

Pollen morphology of the genus *Alchemilla* L. (Rosaceae) in Iran

Marzieh Beygom FAGHIR^{1*}, Farideh ATTAR², Robabeh Shahi SHAVVON¹, Atefeh MEHRMANESH¹

¹Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran

²Central Herbarium of Tehran University and School of Biology, University College of Science, Tehran, Iran

Received: 08.06.2014 • Accepted: 05.10.2014 • Published Online: 16.03.2015 • Printed: 10.04.2015

Abstract: This paper reports the morphological characters of 18 Iranian species of the genus *Alchemilla* using light and scanning electron microscopy. The pollen grains are monad, radially symmetrical, isopolar, or subsipolar; small to medium in size; tri- and tetra-colporate; rectangular to cylindrical (from equatorial view) and triangular to circular (from polar view) in outline; and prolate-spheroidal to subprolate and prolate in shape. The exine ornamentation is psilate and microechinate. Based on the exine sculpturing and microechinate distribution pattern, three main types, four subtypes, and two categories of pollen grains were recognized. We used cluster analysis and principal component analysis to determine the potential contribution of pollen morphological characters to the species relationships. Our findings revealed the significance of palynological evidence in explaining the species relationship. The results of two multivariate analyses showed a close affinity among the seven studied species (*A. amardica*, *A. sericata*, *A. fluminea*, *A. kurdica*, *A. hyrcana*, *A. sedelmeyeriana*, and *A. pesudocartalinica*).

Key words: *Alchemilla*, Rosaceae, palynological characters, numerical analysis, Iran

1. Introduction

The genus *Alchemilla* L. (Rosaceae), with ca. 1000 species, is one of the most species-rich Holarctic plant genera, widespread mainly in western Eurasia. Some species of the genus also show a preference for mountains of South India, Sri Lanka, Java, China, Japan, Africa, and Madagascar (Izmailow, 1981). The genus belongs to a critical and taxonomically difficult group. Several authors divided the genus into different subgenera, sections, groups, subgroups, series (De Candolle, 1825; Focke, 1888; Buser, 1892; Lagerheim, 1894; Rothmaler, 1944; Fröhner, 1995; Kalkman, 2004; Notov and Kusnetzova, 2004; Hayirlioglu-Ayaz and Inceer, 2009), and a large number of micro-species and species complexes. This is most likely due to confusion resulting from frequent apomixis, polyploidization, and hybridization observed in this group (Asker and Jerling, 1992; Sepp, 2000; Hörandl, 2004; Gehrke et al., 2008). The genus was thought to be related to the tribe *Sanguisorbeae* (Juzepczuk, 1941) due to superficial similarity caused by reduction in flower parts (Gehrke et al., 2008). However, its relation to the tribe *Potentilleae* was noted by Schulz-Menz (1964) and has been supported by molecular analysis (Eriksson et al., 1998, 2003). *Alchemilla*, *Aphanes* L., and *Lachemilla* Rydb. are placed in the subtribe *Alchemillinae* based on anther

structure (one elliptic theca) and style position (subbasal to basal) (Soják, 2008). *Alchemilla* L. is represented by 31 species in *Flora Iranica* (Fröhner, 1969) and 24 representatives in *Flora of Iran* (Khatamsaz, 1993). They are mainly distributed in the N and NW but some species grow in W and C Iran. Among them, 14 representatives are endemic to Iran (Fröhner, 1969; Khatamsaz, 1993). The species of the genus are herbaceous perennial plants, preferring open meadows, stony slopes, shady places, river banks, and forest edges of alpine and subalpine regions from 1700 to 3300 m altitudes (Juzepczuk, 1941; Fröhner, 1969; Khatamsaz, 1993). The palynological data of the genus are poorly understood and only limited information focused on pollen morphology of Rosaceae genera was presented (Hebda et al., 1988; Hebda and Chinnappa, 1990). The aim of the current survey was to describe the pollen morphological characters of Iranian species of *Alchemilla* and to determine how these traits relate to species relationship.

2. Materials and methods

In this survey, 18 Iranian species of *Alchemilla* underwent palynological analysis using a light microscope (LM) and scanning electron microscope (SEM). Out of them 16 are included in the numerical analysis. We used pollen grains

* Correspondence: marziehbeygomfaghir@gmail.com

of both fresh (collected from 2010 to 2012, during spring and later summer) and dried herbarium specimens of Guilan University Herbarium (GUH), Tehran University Herbarium (TUH), and Herbarium of the Research Institute of Forests and Rangelands of Iran (TARI). The voucher specimens of newly collected samples were deposited in Guilan University Herbarium (GUH). A list of specimens used in this analysis is presented in Table 1. For LM, flower buds were acetolyzed following the method described by Harley (1992). Prepared slides were studied with an Olympus BH-2 microscope and photographed by

a Nikon Coolpix S10 camera. Measurements were taken from at least 25 grains per species. The pollen characters were measured under a $\times 40$ eyepiece, and are summarized in Tables 2 and 3. For SEM observation, grains were fixed on aluminum stubs 12.5 mm in diameter covered with double-sided cellophane tape and then sputter coated (Emitech k450) with gold. The micrographs were taken using an SEM model VEGA/TESCAN in Razi Metallurgical Research Center (RMRC), Tehran. The pollen terminology in general follows Erdtman (1952), Eide (1981), Ueda and Tomita (1989), and Punt et al. (2007).

Table 1. The species used in the current analysis.

Species	IRAN: Province, Collector, Date	Accession No.
1. <i>A. amardica</i> Rothm	Guilan: Deylaman; Shahe shahidan; Chaichi, Faghir and Shahi; 6. 2012.	4872 (GUH)
2. <i>A. caucasica</i> Buser	Mazandaran: Kojur; Firozabad village; 1700 m; Ghahreman and Attar; 19.6. 1997. Mazandaran: Karaj - Chalus, Zangule Bridge; 3000 m; Nazarian; 10.1997	20598 (TUH) 33155 (TUH)
3. <i>A. citrina</i> Fröhner	Guilan: Deylaman; Shahe Shahidan; Chaichi, Faghir and Shahi; 6.2012.	4876 (GUH)
4. <i>A. condensa</i> Fröhner	Guilan: Masal; Chaichi; 2012. Guilan: Deylaman, Larikhani, 1500 m; Saeidi; 20.5.1993.	4871 (GUH) 18845 (TUH)
5. <i>A. erythropoda</i> Juz..	Mazandaran: Kojour, Firoozabad village; 1700 m; Ghahreman and Attar; 8.1999 Mazandaran: Kojur; Kikuh Mont, Zinoosht Rangeland. 2000–2300 m, Khatamsaz and Gholoizadeh. Gilan: mountain above Damash-east of Rudbar: 1900 m; P.Wendelbo & Ann Ala. Azarbayejan: Shahbil, Kohe Sabalan, 3450 m; Foroughi and Assadi.	20595 (TUH) 751847(TARI) 18232(TARI) 24248(TARI)
6. <i>A. fluminea</i> Fröhner	Guilan: Deylama, Larikhani, 1530 m; Ghahreman and Attar.	18844 (TUH)
7. <i>A. hessii</i> Rothm.	Mazandaran: Kojur, Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1997. Mazandaran: Kandovan; Ghahreman, Augustine and Sheikholeslami; 6.1974.	20600/1 (TUH) 19418 (TUH)
8. <i>A. hyrcana</i> (Buser) Juz.	Guilan: Deylaman; Shahe Shahidan; Chaichi, Faghir and Shahi; 6.2012. Mazandaran: Kojur; Firozabad village; 1700 m; Ghahreman and Attar; 19.6. 1997.	4873 (GUH) 20597 (TUH)
9. <i>A. kurdica</i> Rothm. Bornm.	Guilan: Masal; Khashkhami; Chaichi, Faghir, and Shahi; 6.2012.	4875 (GUH)
10. <i>A. pectinloba</i> Fröhner	Guilan: Deylama; Larikhani; 1530 m; Saeidi; 5.1993	18837 (TUH)
11. <i>A. persica</i> Rothm.	Mazandaran: Tonokabon, Jannat Rudbar, 1600 m; Ghahreman, Attar, and Khatamsaz; 20.6.1997. Azarbijan: Arasbaran, Veighan; Makidan; 1400 m; Ghahreman, Attar, and Hamzehei; 2006. Mazandaran: Karaj to Chalus road, Polezangule bridge, 2600 m; Nazarian; 24.6. 1999. Tehran: Darake; Mobayen; 1969. Mazandaran: Chalus road; Mobayen; 20.4.1965	20603 (TUH) 35575 (TUH) 33440 (TUH) 19419 (TUH) 8603 (TUH)
12. <i>A. plicatissima</i> Fröhner	Guilan: Almas pass; Chaichi, Faghir, and Shahi; 8.2012.	4869 (GUH)
13. <i>A. pseudocartalinica</i> Juz.	Mazandaran: Kojur; Firozabad village; 1700 m; Ghahreman and Attar; 19.6. 1997.	20602 (TUH)
14. <i>A. rechingeri</i> Rothm.	Mazandaran: Kojur; Firozabad village; 1700 m; Ghahreman and Attar; 19.6. 1997.	20601 (TUH)
15. <i>A. retinervis</i> Buser.	Mazandaran: Kojur; Firozabad village, 1700 m; Ghahreman and Attar; 19.6. 1997. Azarbaijan: Maku, Southwest Mountain Church Kennedy, 2400 m to 2650 m; Mozaffarian Asadi 11.9.2009. 30 336(TARI)	20599 (TUH) 336(TARI)
16. <i>A. rigida</i> Buser,	Guilan: Espili; Larikhani; 1510 m; Saeidi; 5.1993. Mazandaran: Kojur; Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1996.	18842 (TUH) 20596 (TUH)
17. <i>A. sedelmeyeriana</i> Juz.	Mazandaran: Kojur; Firozabad village; 1700 m; Ghahreman and Attar; 19.6. 1997. Mazandaran: Firozabad Mont, 2200m, Khatamsaz and gholizade.	20593 (TUH) 57165(TARI)
18. <i>A. sericata</i> Reichen.	Azarbaijan: Kaleibar to Makidi; 1510 m; Ghahreman, Mozaffarian, and Sheikholeslami; 5.1993.	17540 (TUH)

Table 2. Pollen morphology data: Numbers refer to (minimum-) mean \pm standard deviation (-maximum), Polar axis (P), Equatorial axis (E), Polar axis/Equatorial axis (P/E) ratio, Pollen shape (Ps) and Size (Si), Distance between the apices of two ectocolpi/equatorial diameter (d/D), Mesocolpium (Me), Colpus length/polar axis (Cl/P), Number of Colpi (No. Cl), Colpus length (Cl), Spine height (Sh), Spine diameter (Sd), Exine thickness (Et), Prolate-spheroidal (PS), Prolate (P), Subprolate (Subp), Small (S), Medium (M).

Species	P	E=D	P/E	Ps	Si	d	d/D	Me
1. <i>A. amardica</i>	18.92 (19.09 \pm 0.18) 19.35	16.18 (16.3 \pm 0.17) 16.56	1.17	Subp	S	4 (4.07 \pm 0.09) 4.2	0.24	7.78 (8.22 \pm 0.36) 8.6
2. <i>A. caucasica</i>	19.96 (20.14 \pm 0.12) 20.22	18 (18.17 \pm 0.15) 18.38	1.10	PS	S	7 (7.65 \pm 0.55) 8.2	0.42	7.9 (8.15 \pm 0.26) 8.5
3. <i>A. citrina</i>	17.5 (17.81 \pm 0.34) 18.2	12.9 (13.02 \pm 0.12) 13.2	1.36	P	S	3.5 (3.9 \pm 0.27) 4.1	0.29	7.5 (7.92 \pm 0.43) 8.5
4. <i>A. erythropoda</i>	24.24 (24.68 \pm 0.37) 25	15.78 (15.86 \pm 0.057) 15.91	1.55	P	S	4.3 (4.37 \pm 0.07) 4.46	0.27	4.6 (4.7 \pm 0.09) 4.82
5. <i>A. condensa</i>	22.94 (23.15 \pm 0.14) 23.26	14.6 (14.79 \pm 0.16) 15	1.56	P	S	5.2 (5.36 \pm 0.12) 5.5	0.36	4.7 (4.78 \pm 0.08) 4.9
6. <i>A. fluminea</i>	26.71 (26.96 \pm 0.36) 27.5	19.29 (19.39 \pm 0.13) 19.6	1.39	P	M	6.74 (7.14 \pm 0.37) 7.59	0.36	3.7 (3.86 \pm 0.12) 4
7. <i>A. hessii</i>	26.5 (27.42 \pm 0.8) 28.2	18.2 (18.34 \pm 0.12) 18.51	1.49	P	M	4.12 (4.28 \pm 0.16) 4.5	0.23	4.26 (4.35 \pm 0.09) 4.48
8. <i>A. hyrcana</i>	16.08 (16.4 \pm 0.58) 17.28	11.89 (12.19 \pm 0.2) 12.32	1.34	P	S	3.7 (3.87 \pm 0.17) 3.87	0.31	5.15 (5.44 \pm 0.38) 6
9. <i>A. kurdica</i>	22.8 (23.4 \pm 0.4) 23.66	16.2 (16.35 \pm 0.1) 16.45	1.43	P	S	4.8 (4.96 \pm 0.12) 5.1	0.30	5.6 (5.76 \pm 0.16) 5.98
10. <i>A. persica</i>	20 (20.16 \pm 0.14) 20.34	16.1 (16.23 \pm 0.14) 16.43	1.24	Subp	S	5.5 (5.60 \pm 0.096) 5.73	0.34	6.52 (6.63 \pm 0.10) 6.77
11. <i>A. pectinoloba</i>	19 (19.14 \pm 0.16) 19.38	15.8 (16.02 \pm 0.26) 16.4	1.19	Subp	S	3.87 (4.3 \pm 0.39) 4.8	0.26	6.97 (7.47 \pm 0.42) 7.97
12. <i>A. plicatissima</i>	16.65 (16.8 \pm 0.1) 16.89	10.6 (10.79 \pm 0.16) 10.98	1.55	P	S	3.17 (3.23 \pm 0.08) 3.35	0.29	3.62 (3.73 \pm 0.09) 3.83
13. <i>A. pseudocartalinica</i>	21.7 (21.77 \pm 0.07) 21.88	13.86 (14.02 \pm 0.23) 14.38	1.55	P	S	3.51 (4 \pm 0.38) 4.39	0.28	3.75 (3.85 \pm 0.09) 3.98
14. <i>A. rechingeri</i>	24.8 (25.12 \pm 0.27) 25.4	16.7 (16.83 \pm 0.125) 17	1.49	P	S	3.12 (3.28 \pm 0.12) 3.4	0.19	3.6 (3.71 \pm 0.08) 3.8
15. <i>A. retinervis</i>	18.09 (18.23 \pm 0.14) 18.41	13 (13.13 \pm 0.10) 13.25	1.38	P	S	5.01 (5.12 \pm 0.089) 5.21	0.38	6.24 (6.32 \pm 0.089) 6.45
16. <i>A. sedelmeyeriana</i>	14 (14.21 \pm 0.14) 14.34	9.4 (9.57 \pm 0.28) 10	1.48	P	S	2.8 (2.92 \pm 0.09) 3	0.30	2.7 (2.78 \pm 0.08) 2.89
17. <i>A. sericata</i>	20.56 (20.79 \pm 0.3) 21.22	17.27 (17.44 \pm 0.12) 17.55	1.19	Subp	S	4.88 (4.97 \pm 0.09) 5.1	0.28	9 (9.5 \pm 0.4) 9.88
18. <i>A. rigida</i>	14.41 (14.51 \pm 0.09) 14.61	9 (9.65 \pm 0.43) 9.91	1.50	P	S	3.5 (3.91 \pm 0.29) 4.2	0.40	4.28 (4.58 \pm 0.21) 4.75

Table 2. (Continued).

Species	Cl/P	No. Cl	Cl	Sh	Sd	Et
1. <i>A. amardica</i>	0.76	3-4	14.2 (14.65 \pm 0.44) 15.2	0.10 (0.12 \pm 0.02) 0.16	0.16 (0.21 \pm 0.05) 0.28	0.89 (0.95 \pm 0.09) 1.02
2. <i>A. caucasica</i>	0.57	3-4	10.9 (11.52 \pm 0.66) 12.19	0.15 (0.18 \pm 0.02) 0.19	0.12 (0.16 \pm 0.02) 0.18	1.45 (1.90 \pm 0.63) 1.90
3. <i>A. citrina</i>	0.64	3	10.7 (11.5 \pm 0.7) 12.33	0.10 (0.14 \pm 0.02) 0.16	0.18 (0.21 \pm 0.03) 0.25	1.05 (1.13 \pm 0.11) 1.21
4. <i>A. erythropoda</i>	0.80	3-4	19.68 (19.76 \pm 0.09) 19.88	0.29 (0.31 \pm 0.01) 0.33	0.18 (0.25 \pm 0.05) 0.30	0.80 (1.02 \pm 0.31) 1.24
5. <i>A. condensa</i>	0.86	3	19.7 (19.96 \pm 0.22) 20.25	0.09 (0.14 \pm 0.04) 0.19	0.10 (0.19 \pm 0.07) 0.26	1.90 (2.25 \pm 0.49) 2.60
6. <i>A. fluminea</i>	0.77	3	20.41 (20.90 \pm 0.45) 21.5	0.10 (0.14 \pm 0.03) 0.17	0.13 (0.20 \pm 0.06) 0.26	1.17 (1.23 \pm 0.08) 1.29
7. <i>A. hessii</i>	0.87	3	23.15 (24.04 \pm 0.68) 24.8	0.13 (0.14 \pm 0.01) 0.15	0.15 (0.23 \pm 0.07) 0.33	1.83 (1.90 \pm 0.10) 1.98
8. <i>A. hyrcana</i>	0.82	3	12.86 (13.45 \pm 0.61) 14.1	0.10 (0.13 \pm 0.02) 0.17	0.14 (0.17 \pm 0.03) 0.21	1.88 (2.04 \pm 0.22) 2.20
9. <i>A. kurdica</i>	0.84	3	19 (19.71 \pm 0.51) 20.2	0.12 (0.13 \pm 0.02) 0.16	0.12 (0.16 \pm 0.04) 0.22	0.94 (1.05 \pm 0.16) 1.17
10. <i>A. persica</i>	0.70	3	14 (14.15 \pm 0.13) 14.32	-	-	1.05 (1.17 \pm 0.17) 1.30
11. <i>A. pectinoloba</i>	0.86	3	16.14 (16.59 \pm 0.34) 16.96	0.19 (0.20 \pm 0.01) 0.21	0.14 (0.21 \pm 0.05) 0.28	1.15 (1.24 \pm 0.13) 1.34
12. <i>A. plicatissima</i>	0.93	3-4	14.3 (15.71 \pm 1.26) 16.85	0.14 (0.17 \pm 0.02) 0.21	0.12 (0.17 \pm 0.03) 0.21	1.41 (1.53 \pm 0.16) 1.65
13. <i>A. pseudocartalinica</i>	0.81	3	17 (17.67 \pm 0.89) 19	0.11 (0.12 \pm 0.01) 0.10	0.15 (0.19 \pm 0.03) 0.23	1.67 (2.23 \pm 0.79) 2.80
14. <i>A. rechingeri</i>	0.79	3	19.5 (19.96 \pm 0.7) 21	0.11 (0.16 \pm 0.03) 0.19	0.18 (0.21 \pm 0.03) 0.25	1.05 (1.19 \pm 0.20) 1.34
15. <i>A. retinervis</i>	0.70	3	12.5 (12.78 \pm 0.20) 13	-	-	0.90 (1.22 \pm 0.45) 1.22
16. <i>A. sedelmeyeriana</i>	0.80	3	11.24 (11.49 \pm 0.34) 12	0.09 (0.13 \pm 0.01) 0.12	0.13 (0.15 \pm 0.01) 0.17	1.82 (1.92 \pm 0.14) 2.03
17. <i>A. sericata</i>	0.88	3	18 (18.43 \pm 0.33) 18.72	0.13 (0.16 \pm 0.03) 0.21	0.17 (0.21 \pm 0.04) 0.26	1.73 (1.85 \pm 0.17) 1.98
18. <i>A. rigida</i>	0.81	3	11.62 (11.84 \pm 0.21) 12.1	0.09 (0.11 \pm 0.02) 0.15	0.10 (0.12 \pm 0.02) 0.15	1.08 (1.13 \pm 0.07) 1.19

Table 3. Species grouping based on sculpturing type.

Species	Sculpturing type	
T I Subtype A	Microechinate distribution	
1. <i>A. citrina</i>		
2. <i>A. pectinoloba</i>		
3. <i>A. erythropoda</i> *	Circular	Type I
4. <i>A. hessii</i>		
5. <i>A. pseudo-cartalinica</i>		Microechinate only in vicinity of colpi
T I Subtype B		
6. <i>A. condensa</i>		
7. <i>A. rigida</i>	Striate	
TII Subtype A		
Category 1		
8. <i>A. kurdica</i>		
9. <i>A. hyrcana</i>	Striate	Type II
10. <i>A. sericata</i>		
Category 2		
11. <i>A. sedelmeyeriana</i>		Microechinate in vicinity of colpi and mid-intercolpium / pole psilate
12. <i>A. fluminea</i>	Circular	
TII Subtype B		
13. <i>A. plicatissima</i> *		
14. <i>A. caucasica</i> *	Arranged in 2–3 rows	
15. <i>A. amardica</i> *		
TIII		Type III
16. <i>A. rechingeri</i>		Microechinate in vicinity of colpi, mid-intercolpium and poles

2.1. Data analysis

In the current survey, two numerical analyses including cluster analysis and principal components analysis (PCA) were carried out. In these analyses 13 quantitative and quantitative evidences comprising the mean of quantitative and coded qualitative characters, as binary/multistate characters, were involved. The standardized variables were employed for multivariate statistical analysis. The palynological characters and character states used in the numerical analysis of 16 species of *Alchemilla* in Iran are presented in Table 4. Cluster analysis (CA) was undertaken using average taxonomic distance (Euclidean distance matrix) and unweighted pair-group method with arithmetic mean (UPGMA) clustering procedures (SIMINT, SAHN, and TREE). The values of each character were standardized and the cophenetic correlation coefficient was determined to find out to what extent the cluster analysis fits the distance matrix. PCA was conducted (for analyzing multivariate data) using the general linear model (GLM) in Minitab statistical software (Ryan and Joiner, 2001).

3. Results

3.1. General pollen morphology

The microphotographs taken by LM (Figure 1) and SEM (Figures 2–4) revealed interesting pollen morphological characters of the studied species. The pollen grains are isopolar to subisopolar radially symmetrical monads, prolate spheroidal ($P/E = 1.11-1.12$) (Figure 1A), subprolate ($P/E = 1.19-1.32$) (Figure 1B), and prolate ($P/E = 1.38-1.79$) (Figures 1C–1F) in shape. The outline of pollen varies from rectangular to cylindrical in equatorial view (Figures 1A–1F) and triangular to subcubic in polar view (Figures 1G–1L). The pollen grains are small to medium based on Erdtman (1952), with tri- and tetracolporate apertures (Table 2). The minimum and maximum polar axis varies from 14.21 μm (in *A. sedelmeyeriana*) to 27.42 μm (in *A. hessii*). The minimum equatorial axis (9.57 μm) is reported in *A. sedelmeyeriana* and the maximum equatorial axis is found (19.39 μm) in *A. fluminea*. The minimum P/E ratio (1.1) and maximum P/E ratio (1.56) are identified in *A. caucasica* and *A. condensa*,

Table 4. Palynological character and character states used in numerical analysis of 16 species of *Alchemilla* in Iran.

Characters	Character states	No.
	Polar axis (P)	1
	Equatorial axis (E or D)	2
	Polar axis/Equatorial axis ratio(P/E)	3
	Distance between the apices of two ectocolpi (d)	4
	Apocolpium index (d/D)	5
	Mesocolpium (Me)	6
	Colpus length/Polar axis (Cl/P)	7
	Colpus length (Cl)	8
	Exine thickness (Et)	9
	Number of colpi (No. Cl)	10
0 Prolate		
1 Spheroidal	Pollen shape (Ps)	11
2 Subprolate		
0 Isopolar		
1 Subisopolar	Polarity (Pol)	12
0 T1 Subtype A		
1 TI Subtype B		
2 TII Subtype A category 1	Sculpturing types (Sculp)	13
3 TII Subtype A category 2		
4 TII Subtype B		
5 TIII		

respectively. The mesocolpium length ranges from 2.78 μm (in *A. sedelmeyeriana*) to 9.5 μm (in *A. sericata*). The minimum and maximum colpus length is measured in *A. sedelmeyeriana* (11.49 μm) and *A. hessii* (24.04 μm). The exine thickness varies from minimum 0.95 μm in *A. amardica* to maximum 2.25 μm in *A. condensata*. The ratio of colpus length and polar axis varies from 0.57 in *A. caucasica* to 0.93 in *A. plicatissima*. The apocolpium index (AI) (Punt et al., 2007) or d/D: the ratio of the distance between the apices of two ectocolpi of a zonocolpate pollen grain (d) to its equatorial diameter (D) ranges over an interval of 0.19 μm in *A. rechingeri* to 0.42 μm in *A. caucasica*. The minimum (0.11 μm in *A. rigida*) and maximum (0.31 μm in *A. erythropoda*) spine height were also measured. *A. sedelmeyeriana* and *A. erythropoda* had the minimum (0.15 μm) and maximum (0.25 μm) spine diameter, respectively (Table 2).

3.2. Exine sculpture types

We observed three main types of exine sculpturing (Table 3).

Type I: Microechinate only in vicinity of colpi/poles and mid-intercolpium psilate. Based on the microechinate distribution pattern the first type is further divided into 2 subtypes:

TI subtype A: consists of the species with circular microechinate distribution pattern, which includes *A. pseudocartalinica* (Figures 2A–2C), *A. hessii* (Figure 2D), *A. citrina* (Figures 2E, 2F), *A. pectinoloba* (Figures 2G–2I), and *A. erythropoda* (Figures 2J–2L).

TI subtype B: comprises the species with striate microechinate distribution pattern. This subtype is recorded in *A. condensata* (Figures 3A, 3B) and *A. rigida* (Figure 3C).

Type II: Microechinate in vicinity of colpi, mid-intercolpium/poles psilate

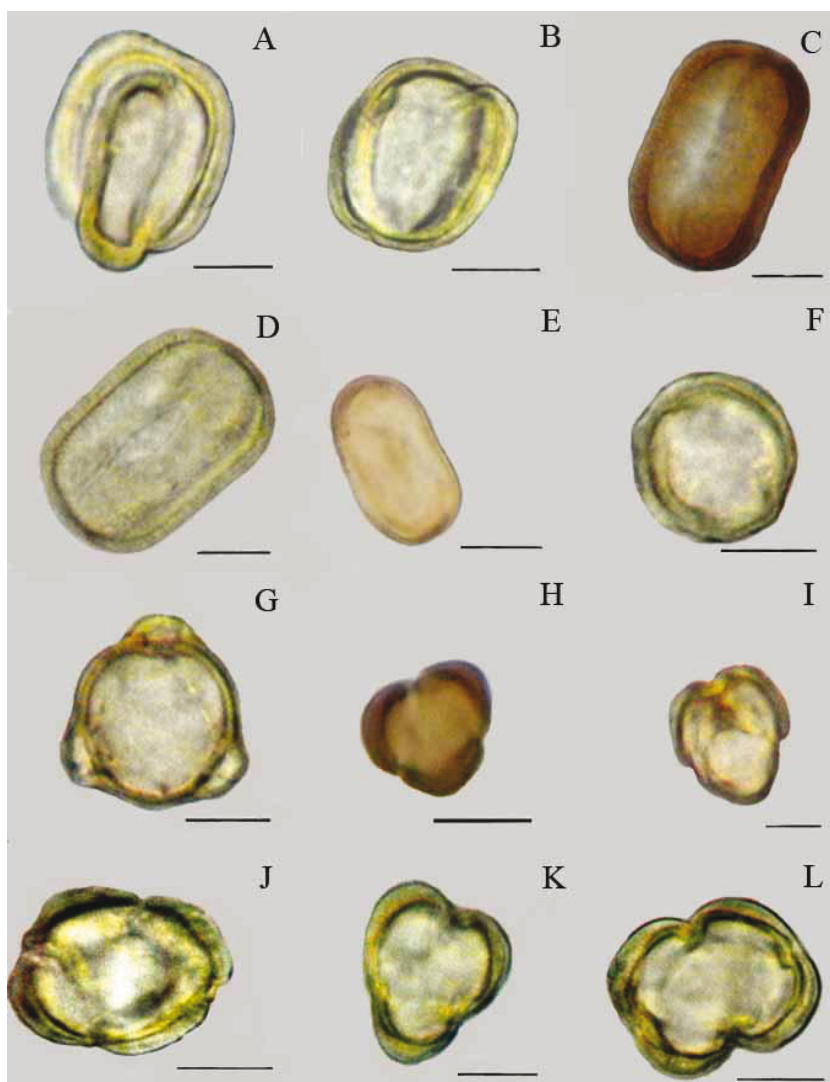


Figure 1. A–L. LM micrographs of pollen grains in species of *Alchemilla*: *A. caucasica* A and J; *A. plicatissima* B, K, L; *A. condensata* C; *A. kurdica* D; *A. erythropoda* E; *A. rechingeri* F, G; *A. retinervis* H; *A. amardica* I (scale bar = 2.5 μ m).

This type is further divided into two subtypes:

TII subtype A: Microechinate in vicinity of colpi and mid-intercolpium. Based on the microechinate distribution pattern this subtype is divided into 2 categories:

The first category is composed of species with a striate microechinate distribution pattern and includes three species: *A. kurdica* (Figure 3D), *A. hyrcana* (Figures 3E, 3F), and *A. sericata* (Figures 3G, 3H). The second category includes species with a circular microechinate distribution pattern, which is found in *A. sedelmeyeriana* (Figures 3I, 3J) and *A. fluminea* (Figures 3K, 3L).

TII subtype B: Three rows of microechinae in vicinity of colpi and mid-intercolpium. This type is observed in three tetracolporate species of *A. plicatissima* (Figures

4A–4E), *A. caucasica* (Figures 4F–4H), and *A. amardica* (Figures 4I, 4J).

Type III: In this type the mid-intercolpium, colpi edges, and poles are covered by microechinae. This was recorded in *A. rechingeri* (Figures 4K, 4L).

3.3. Cluster analysis

The UPGMA phenogram and all OTUs in this survey are presented in Figure 5.

The cophenetic correlation was 75.75%, indicating a reasonable percent of the data similarity matrix transferred to the phenogram (Rohlf, 1993). Three clusters and branches were formed: A) a cluster is divided into three subgroups: A1) a branch with *A. amardica*, *A. pectinoloba*, and *A. sericata*, A2) a branch

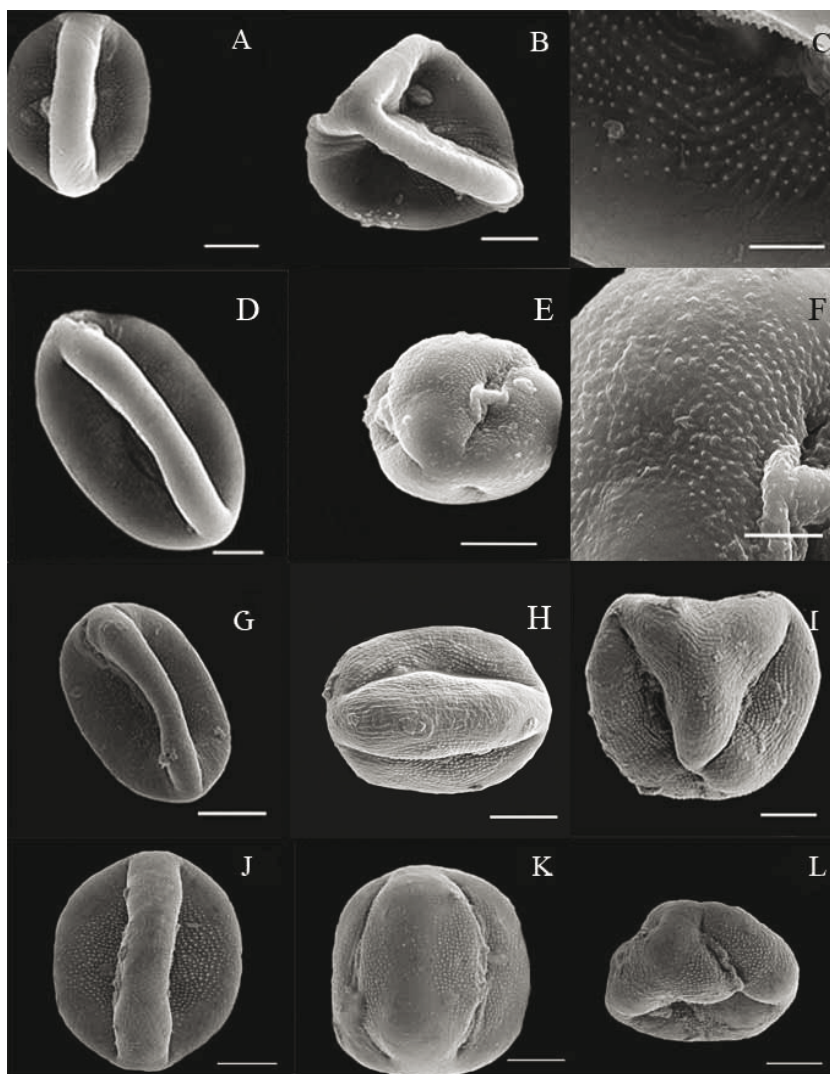


Figure 2. A–L. SEM micrographs of pollen grains in species of *Alchemilla*: *A. pseudocartalinica* A–C; *A. hessii* D; *A. citrina* E, F; *A. pectinoloba* G–I; *A. erythropoda* J, L (scale bar = 5 μ m for A, B, D, E–L; 2 μ m for C, F).

with *A. erythropoda*, *A. rechingeri*, *A. fluminea*, *A. kurdica*, and *A. hessii*, and A3) a branch with *A. condensata*; B) a branch with *A. citrina*, *A. rigida*, *A. hyrcana*, *A. pseudocartalinica*, *A. plicatissima*, and *A. sedelmeyeriana*; and C) a branch with *A. caucasica* at the base of the phenogram.

3.4. Principal component analysis (PCA)

Eigen analysis results from the PCA and loading scores of three principal components are presented in Tables 5 and 6 and a score plot is shown in Figure 6. The three components describe 89.9% of the pollen morphological character variation between 16 individuals.

The projections of the loadings defined by the first two principal components describe the position of 16 studied species. The first principal component (PC1) scoring system is dominated by equatorial axis

(E), polar axis/equatorial axis ratio (P/E), ratio of the distance between the apices of two ectocolpi (d), and mesocolpium index (Me). Along the first axis a group of 10 species: *A. amardica*, *A. pectinoloba*, *A. sericata*, *A. erythropoda*, *A. rechingeri*, *A. fluminea*, *A. kurdica*, *A. hessii*, *A. condensata* and *A. caucasica* with 31.6% of the total variation was segregated. The second principal component (PC2) scoring system is dominated by polar axis (P) and colpus length (CL) (Table 6). In the second axis, a group of *A. citrina*, *A. rigida*, *A. hyrcana*, *A. pseudocartalinica*, *A. plicatissima*, and *A. sedelmeyeriana*, with 29.8% of the total variation was formed. The third principal component (PC3) scoring system is dominated by polarity (Polr), number of colpi (ncol), and sculpturing (Sculp) (Table 6).

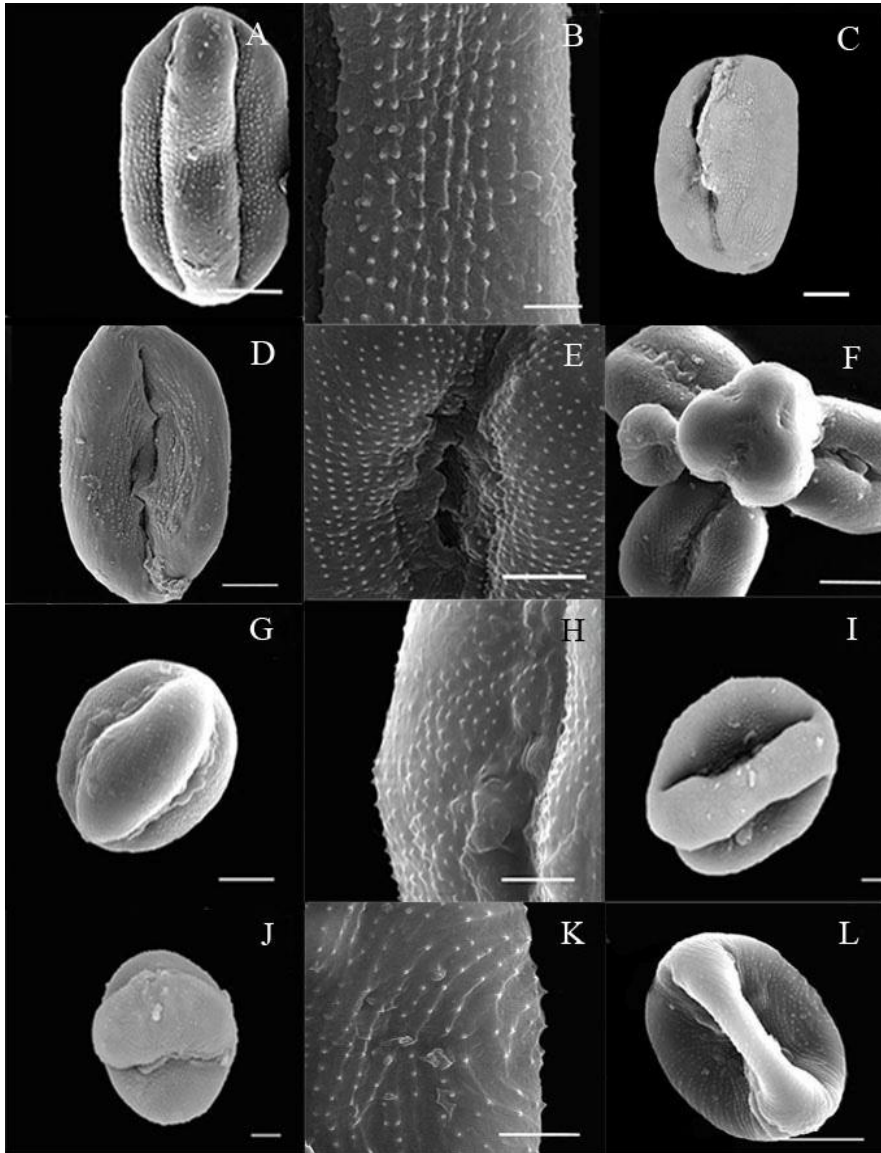


Figure 3. A–L. SEM micrographs of pollen grains in species of *Alchemilla*: *A. condensata* A, B; *A. rigida* C; *A. kurdica* D; *A. hyrcana* E, F; *A. sericata* G, H; *A. sedelmeyeriana* I, J; *A. fluminea* K, L (scale bar = 5 μm for A, C, D, F, G, I, J, L; 2 μm for B, E, H, K).

4. Discussion

The current results revealed the most important palynological characters within the genus *Alchemilla*. They include pollen polarity, outline, shape, and size; colpi length and number; and exine thickness and sculpturing. Based on our findings, the studied species have either isopolar and subsipolar pollen. Hebda et al. (1988) identified the isopolar pollen of *A. occidentalis* Nutt.

The pollen shape varies from prolate-spheroidal (Figure 1A) to subprolate (Figure 1B) and prolate (Figures 1C–1F). The prolate shape is the most dominant type and present among 14 studied species, while subprolate and

prolate-spheroidal shapes are the least common types. Murbeck (1901) and Strasburger (1905) reported variation in shape of some species, especially *A. vulgaris*, *A. alpina*, and *A. glabra*.

Regarding pollen size, the small grains are the most dominant (found in 22 species), whereas the medium pollen is recorded in two species: *A. fluminea* and *A. hessii*. These pollen morphological data are in agreement with the previous studies by Hebda et al. (1988) and Hebda and Chinnappa (1990). The size of the pollen grains can be helpful to distinguish some species, especially *A. hessii* for the largest and *A. sedelmeyeriana* for the smallest pollen grains.

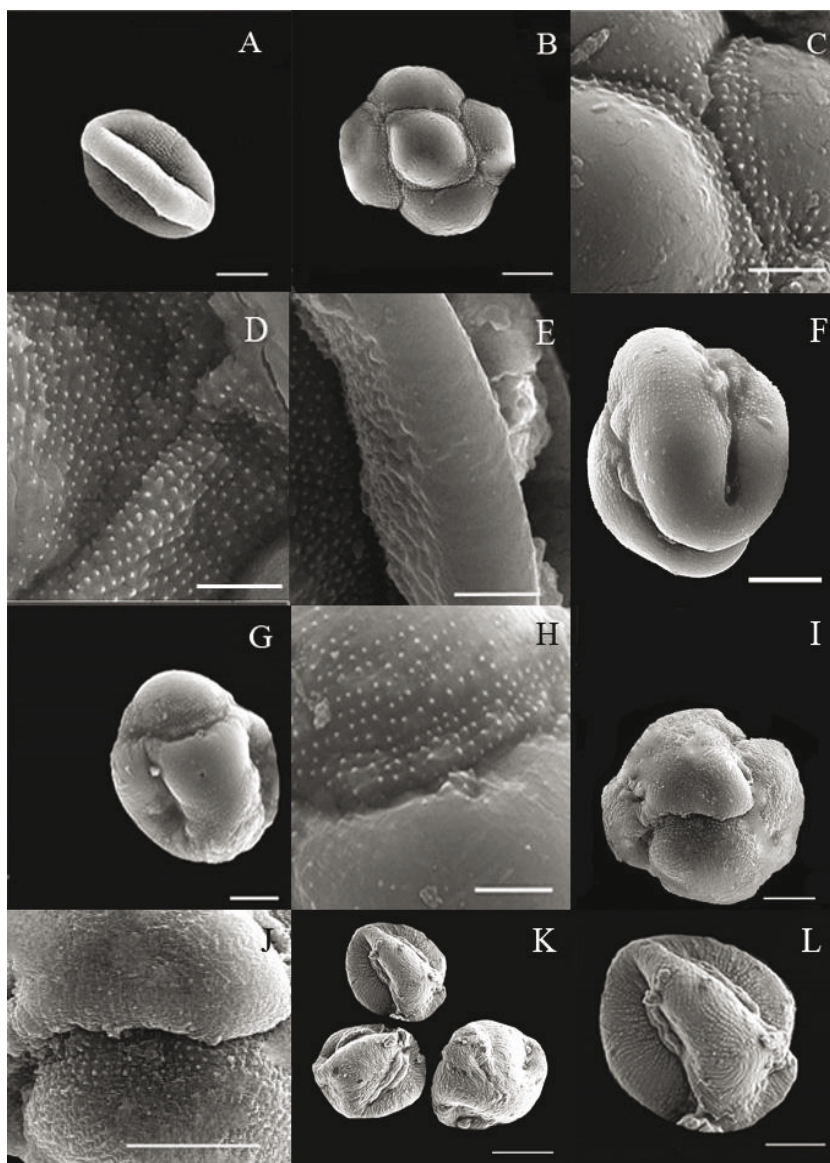


Figure 4. A–L. SEM micrographs of pollen grains in species of *Alchemilla*: *A. plicatissima* A–E; *A. caucasica* F–H; *A. amardica* I, J; *A. rechingeri* K, L (scale bar = 5 μ m for A, B, F, G, I–L; 2 μ m for C–E, H).

Previously, 3-colporate pollen was reported in some species of the genus, e.g., *A. occidentalis* (Hebda et al., 1988). We identified 3-colporate pollen grains in the majority of the studied species (Figures 1G–1I). However, in some species, especially *A. amardica* and *A. erythropoda* (with 30%), *A. caucasica* (with 60%–65%) (Figure 1H), and *A. plicatissima* (with 60%–70%) (Figures 1K, 1L) 4-colporate pollen grains were also recorded.

The colpus usually spans 80%–90% of the distance between poles (Hebda et al., 1988; Hebda and Chinnappa, 1990). In this survey, based on the colpus length, three main groups including 11–14 μ m, 15–19 μ m, and 20–25

μ m were recognized. Each of the first and second groups includes 8 representatives and the third one consists of two species.

The colpus membrane and margin are occasionally covered by microechinae (Figures 4D, 4E). The pore area is not distinct, because the pore is buried in the colpus showing microechinae (spinules) (Figures 3E, 3F). The endopore usually is slit-like in the colpus floor, e.g., in *A. rigida* and *A. kurdica* (Figures 3C, 3D) and the operculum is either fully absent or poorly formed, e.g., *A. citrina* (Figures 2E, 2F) and *A. sericata* (Figure 3H). *A. sedelmeyeriana* (Figure 3J) and

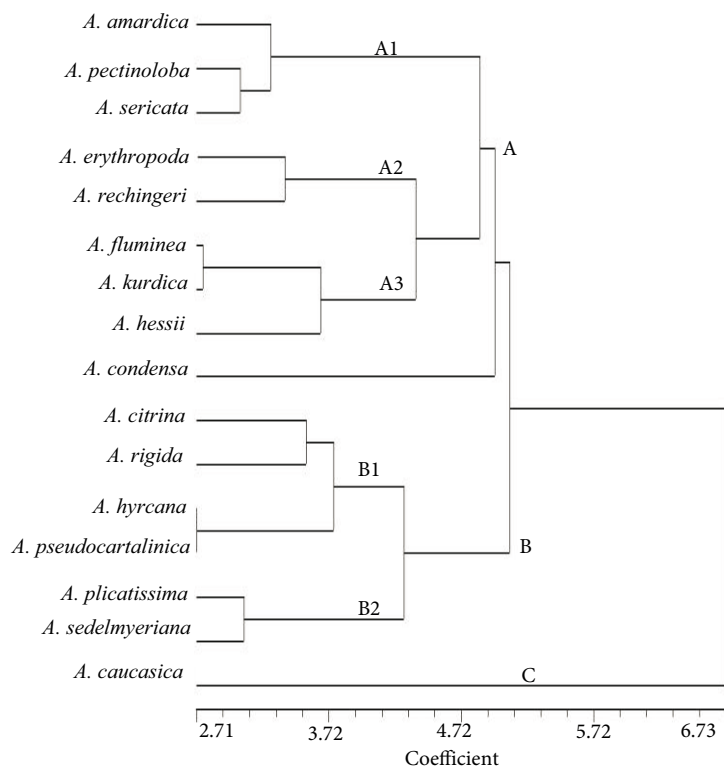


Figure 5. Phenogram of the 16 OTUs studied, clustering with UPGMA method.

Table 5. Eigen analysis of the correlation matrix.

	PC1	PC2	PC3
Eigenvalue	3.1661	2.9822	2.2538
Proportion	0.244	0.229	0.173
Cumulative	0.244	0.473	0.646

A. rechingeri (Figures 4K, 4L). These pollen grains' morphological data are consistent with previous studies by Hebda et al. (1988) and Hebda and Chinnappa (1990).

Thick exine is one of the palynological characteristics of the genus (Hebda and Chinnappa, 1990). Based on exine thickness 3 groups were recorded including 9 species with 0.95–1.24 μm , 4 species with 1.53–1.98 μm , and 3 species with more than 2 μm (2.04–2.25 μm).

The thickness of the exine is increased especially at the center of intercolpia, which is responsible for giving a subrectangular outline of the pollen in equatorial view and shifting the apertures to an interangular position (Reitsma, 1966). However, in *A. rechingeri* (Figure 1G) the exine is thickened at the corners and the pores are located at angles.

Ueda and Tomita (1989) reported the importance

of exine sculpture types in the family Rosaceae. Later, Hebda and Chinnappa (1990) divided the Canadian rosaceous pollen types into two broad categories and placed *Alchemilla* in the second category, containing psilate with microperforations sculpturing patterns. The exine sculpturing analysis of the present study revealed three main types, 4 subtypes, and 2 categories. However, the exine sculpturing type classes do not support a close relationship between the species and is of restricted taxonomic value.

In some species, especially in *A. persica*, rarely free functional pollen grains were collected. Occurrence of nonfunctional pollen was primarily recorded by Murbeck (1901) and Strasburger (1905) in *A. vulgaris*, *A. alpina*, and *A. glabra*.

Table 6. The variable loading scores for each principal component (PC1–PC3) for 16 species of the genus *Alchemilla* in Iran (the dominant coefficients for PC1, PC2, and PC3 scoring systems are underlined).

Variable	PC1	PC2	PC3
P	0.205	<u>-0.496</u>	-0.136
E	<u>0.445</u>	-0.326	-0.099
<u>P/E</u>	-0.443	-0.227	-0.068
d	<u>0.444</u>	-0.035	0.103
<u>d/D</u>	0.110	0.267	0.264
Me	<u>0.418</u>	0.163	0.159
<u>cl/p</u>	-0.287	-0.305	0.039
Cl	0.049	<u>-0.568</u>	-0.084
Et	-0.086	0.003	0.184
PS	0.160	-0.113	0.250
Pol	-0.087	0.167	<u>-0.540</u>
<u>NO.Cl</u>	0.205	0.085	<u>-0.506</u>
sculp	0.114	0.182	<u>-0.457</u>

Abbreviations used in Table 6: Polar axis (P), Equatorial axis (E), Polar axis/Equatorial axis (P/E), Pollen shape (Ps), Distance between the apices of two ectocolpi/equatorial diameter (d/D), Mesocolpium (Me), Colpus length/polar axis (Cl/P), Colpus length (Cl), Exine thickness (ET), Number of Colpi (No. Cl), Pollen shape (PS), Polarity (Pol), Sculpturing types (Sculp).

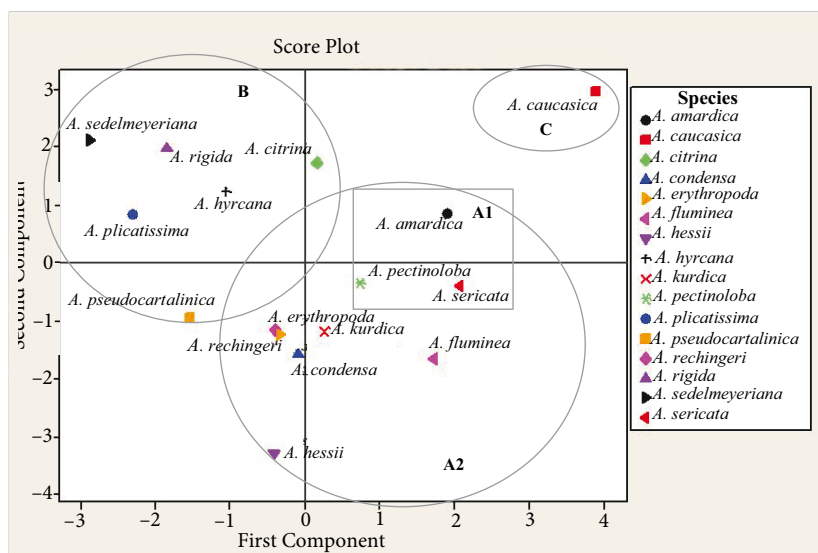


Figure 6. Principal components analysis score plot expressing the pollen morphological variation of 16 species of *Alchemilla* from Iran.

Generally, our findings revealed congruence between the UPGMA clustering and PCA analyses and the studied species were arranged into three groups: A) *A. amardica*, *A. pectinoloba*, *A. sericata*, *A. erythropoda*, *A. rechingeri*, *A. fluminea*, *A. kurdica*, *A. hessii*, and *A. condensa*; B) *A. citrina*, *A. rigida*, *A. hyrcana*, *A. pseudocartalinica*, *A. plicatissima*, and *A. sedelmeyeriana*; and C) *A. caucasica* (Figures 5 and 6).

The representatives of each group share some morphological evidence. For example, in subgroup A1, *A. amardica*, *A. pectinoloba*, and *A. sericata* are characterized by having a hairy hypanthium shorter than sepals. Among them, *A. amardica* and *A. sericata* have been considered as closely related species because their petiole is covered by appressed hairs (Fröhner, 1969; Khatamsaz, 1993).

There are several morphological affinities between different species of the large group A, e.g., all these representatives (except *A. hessii*) have a hypanthium shorter than sepals; *A. rechingeri*, *A. fluminea*, *A. kurdica*, and *A. hessii* share a glabrous hypanthium; *A. erythropoda* and *A. condensa* have a hairy hypanthium; and *A. erythropoda* resembles *A. rechingeri*, by having declinate hairs on the petiole of radical leaves. Among them, *A. kurdica* and *A. fluminea* also have been treated as closely allied species for having hairless stem, leaves with 7 lobes, and 7 to 9 teeth on each lobe by Fröhner (1969) and Khatamsaz (1993). These authors also considered the two endemic of Iran, *A. condensa* and *A. amardica*, as related species for possessing a hypanthium covered by dense appressed hairs. *A. condensa* differs from *A. amardica* in its broad leaf lobes and triangular sepals.

There are controversies regarding the relationship between 6 representatives of the second group (B). Based on Fröhner (1969) *A. citrina* resembles *A. gigantodus* (for its common erecto-patent hairs on the petiole of radical leaves), while according to Khatamsaz (1993) it has more common morphological features with *A. rigida* (for having

hypanthium shorter than sepals and all parts covered by hairs) and *A. caucasica* (by erecto-patent hairs on the petiole of radical leaves). However, earlier, Juzepczuk (1941) treated *A. rigida* and *A. sericata* in the first series and *A. caucasica* in the second series of the section Pubscentes Buser. and subgenus Pes-Leonis Juz. He also arranged *A. hyrcana*, *A. sedelmeyeriana*, and *A. pseudocartalinica* in the section Vulgares Buser. (for sharing pedicels varying in length; inner sepals shorter than or as long as hypanthia, and glabrous like hypanthia or more or less hairy). *A. hyrcana* and *A. pseudocartalinica* resemble each other by their hairless pedicel and hypanthium. According to *Flora Iranica* and *Flora of Iran*, *A. hyrcana* and *A. sedelmeyeriana* was treated as related species because both share a thin stem, hairy leaf (on both sides), and more or less hairy hypanthium (Fröhner, 1969; Khatamsaz, 1993).

In addition, Khatamsaz (1993) regarded three endemics species (*A. plicatissima*, *A. amardica*, and *A. condensa*) as closely related for possessing erecto-patent hairs covering all parts.

In conclusion, our findings revealed the palynological characteristics (e.g., pollen polarity, size, exine sculpturing) of the genus *Alchemilla*. These pieces of evidence are reliable criteria for explaining species relationships. The current numerical analysis supports the relationship between some species, especially *A. amardica*, *A. sericata*, *A. fluminea*, *A. kurdica* (of group A); *A. hyrcana*, *A. sedelmeyeriana*, and *A. pseudocartalinica* (of group B), but its application is restricted for others.

Acknowledgments

This study was supported by grants from research assistance of Guilan University, Rasht, Iran. We would like to thank Rahmani from Razi Metallurgy Research Institute (Tehran, Iran) for taking the SEM photographs.

References

- Asker SE, Jerling L (1992). Apomixis in Plants. Boca Raton, FL, USA: CRC Press.
- Buser R (1892). Nouvelle classification du genre *Alchemilla*. Bull Soc Bot Lyon 10: 34–35 (in French).
- De Candolle AP (1825). *Alchemilla*. In: Prodr. Syst. Nat. Regn. Veg., vol. 2, Treuttel et Würtz, Paris, p. 590.
- Eide F (1981). Key for northwest European Rosaceae pollen. Grana 20: 101–118.
- Erdtman G (1952). Pollen morphology and plant taxonomy. Angiosperms. Stockholm, Sweden: Almqvist and Wiksells.
- Eriksson T, Donoghue MJ, Hibbs MS (1998). Phylogenetic analysis of *Potentilla* using sequences of nuclear ribosomal internal transcribed spacers (ITS), and implications for the classification of Rosoideae (Rosaceae). Plant Syst Evol 212: 155–179.
- Eriksson T, Hibbs MS, Yoder AD, Delwiche CF, Donoghue MJ (2003). The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the trnL/F region of chloroplast DNA. Int J Plant Sci 164: 197–211.
- Focke WO (1888). *Alchemilla*. In: Engler A, Prantl K, editors. Die Natürlichen Pflanzenfamilien. Vol. 3. Berlin. p. 43 (in German).

- Fröhner S (1969). *Alchemilla* (Rosaceae) *Flora Iranica* (ed. K. H. Rechinger). Vol. 66/30, pp. 124–147.
- Fröhner SE (1995). *Alchemilla*. In: Scholz H, Conert HJ, Jäger EJ, Kadereit JW, Schultze-Motel W, Wagenitz G, Weber HE, editors. Hegi: Illustrierte Flora von Mitteleuropa, vol. 4 Teil 2B. Berlin, Germany: Verlag Paul Parey, pp. 13–242 (in German).
- Gehrke B, Bräuchler C, Romoleroux K, Lundberg M, Heubl G, Eriksson T (2008). Molecular phylogenetics of *Alchemilla*, *Aphanes* and *Lachemilla* (Rosaceae) inferred from plastid and nuclear intron and spacer DNA sequences, with comments on generic classification. *Mol Phylogenet Evol* 47: 1030–1044.
- Harley MM (1992). The potential value of pollen morphology as an additional taxonomic character in subtribe Ociminae (Ocimeae, Nepetoideae, Labiatae). In: Harley RM, Reynolds T, editors. *Advances in Labiatae Science*. Richmond, UK: Royal Botanic Gardens, Kew, pp. 125–138.
- Hayirlioglu-Ayaz S, Inceer H (2009). Three new *Alchemilla* L. (Rosaceae) records from Turkey. *Pak J Bot* 41: 2093–2096.
- Hebda RJ, Chinnappa CC (1990). Studies on pollen morphology of Rosaceae in Canada. *Rev Palaeobot Palynol* 64: 103–108.
- Hebda RJ, Chinnappa CC, Smith BM (1988). Pollen morphology of the Rosaceae of western Canada. II. *Dryas*, *Fragaria*, *Holodiscus*. *Can J Bot* 66: 595–612.
- Horandl E (2004). Comparative analysis of genetic divergence among sexual ancestors of apomictic complexes using isozyme data. *Int J Plant Sci* 165: 615–622.
- Izmailow R (1981). Karyological studies in species of *Alchemilla* L. from the Calycinae Bus. (Section Brevicaulon Rothm.). *Acta Biol Cracov Bot* 23: 117–180.
- Juzepczuk SW (1941). *Alchemilla* L. In: Komarov VL, editor. *Flora U.S.S.R.*, Izd. Akad. Nauk. S.S.S.R., Moskva. -Leningrad 13: 289–410.
- Kalkman C (2004). Rosaceae: 13. *Alchemilla* group. In: Kubitzki K, editor. *Flowering Plants. Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales*, vol. 6. Berlin, Germany: Springer, pp. 371–372.
- Khatamsaz M (1993). *Flora of Iran: Rosaceae*. Research Institute of forests and rangeland, Tehran, Iran. Vol. 6, pp. 88–140.
- Lagerheim NG (1894). Ueber die andinen *Alchemilla*-Arten. *Kongl. Svenska Vetensk Acad Handl* 51: 15–18.
- Murbeck S (1901). Pathogenetische Embriebildung in der Gattung *Alchemilla* Lund Univ. Arssk. 36. Afd. 2. Nr.7.1-41.
- Notov AA, Kusnetzova TV (2004). Architectural units, axiality and their taxonomic implications in *Alchemillinae*. *Wulfenia* 11: 85–130.
- Punt W, Hoen PO, Blackmore S, Nilsson S, Thomas AL (2007). Glossary of pollen terminology. *Rev Palaeobot Palynol* 143: 1–81.
- Reitsma, T.J (1966). Pollen morphology of some European Rosaceae. *Acta Bot Neerl* 15: 290–307.
- Rohlf FJ (1993). NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.80. Exeter Software: Setauket, New York.
- Rothmaler W (1944). Zur nomenklatur der Europäischen *Alchemilla*-Arten. *Svenska Botaniska Tidskrift* 38: 102–112 (in German).
- Ryan BF, Joiner BL (2001). *MINITAB Handbook*, Fourth Edition, Duxbury.
- Schulz-Menz GK (1964). Rosales. In: Melchior H, editor. *A. Engler's Syllabus der Pflanzenfamilien*. Berlin, Germany: Borntraeger (in German).
- Sepp S, Bobrova VK, Troitsky AK, Glazunova KP (2000). Genetic polymorphism detected with RAPD analysis and morphological variability in some microspecies of apomictic *Alchemilla*. *Ann Bot Fennici* 37: 105–123.
- Soják J (2008). Notes on *Potentilla* XXI. A new division of the tribe *Potentilleae* (Rosaceae) and notes on generic delimitations. *Bot Jahrb für Syst* 127: 349–358.
- Strasburger E (1905). Die Apogamic der *Eualchemillen* und allgemeine Gesichtspunkte die sich aus ihr ergeben. *Jahrb. Wiss Bot* 41: 88–164 (in German).
- Ueda Y, Tomita H (1989). Morphometric analysis of pollen exine patterns in roses. *J Japan Soc Hort Sci* 58: 211–220.