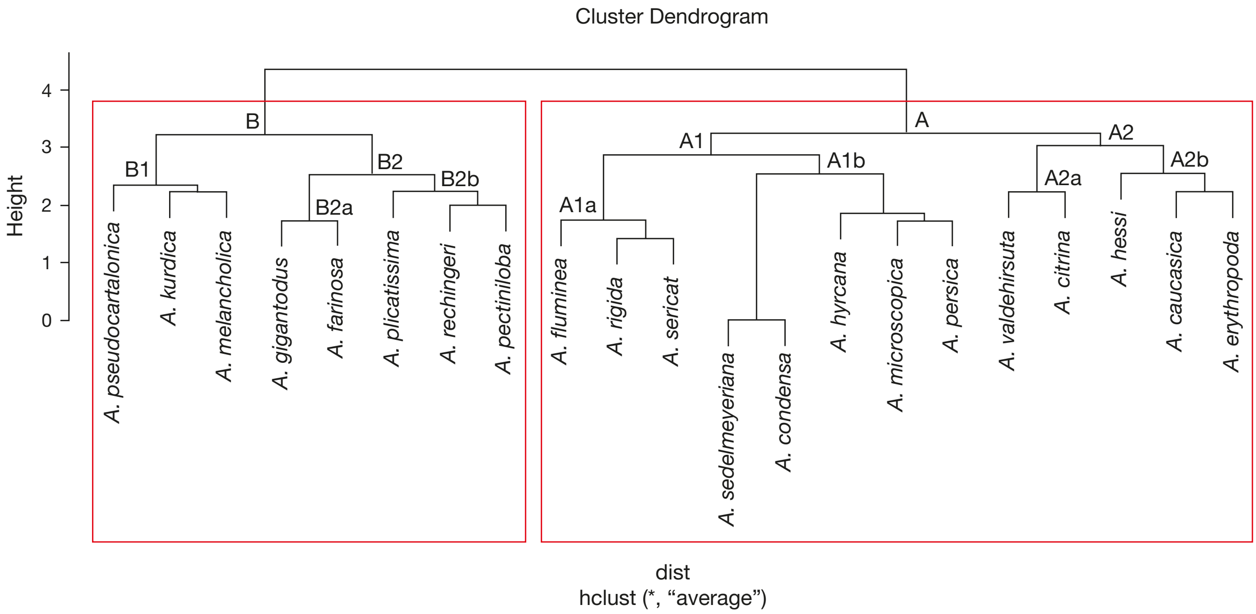

FIG. 7. — UPGMA dendrogram of the 21 of Iranian species of *Alchemilla* L. based on achene micromorphological characters.

variation. Achene length and achene width were important variables composing PC2, and contributed positively to it. The third principal component constituted 14% of the total variation Achene base type and hilum position contributed to PC3. These most effective PCs could explain the total variation observed to a large degree. Therefore, principal component analysis would be a very suitable technique for grouping (Lezzoni & Pritts 1991).

The two-dimensional plot of the first two axes (51.41% total variation explained) displayed two distinct groups (Fig. 8). Group A includes 10 representatives formed based on their similar achene, outer anticlinal wall, achene length, hilum length, and achene base type. While, group B comprises 11 species, distinguished by characters such as achene sculpturing type, epidermal cell wall type, anticlinal cell wall, epidermal cell shape, achene surface type, apex type, achene width, and hilum position.

DISCUSSION

Achene micromorphological characters have been shown to provide useful taxonomic characteristics in the family Rosaceae and its genera (Barthlott 1981; Dowidar *et al.* 2003; Tantawy & Naseri 2003; Faghir *et al.* 2015). However, such studies are lacking in *Alchemilla*. The present study revealed that there are considerable differences among the studied species. Following achene characters were recognized to be reliable in the interspecific classification of *Alchemilla*: achene width, base, surface, sculpturing, anticlinal cell wall, outer periclinal layer, epidermal cell wall types and shape, and hilum position. The result of multivariate analysis showed that species combination of the two major groups (A and B) resulted from PCA were mostly consistent with UPGMA analysis. The resulting species relationships based on cluster analysis are in contrary with the current classifications (Fröhner 1969; Khatamsaz 1993), except for *A. caucasica, A. erythropoda* (subcluster A2), *A. rigida* and *A. sericata* (subcluster A1a) which belong to the subgen. *Pes-Leonis* Juz., sect. *Pubescentes* Buser.

A. rigida and *A. sericata* along with *A. fluminea* clustered together due to their ovoid base, achene apex, hilum position, colliculate sculpturing, depressed anticlinal cell wall, raised outer periclinal layer, similar hilum length (0.14-0.19 mm), and dimension (1.5-2.1× 1.2-1.6 mm). Morever *A. rigida* and *A. sericata* resemble in terms of their hypanthium, pedicels and leaflets hair types (Fröhner 1969; Khatamsaz 1993; Faghir *et al.* 2014b), distribution and ecology (growing in rocky and stony pastures of the alpine and subalpine zones, from Alborz Mountain range) (Khatamsaz 1993) as well as palynological characteristics (having 3- colporate, small pollen in size) (Faghir *et al.* 2015). They have been classified in series *Sericatae* Juz., due to their identical appressed hairs on stem and petiole of radical leaves, leaves color and stem type (Juzepczuk 1941). In the same cluster, *A. fluminea* showed an isolated position possibly due to its micro- (achene sculpturing, epidermal cell shape, and wall type) and macromorphological characters (especially hypanthium glabrous or with very few hairs) (Khatamsaz 1993). *A. fluminea* is Iranian endemic, distributed in Irano-Turanian phytogeographical regions, growing in N and NW Iran at 1800 to 3750 m alt. (Khatamsaz 1993).

Despite their distinct morphological differences (e.g. presence or absence of hair on hypanthium, the length ratio of calyx/ hypanthium), the three Iranian endemics, *A. hyrcana, A. microscopica,* and *A. persica* were found in the same clusters (subcluster A1b). Among them, *A. hyrcana* is characterized by its glabrous hypanthium and almost glabrous stem; *A. microscopica* is recognized by its short stem (5-7 cm),

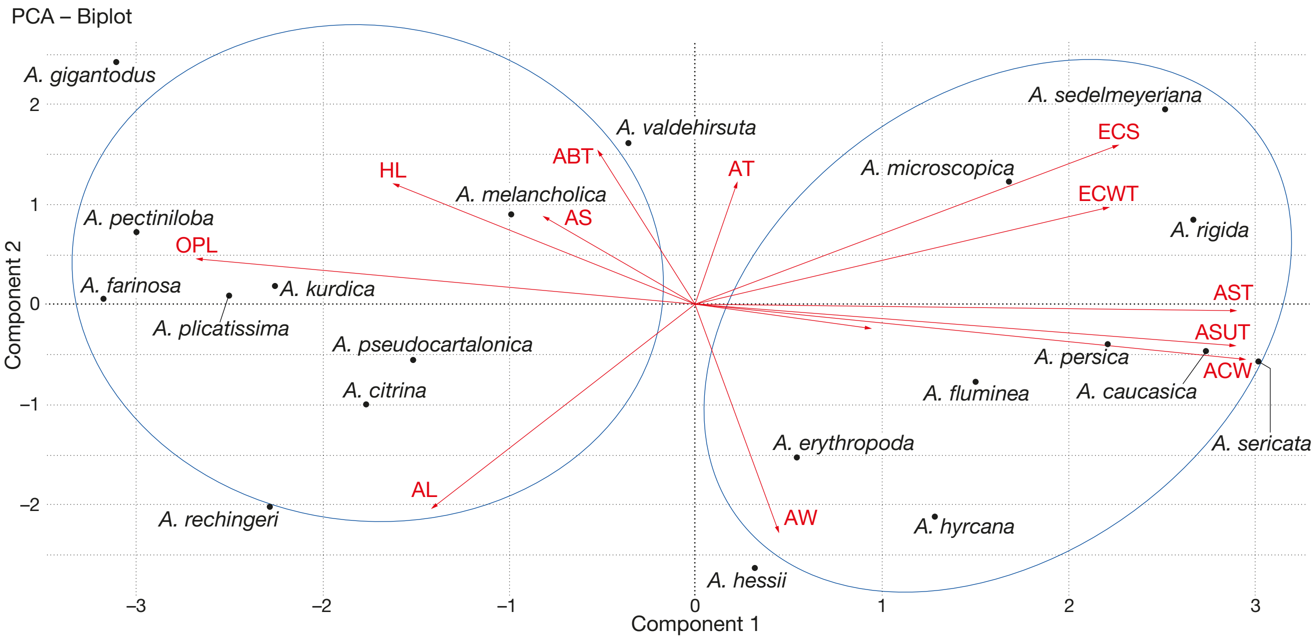

Fig. 8. — Bi plot of the PCA analysis of 21 Iranian species of *Alchemilla* L. based on achene micromorphological characters.

small leaf teeth, the densely hairy hypanthium, and glabrous pedicel; while *A. persica* is identified by its calyx longer than hypanthium, hypanthium hairy at the base, petiole with only sparsely to subdensely erect-patent hairs characterizes this species (Fröhner 1969; Khatamsaz 1993). In addition, *A. persica* and *A. oxysepala* Juz have been considered as allied species, because of their stem height, few numbers of leaves on upper part of the stem, and trichomes type of leaf surface (Fröhner 1969). However, according to Khatamsaz (1993) *A. persica* is more related to *A. hessii* due to some morphological affinities (e.g. calyx shorter than hypanthium, erect-patent hairs on the stem and petiole of radical leaves).

A.condensa and *A. sedelmeyeriana* (in cluster A, subcluster A1b) showed identical surface and sculpture type, hilum position, achene base, anticlinal and periclinal and epidermal cell wall type. But, their palynological (e.g. especially exine sculpturing type) (Faghir *et al.* 2015), leaf micromorphological (e.g. epicuticular wax types) (Faghir *et al.* 2014b) and morphological characters (especially stem, petiole and hypanthium hair types) (Fröhner 1969) contradict the results of this study.

The two representatives of subcluster A2a, *A. valdehirsuta* and *A. citrina* clustered based on achene sculpturing, surface pattern, achene shape, hilum position, anticlinal wall, and epidermal cell wall and achene width. *A. citrina* is thought to be related to *A. gigantodus* for its pedicel with sericeous hairs and acute leaflet teeth) (Fröhner 1969). Its erect calyx having densely smooth hairs (Khatamsaz 1993) and petiole with pilose-subsericeous hairs of 0.85-0.90 mm long (Faghir *et al.* 2014b) identify this Iranian endemic. While *A. valdehirsuta* is characterized by its petiole with densely pilose hairs of 1.58-1.64 mm long (Faghir *et al.* 2014b), pedicel glabrous or scarcely hairy, dense hairs on both sides of leaf or epicalyx lanceolate- narrowly ovate (Fröhner 1969; Khatamsaz 1993). Some authors classified the latter species near *A. surcolosa* due its similar number of leaflet teeth, stem height and blade dimension (Fröhner 1969; Khatamsaz 1993). While others placed it in the sect. *Vulgare* Burser, along with *A. sedelmeyeriana, A. vulgaris, A. hyrcana,* and *A. pseudocartalinica* (Juzepczuk 1941).

A. caucasica and *A. erythropoda* are joined as allied species because of their achene micromorphological similarities e.g. achene apex, hilum position, sculpturing type, anticlinal cell wall, outer periclinal layer, achene length, and surface type. This is supported by their other similar characters such as pedicels, calyx and hypanthium hair types and epicalyx shape (Fröhner 1969; Khatamsaz 1993; Faghir *et al.* 2017), flexuose (villose) hairs on the leaf surface (Faghir *et al.* 2014a), 3-4- colporate and small pollen (Faghir *et al.* 2015) arranged *A. caucasica, A. erythropoda* in series *Flabellatae* Juz., while, Kalheber (1994) classified them in sect. *Alchemilla*, subsect. *Heliodrosium*, and series *Pubescentes.* based on their stems and petioles hair types, leaves incision, hairy pedicels (Kalheber 1994). Both species grow on alpine meadows and pastures of N and NW Iran, in the Hyrcanian mountainous regions from 2000-3380 m alt. (Khatamsaz 1993). The current present result supports the previous classification.

In subcluster A2b, *A. hessii* displayed an isolated position. However, former studies exhibited its similar anatomical (e.g. extraxylary fibers in petiole) and morphological characterstics (especially sepals longer than hypanthium and epicalyx longer than or as long as sepals), which associate it with *A. persica* (Faghir *et al.* 2016, 2017), and *A. epipsila* (Fröhner 1969; Khatamsaz 1993).

A. pseudocartalinica, A. kurdica, and *A. melancholica* (from subcluster B1) clustered due to their similar epidermal cell wall, hilum position, achene base, and achene length. The first species showed an isolated position, due to its reticulate-foveate sculpturing, rectangular-polygonal epidermal cell shape, raised anticlinal wall and depressed outer periclinal wall type. This result is consistent with the former leaf micromorphological study (Faghir *et al.* 2014a). The latter species is an Irano-Turanian floristic element, growing in the forest and subalpine meadows of NW and W Iran at 1700-3700 m alt. (Fröhner 1969; Khatamsaz 1993). We find *A. pseudocartalinica* in the series *Vulgares*, subsect. *Heliodrosium* and sect. *Alchemilla* of Hayirioglu-Ayaz & Kalheber's (2002) taxonomic treatment. The alliance of *A. kurdica* and *A. melancholica* contradicts the previous macro-micromorphological studies. Its glabrous stem or with few appressed to erect hairs, scattered hairs on the leaf surface, sparsely to subdensely erect-patent hairs on the petiole identify *A. kurdica.* Whereas, *A. melancholica* is characterized by its hairy stem, declined hairs on the petiole and densely hairy leaf surfaces (Fröhner 1969; Khatamsaz 1993; Faghir *et al.* 2014a).

In subcluster B2a, *A. gigantodus* and *A. farinosa* were separated from three other species based on some diagnostic evidences such as subbasal hilum position, obtuse achene base, polygonal epidermal cell, raised anticlinal wall and depressed outer periclinal layer, hilum length and achene length. According to previous studies, these two species displayed leaf micromorphological (e.g. membranous platelets on both sides of leaf) and anatomical affinities (e.g. oval-shaped stoma, guard cells with wavy walls, and 4-6 adjacent epidermal cells) (Faghir *et al.* 2014b, 2015). According to Fröhner (1969), *A. gigantodus* associates with *A. citrina* (especially for its erect-patent hairs on the petiole of radical leaves), while *A. farinosa* correlates to *A. rigida* (for having hypanthium shorter than sepals and all parts covered by hairs) and *A. caucasica* (by erect-patent hairs on the petiole of radical leaves). The three Iranian endemics *A. plicatissima,* A. *pectiniloba,* and *A. rechingeri* (subcluster B2b), resemble micromorphologically especially due to their achene and epidermal cell shape, and epidermal cell wall type. Among them, *A. plicatissima* showed an isolated position, because of its obtuse achene base and acute curved apex. The latter species grows in N Iran, from 1800-2000 m alt., preferring open sunny alpine meadows. *A. plicatissima* is distinguished by its short height (3-10 cm in height), weak stem, 7-9 leaflet teeth, and petiole with densely pilose hairs of 0.94-1 mm long (Faghir *et al.* 2014b). It was considered as a close relative of *A. pectiniloba,* because of some morphological similarities e.g. calyx shorter than or as long as hypanthium, epicalyx shorter than calyx, dense hairs covering all parts (Fröhner 1969). However, Khatamsaz (1993) described its association with *A. valdehirsuta* and *A. surcolosa* based on their identical petiole hairs.

A. pectiniloba and *A. rechingeri* were assembled in a small cluster, based on their identical achene characteristics (surface, shape, apex and base type, hilum position, anticlinal cell wall, epidermal cell shape and type). Both species are the Hyrcanian mountainous floristic elements, growing in different altitudes of Alborz Mountains. *A. pectiniloba* occurs from 2000-2400 m alt., while *A. rechingeri* prefers higher altitude, from 2300-3400 m (Khatamsaz 1993). However, the present result is not in agreement with current classifications (Fröhner 1969; Khatamsaz 1993) and the previous results obtained from leaf, flower and pollen in micro-macromorphological studies (Faghir *et al.* 2014a, 2015, 2017). The results displayed a close affinity among the studied species. However, this was not consistent with the current classification, except for four species (*A. sericata, A. rigida, A. caucasica* and, *A. erythropoda*). These results slightly support the previous classifications and suggest the importance of the achene micromorphology of the genus *Alchemilla* as a taxonomic character to species-level classification. This study suggests further research on the achene anatomy and evolutionary trend of anatomical and morphological characters of *Alchemilla* achene for a better understanding of the relationships among species.

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