Foliar epidermis micromorphology of the genus *Alchemilla* (*Rosaceae*) in Iran

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Abstract. The foliar epidermis micromorphology of 24 species of *Alchemilla* (*Rosaceae*) was studied by scanning electron microscopy (SEM). The trichomes were examined on both abaxial and adaxial leaf surfaces. Two trichome type classes were recognized: the straight cylindrical appressed-subappressed and the straight cylindrical – flat ribbon-shape hairs. The first type was dominant and consisted of two subtypes. Glandular trichomes were either one-celled stalk with capitated head cell or a three-four-celled stalk with subglobular head cell. Epicuticular waxes were composed of two main types, including film (the crust, smooth layer) and crystalloids (membrane and irregular, rosette, aggregate platelets and plates). In all examined species, the stomata were situated lower than the epidermal layer and their shape varied from oval to round and elliptical. Three types of epicuticular wax distribution on the stomata surface were recorded. The foliar epidermis micro morphological characters proved to play an outstanding role in the taxonomy and classification of the genus. A key to Iranian species based on foliar epidermal characters is presented.

Key words: *Alchemilla,* epicuticular wax, stomata, trichomes

Introduction

Alchemilla L. is a large genus (with c. 1000 species) in the tribe *Potentilleae* (*Rosaceae*), subtribe *Alchemillinae,* (Notov & Kusnetzova 2004; Soják 2008). It is distributed in the Holarctic, especially in West Eurasia (Gehrke & al. 2008). However, it also grows in the mountains of East Africa, South India, Sri Lanka, and Java (Izmailow1981). Fröhner's (1969) treatment of *Alchemilla*, revealed 31 species in the area covered by *Flora Iranica*. This genus has 24 species in Iran. Of these, 14 are endemic to Iran (Fröhner 1969; Khatamsaz 1993). The Iranian species of *Alchemilla* are mainly distributed in the north and northwest of the country. However, there are some species that grow in the west (e.g. *A. kurdica*, *A. persica* and *A. pseudocartalinica*) and centre (e.g. *A. hessii*, *A. rechingeri* and *A. fluminea*) of Iran, too (Fröhner 1969; Khatamsaz 1993). The representatives of this genus are perennial herbs with a woody rhizome (Pawlowski & Walters 1972), commonly growing in open damp grasslands, in shady places, on river banks, forest edges, alpine and subalpine meadows, and stony mountain slopes (Fröhner 1969; Khatamsaz 1993).

With its critical and taxonomically difficult group, this genus forms an interesting object for taxonomical surveys. They were initiated by the classical survey of Linnaeus (1753), Focke (1888) and Lagerheim (1894) and continued by other botanists, such as Juzepczuk (1941), Rothmaler (1944), Pawlowski (1972), and followed by the recent researches of Hayirlioglu-Ayaz (2000), Kalkman (2004), Notov & Kusnetzova, (2004), Hayirlioglu-Ayaz & Inceer (2009), as well as by the molecular survey of Gehrke & al. (2008). Several researchers examined the foliar wax nanopattern structure and its multifunctional properties (e.g. water repellency, self-cleaning, Lotus effect and superhydrophobicity: Riding & Percy 1985; Neinhuis & Barthlott 1997; Wagner & al. 2003; Mock & al. 2005; Shirtcliffe & al. 2009, Koch & al. 2009; Kim 2012) by using different species of *Alchemilla* (especially *A. di-*

plophylla, A. vulgaris and *A. mollis*). However, taxonomic importance of the foliar micromorphological features was seldom studied in this genus (Barthlott & al. 1988). These criteria proved to be diagnostically useful in different families (Metcalfe & Chalk 1950; Barthlott & al. 1998), and especially in *Rosaceae* (e.g. Eriksen & Yurtsev 1999; Wissemann 2000; Kolodziejek 2008; Ganeva & Uzunova 2010; Faghir & al. 2010).

The main aims of the current research are to provide a detailed account of the foliar epidermis structure in the Iranian species of *Alchemilla* and strong evidence on their taxonomic implication.

Table 1. Grouping of species based on trichome types, (glands are indicated by*).

Species		Group
TI subtype A		
1. *A. amardica**	Appressed-subappressed (Pilose, sericeous,hirsute)	**Type I** Straight, cylindrical
2. *A. citrina*		
3. *A. condensa*		
4. *A. farinosa*		
5. *A. hyrcana*		
6. *A. gigantodus**		
7. *A. pectinoloba*		
8. *A. persica*		
9. *A. rechingeri*		
10. *A. retinervis*		
11. *A. rigida*		
12. *A. sedelmeyeriana*		
TI subtype B		
13. *A. caucasica**	Flexuose (villose)	
14. *A. erythropoda**		
15. *A. surculosa*		
16. *A. melancholica*		
17. *A. plicatissima**		
18. *A. valdehirsuta*		
TII		
19. *A. kurdica**	Appressed-subappressed	**Type II** Straight, cylindrical and flat, twisted ribbon-shaped
20. *A. fluminea*		
21. *A. hessii**		
22. *A. pseudocartalinica*		
23. *A. sericata*		
24. *A. microscopica*		

Material and methods

In the current study, we used well-developed basal leaves, both fresh (collected from 2010 to 2012, in spring and late summer) and from dried herbarium specimens of the Guilan University Herbarium (GUH), Tehran University Herbarium (TUH) and Herbarium of the Research Institute of Forests and Rangelands of Iran (TARI). Voucher specimens of each newly collected species was deposited in the Guilan University Herbarium (GUH). *Flora Iranica* (Fröhner 1969) and *Flora of Iran* (Khatamsaz 1993) were the principal reference books for identification. The species samples are listed in Appendix 1, with nomenclatural authorship for all species. For scanning electron microscopy (SEM), leaves (or portions of them) were taken from the central lobe. They were washed with water detergent for about three hours in a water bath at 45 °C and then dried out. Dried materials were mounted on SEM stubs by double-sided adhesive tape of silver paint and finally coated with gold with a sputter coater. Scanning electron microscopy was carried out by a Vega Tescan Razi instrument. The terminology of trichomes, wax ultra-structures and types follows mainly that of Fröhner (1969), Juzepczuk (1941) and Barthlott & al. (1998).

Results

Trichomes

Scanning electron microscopy revealed two types of leaf trichomes (Table 1) (Fig. 1 A-B).

Type I: straight, cylindrical appressed-subappressed hairs. This type comprises to two subtypes.

T1 subtype A: includes spare to moderately dense pilose, sericeous, hirsute hairs (found in 12 species of Table 1) (Fig. 1 C-D). The straight trichomes consist of a unicellular body 0.8–2 mm long and a basal stalk of 5–7 cells forming a small cushion. They are either smooth or verrucose. T1 subtype B: includes relatively dense flexuose (villose) hairs (found in six species of Table 1) (Fig. 1 E-F). The flexuose (villose) hairs are fairly long, soft, straight, or interwoven ascending.

Type II: straight, cylindrical-flat ribbon-shaped hairs (observed in six species of Table 1), (Fig. 1 G-H). The ribbon-shaped trichomes are long, soft, twisted ascending.

Two types of glandular trichomes were recorded: 1) one-celled stalk with capitated head cell (e.g. in *A. amardica* (Fig. 2 A), *A. caucasica*); 2) 3–4 celled stalk with subglobular–cylindrical head cell (e.g. in *A. erythropoda, A. gigantodus* (Fig. 2 B), *A. hessii, A. plicatissima*). In many species the glands were not distinguishable due to dense trichomes and wax deposits.

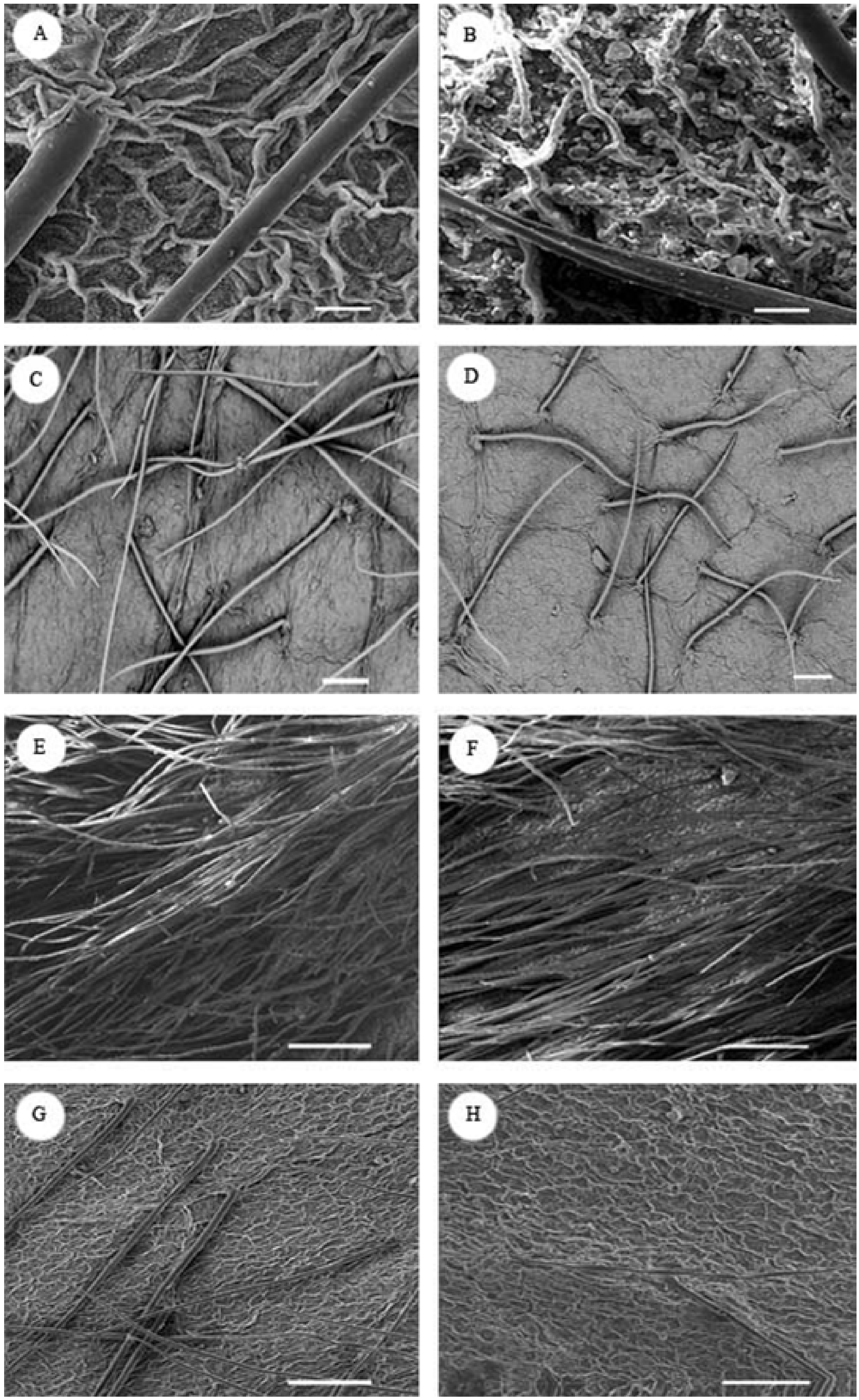

Fig. 1. *A. fluminea* (A-G), *A. sericata* (B), *A. hyrcana* (C), *A. rechingeri* (D), *A. caucasica* (E), *A. erythropoda* (F) , *A. kurdica* (H), A: scale bar = 10 µm, B: scale bar =20 µm; C-D: scale bar = 100 µm, E-H: scale bar = 200 µm.

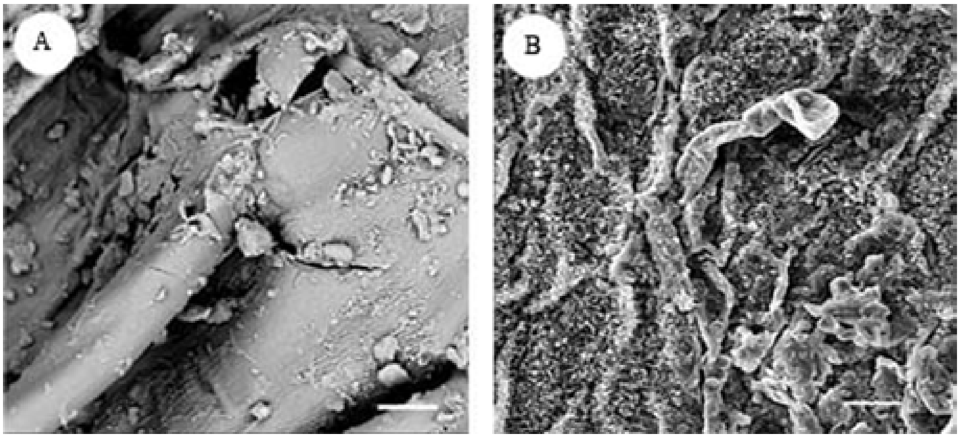

Fig. 2. *A. amardica* (arrow indicates the gland) (A), *A. gigantodus* (B), A: scale bar = 10 µm, B: scale bar = 20 µm.

Waxes

Wax characteristic features are presented in Table 2. Two main types of epicuticular waxes were identified, as follows:

1. Layers and crusts (including smooth layer and crust)
2. Crystalloid platelets (including membranous, irregular, aggregate and rosette platelets).

Smooth layers: continuous covering, less than 1 µm thick, without any prominent surface sculpturing (Fig. 3 A-B)

Platelets and plates: Flat crystalloids attached to the surface by their narrow side and at different angles. Their margin may be entire or irregular.

Granules: irregular, mostly isodiametric, often rounded crystalloids (Fig. 3 C).

Entire platelets: flat crystalloids with entire margin and regular shape, usually 1–3 µm in height,

protruding ± perpendicularly from the surface, often semicircular and arranged into characteristic orientation patterns (Fig. 3 D).

Membranous platelets: flat, membranous, usually interconnected crystalloids protruding from the surfaces at an acute angle, often with threadlike extensions (Fig. 3 E).

Irregular plateletes: flat crystalloids with irregular margin, 1–10 µm in height, protruding perpendic-

Table 2. Laminar wax patterns type.

Wax pattern on the upper surface	Wax pattern on the lower surface	Species
Smooth layer with scattered platelets	Smooth layer with scattered platelets	1. *A. kurdica*
Smooth layer with scattered platelets	Smooth layer with scattered platelets	2. *A. hessii*
Smooth layer with scattered platelets	Smooth layer with scattered platelets	3. *A. sedelmeyeriana*
Crust with granules	Crust with scattered granules	4. *A. melancholica*
Smooth layer with granules and platelets	Membranous platelets	5. *A. hyrcana*
Smooth layer with granules and platelets	Membranous platelets	6. *A. amardica*
Crust	Membranous platelets	7. *A. persica*
Membranous platelets	Membranous platelets	8. *A. rechingeri*
Membranous platelets	Membranous platelets	9. *A. farinosa*
Membranous and scattered irregular platelets	Membranous platelets	10. *A. surculosa*
Membranous and scattered irregular platelet	Membranous platelets	11. *A. pectinoloba*
Membranous platelets	Crust with irregular platelets	12. *A. citrina*
Scattered membranous, irregular platelets and granules	Membranous platelets	13. *A. fluminea*
Dense irregular platelet and granules	Membranous platelets	14. *A. sericata*
Dense irregular platelets	Crust with membranous platelets	15. *A. erythropoda*
Membranous platelets	Crust and membranous platelets	16. *A. microscopica*
Crust with membranous platelets	Crust with membranous and scattered irregular platelet	17. *A. plicatissima*
Crust with scattered irregular platelet	Crust with scattered irregular platelets	18. *A. retinervis*
Crust with scattered irregular platelet	Crust with round non-entire platelets and plates	19. *A. pseudocartalinica*
Crust	Rosette and aggregated platelets	20. *A. caucasica*
Crust with aggregate and round non-entire platelets	Crust with rosette and aggregated platelets and plates	21. *A. rigida*
Membranous and scattered irregular platelet	Dense irregular platelets	22. *A. condensa*
Membranous and scattered irregular platelet	Dense irregular platelets	23. *A. gigantodus*
Irregular platelets	Dense irregular platelets	24. *A. valdehirsuta*

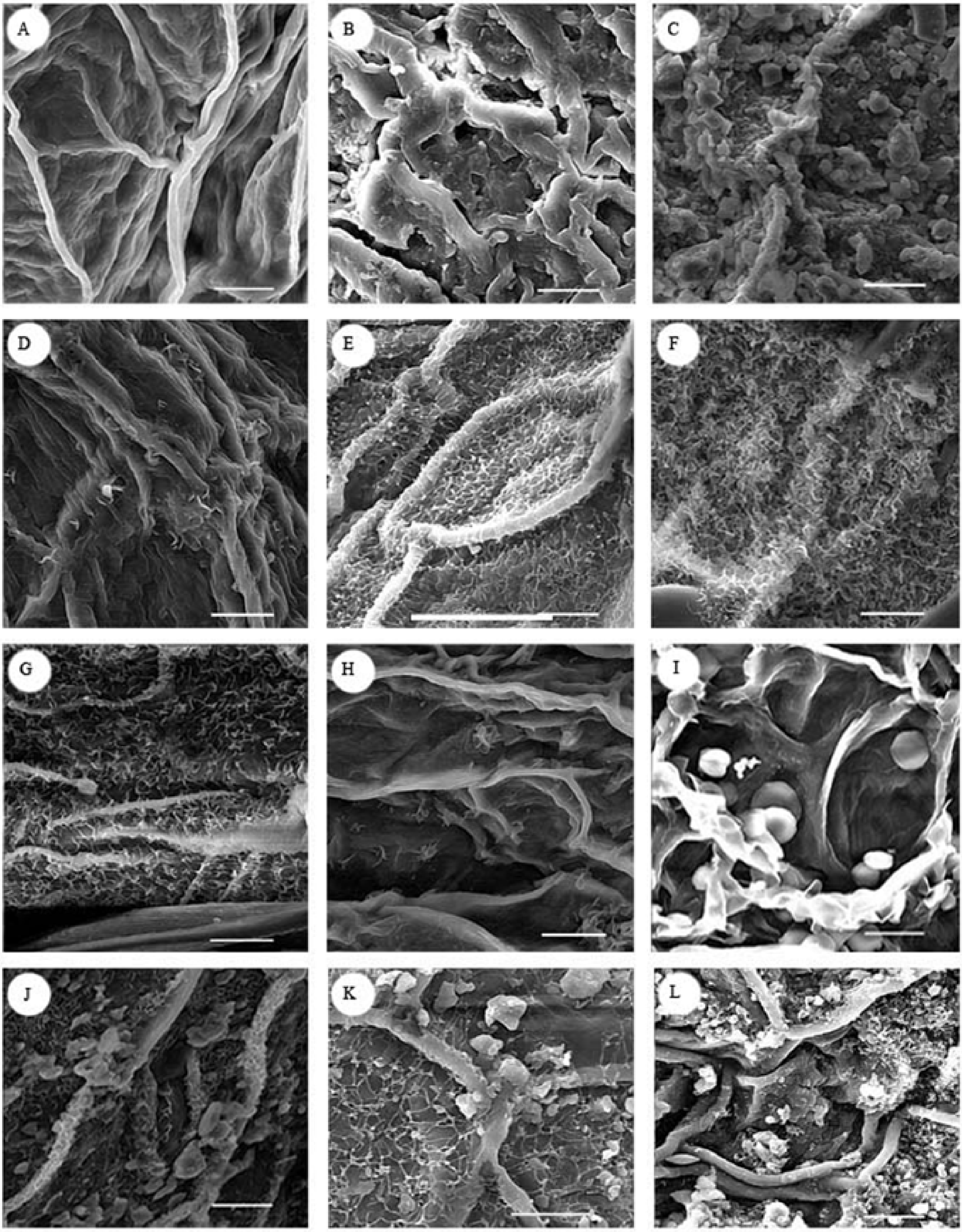

Fig. 3. *A.hessii* (A), *A. erythropoda* (B and F), *A. sericata* (C), *A. sedelmeyeriana* (D), *A. persica* (E), *A. caucasica* (G), *A. rigida* (H and J), *A. pseudocartalinica* (I), *A. condensa* (K), *A. retinervis* (L); A-B, E, G-H, J-L – leaf lower surface; C-D, F, I – leaf upper surface, scale bar = 10 μm.

ularly from the surface. Occasionally arranged in characteristic orientation patterns (Fig. 3 F).

Rosette plateletes*:* platelets arranged into multiple, more or less radially assembled clusters evenly spread over the epidermal surface (Figs. 3 G-H)

Plates: Flat, often polygonal crystalloids, with distinct edges, commonly found over veins of leaves, often imbricated like tiles and seldom orientated in a particular direction (Fig. 3 I).

On the basis of our findings four types of syntopism were identified (Table 2):

1) Rosette and aggregated platelets (Fig. 3 G).
2) Aggregate, irregular, round non-entire platelets and plates (Fig. 3 J).
3) Membranous and scattered irregular platelets (Fig. 3 K).
4) Granules and membranous platelets (Fig. 3 L).

According to the current analysis, platelets were the most dominant wax structure (in 24 species) and membranous platelets were the most abundant wax pattern (in 18 species) in this genus. Out of 24 studied representatives, 11 species have similar wax pattern on the eider side of the leaf surfaces. While the remaining 13 species have different wax patterns on the lower and upper leaf surfaces (Table. 2).

Stomata

Stomata were lower than the epidermal surface in all examined species. Three types of wax distribution on the stomata rim, guard cell and subsidiary cell surface were identified:

Type I: rim and pore without wax and guard cell covered by wax, e.g. (Fig. 4 A-B).

Type II: rim and guard cell covered by wax, pore without wax (Fig. 4 C-D).

Type III: rim, guard cell and pore covered by wax (Fig. 4 F-E).

The stomata wax Type II is the most abundant and occurs both on the upper (13 species) and lower (11 species) sides of the leaf surfaces. On the contrary, Type III is less dominant and found on the leaf upper surface of *A. microscopica* and *A. gigantodus,* and lower surface of *A. sericata* and *A. farinosa*.

The stomata shape varies from oval (e.g. *in A. fluminea* and *A. condensa*) to round (e.g. in *A. pectinoloba*) and elliptical (e.g. in *A. sericata*). In some species, e.g. *A. kurdica* (Fig. 4 C), stomatal chimneys derived from smooth layers and crusts (Barthlott & al. 1998) were recognized.

Discussion

Our survey into epidermal structures revealed the taxonomical importance of micro morphological characters. The hair types in the genus *Alchemilla* follow the general characteristic features of the family *Rosaceae* (Metcalfe & Chalk 1950; Eriksen & Yurtsev 1999; Wissemann 2000; Kolodziejek 2008; Ganeva & Uzunova 2010; Faghir & al. 2010) and are a diagnostic character in taxonomy of the genus (Swan & Walters 1988). Juzepczuk (1941) divided this genus into two subgenera (Argentaria Juz and Pes-Leonis Juz.), three sections, and several artificial groups and subgroups based on some diagnostic morphological characters, especially the trichome types (Juzepczuk 1941). For example, he placed *A. sericata* and *A. rigida* in the Saricatae Juz. series (different plant parts covered by appressed hair)*, A. caucasica* and *A. erythropoda* in the Flabellate Juz. series (different plant parts covered by ascending hairs) and *A. valdehirsuta* in the Vulgares Buser section, group Hirsutae H. Lindb fil., subgroup Barbulatae Juz. and Valdehirsutae Juz. series. In their taxonomical treatment, Fröhner (1969) and Khatamsaz (1993) used the trichome types for separating the different species. However, the presence of ribbon-shaped hairs was ignored in the earlier studies (Juzepczuk 1941; Fröhner 1969; Khatamsaz 1993).

On the basis of the current analysis, trichome types are constant in the different populations. However, the length, size and amount of leaf hairiness (in the same plant) varies between the young and old leaves and the leaves produced in early and later growing seasons (Juzepczuk 1941). Therefore, this evidence is of a more restricted taxonomic value (Metcalfe & Chalk 1950). Many studied species (Table 1) possess moderate to dense hairs, which develop smooth, shinning silky leaf surfaces (Swan & Walters 1988). These hairs protect the leaf against excessive insulation, (Mensah 2012), increase light reflectance from the leaf surface, provide surface ventilation (Haberlandt 1928), and help the leaf in collecting raindrops (Shirtcliffe & al. 2009). The plants that collect water droplets are mostly xerophytes or, like the *Alchemilla* species, live on limited resources (Shirtcliffe & al. 2009). This includes the species that we have collected from the alpine and

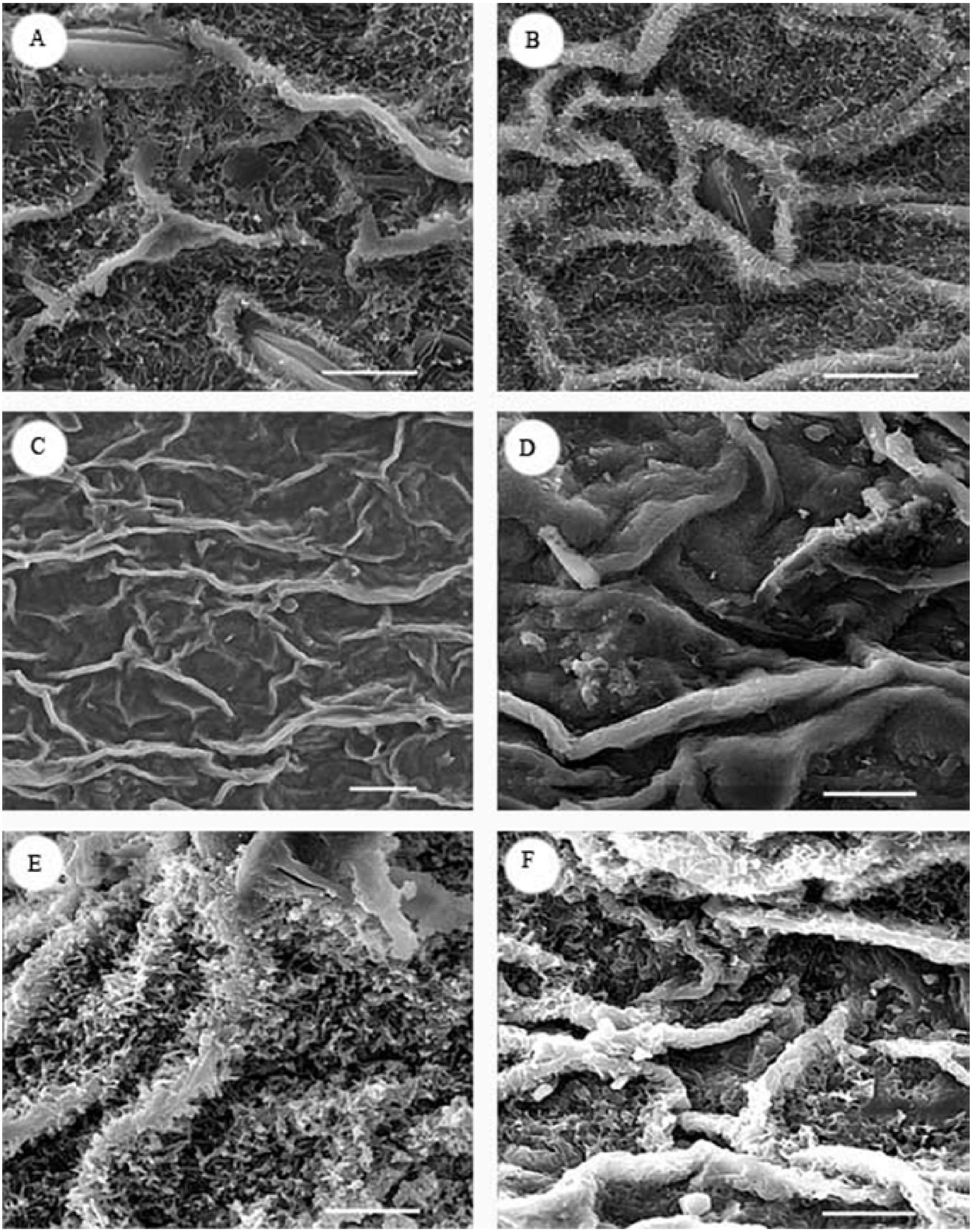

Fig. 4. *A. surculosa* (A), A. *fluminea* (B), *A. kurdica* (arrow indicates the stomata chimney) (C), *A. retinervis* (D), *A. gigantodus* (E), *A. microscopica* (F) – stomata are on the lower surface, scale bar = 10 μm.

subalpine regions (1700–3300 m a.s.l.), especially from the stony mountain slopes (e.g. *A. plicatissima* and *A. caucasica*), rock outcrops in the forests (e.g. *A. erythropoda*), and which thrive in cracks of the rocks (e.g. *A. rechingeri* and *A. sericata*) and even on tree bark (e.g. *A. sedelmeyeriana*).

Epicuticular wax data on genus *Alchemilla* are reported primarily in some broader studies on the significant role of epicuticular wax in classification of the order Rosales (Fehrenbach & Barthlott 1988). Micromorphology of leaf epicuticular waxes in the Iranian species of *Alchemilla* is typical of that of the *Rosaceae* family (Fehrenbach & Barthlott 1988; Wissemann 1998; Neinhuis & Barthlott 1997). Superimposed wax structures (Koch & al. 2009) composed of layers, crusts and crystalloids platelets (including membranous, irregular, aggregate, rosette platelets and plates) have been reported earlier in other genera of the *Rosaceae* family (Wissemann 2000; Jetter & Schaffer 2001; Gostin & Adumitresei 2010). Membranous platelets (Barthlott & al. 1998) or triangular rodlets (Wissemann 2000) are the dominant wax type found in the studied representatives.

Of the studied species, 12 possess the same type of wax distribution on the stomata rim, pore and guard cell on either side of the leaf surfaces. This includes seven species (A. *kurdica*, *A. pseudocartalinica*, *A. retinervis*, *A. hessii, A. melancholica*, *A. sedelmeyeriana* and *A. rigida*) of Type II, three species (*A. valdehirsuta*, *A. surculosa* and *A. rechingeri*) of Type I and two species (*A. microscopica* and *A. gigantodus*) of Type III. The other 12 species have different type of wax pattern on the rim, pore and guard cell of the upper and lower side of the leaf surfaces. This includes six species (*A. amardica*, *A. caucasica*, *A. condensa, A. fluminea*, *A. pectinoloba* and *A. persica*) with Type I on the lower and Type II on the upper side of the leaves; two species (*A. sericata* and *A. farinosa*) with Type I on lower and Type III on the upper side, and three species (*A. plicatissima* and *A. erythropoda* and *A.citrina*) with Type II on lower and Type III on the upper leaf surface. *A. hyrcana* with Type II on the lower and Type I on the upper, and *A. citrina* with Type III on the lower and Type II on the upper leaf surface were also observed.

The presence of trichomes and epicuticular wax crystals among the studied species has strong correlation with their different habitats. For species that grow in open, hot, and arid habitats, the trichomes (shading the leaf surface from UV light) reduce both the heat load and absorbance of solar radiation, and the epicuticular wax crystals (scatter light, giving the leaf surface a glaucous, grayish appearance, limit the penetration of UV light, as well as photosynthetically active radiation) protect the plant body from the dry atmosphere and UV rays (Anurag & al. 2009). *A. caucasica*, *A. erythropoda, A. melancholica*, *A. plicatissima*, *A. sericata,* and *A. valdehirsuta* have been collected in open sunny alpine meadows and possess both dense hairs and wax covering. *A. condensa, A. farinosa*, *A. gigantodus* and A. *rigida*, *A. rechingeri*, and *A. retinervis* have spare to moderately dense hairs, but benefit from dense epicuticular wax covering. These species are distributed on stony mountain slopes or in open arid habitats. In contrast, *A. amardica*, *A. citrina*, *A. hyrcana*, *A. pectinoloba,* and *A. sedelmeyeriana* prefer shady places. These species have been collected under the thorny bushes of *Berberis* sp., or under the large canopy of *Fagus orientalis*.

Furthermore, some species of *A. kurdica*, *A. fluminea*, *A. hessii*, *A. sericata, A. pseudocartalinica,* and *A. microscopica* possess a mixed type of trichomes (straight, cylindrical and flat, twisted ribbon-shape, appressed-subappressed), in which the flat flexible hairs have additional effect on superhydrophobicity of the leaf surface (Shirtcliffe & al. 2009).

The current leaf epidermal micromorphology supports the earlier taxonomic findings (Juzepczuk 1941; Fröhner 1969; Khatamsaz 1993) and confirms the taxonomic importance and usefulness of micromorphological features of the leaf surfaces within the studied taxa. An identification key for the Iranian species of *Alchemilla* is presented on the basis of foliar epidermal characters.

Key to the species of genus *Alchemilla* based on the leaf epidermis features

1- Trichome straight, cylindrical, appressed-subappressed (Type I) 2

- Trichome straight, cylindrical, appressed-subappressed and flat ribbon-shaped (Type II) 19

2- Spare to moderately dense trichomes (pilose, sericeous, hirsute) 3

- Relatively dense flexuose (villose) trichomes 14

3- Smooth layer with scattered wax platelets or granules on one or both surfaces 4

- Membranous and irregular platelets, plus wax crusts on one or both surfaces 6

4- Both abaxial and adaxial surfaces covered by a smooth layer and by scattered wax platelets ....1. *A. sedelmeyeriana*

- Only adaxial surface coved by a smooth layer, granules and platelets/membranous platelets on the abaxial surface 5

5- Stomata rim and pore without wax, guard cell covered by wax. .... 2. *A. amardica*

- Stomata rim and guard cell covered by wax, pore without wax .... 3. *A. hyrcana*

6- Membranous platelets present on both adaxial and abaxial sides 7

- Membranous, irregular, aggregated, rosette platelets and wax crusts, present on one or both sides 8

7- Stomata rim and guard cell covered by wax, pore without wax .... 4. *A. rechingeri*

- Stomata rim and pore without wax, guard cell covered by wax .... 5. *A. farinosa*

8- Either membranous or irregular platelets found on the abaxial side 9

- Wax crusts with irregular, aggregated and rosette platelets found on the abaxial side 12

9- Only irregular platelets on the abaxial surface 10

- Only membranous platelets on the abaxial side 11

10- Stomata and pore without wax, guard cell covered by wax .... 6. *A. condensa*

- Stomata rim and guard cell and pore covered by wax .... 7. *A. gigantodus*

11- Adaxial surface with wax crust .... 8. *A. persica*

- Adaxial surface with membranous and scattered irregular platelets .... 9. *A. pectinoloba*

12- Aggregated, rosette and round non-entire platelets absent/crust and irregular platelets present 13

- Aggregated, rosette and round non-entire platelets and plates present .... 10. *A. rigida*

13- Crust with irregular platelets on both sides .... 11. *A. retinervis*

- Crust with irregular platelets on abaxial/ membranous platelets on the adaxial surface .... 12. *A. citrina*

14- Crust with granules, membranous, irregular, aggregated and rosette platelets on one or both abaxial and adaxial sides 15

- Only membranous or irregular platelets on one or both abaxial and adaxial sides 16

15- Crust with granules on both sides, membranous and irregular platelets absent .... 13. *A. melancholica*

- Crust with aggregated and rosette platelets on the abaxial / wax crust on the adaxial side, granules, membranous and irregular platelets absent .... 14. *A. caucasica*

16- Only irregular platelets on eider side, membranous platelets absent .... 15. *A.valdehirsuta*

- Both membranous and irregular platelets present 17

17- Only dense irregular platelets on the abaxial side .... 16. *A. erythropoda*

- Membranous, irregular platelets and wax crust on the abaxial side 18

18- Only membranous platelets on the abaxial/adaxial side covered by membranous and scattered irregular platelets .... 17. *A. surculosa*

- Membranous and scattered irregular platelets on the abaxial side/ crust with membranous platelets on the adaxial side .... 18. *A. plicatissima*

19- Smooth layer and scattered wax platelets on both abaxial and adaxial sides 20

- Smooth layer absent, crusts, granules, membranous and irregular platelets present on one or both sides 21

20- Gland one-celled stalk with capitated head cell, or without gland .... 19. *A. kurdica*

- Gland 3-4-celled stalk with sub globular-cylindrical head cell .... 20. *A. hessii*

21- Only membranous platelets on the abaxial side 22

- Crust with membranous platelets or round non-entire plates on the abaxial side 23

22- Scattered membranous, irregular platelets and granules on the adaxial sides .... 21. *A. fluminea*

- Dense irregular platelets and granules on the adaxial sides .... 22. *A. sericata*

23- Crust and membranous platelets on the abaxial/ membranous platelets on the adaxial side .... 23. *A. microscopica*

- Crust and scattered irregular platelets on the upper surface/ crust and round non-entire platelets and plates on the lower surface, ... 24. *A. pseudocartalinica.*

Appendix 1.

	Iran: province, collector, date	Accession No.
1. *A. amardica* Rothm.	Guilan: Deylaman; Shahe shahidan; Chaichi, Faghir and Shahi; 6.2012.	4872 (GUH)
2. *A. caucasica* Buser	Mazandaran: Karaj-Chalus road; Pol-e Zanguleh, 3000m; Nazarian; 2.8.1999. Mazandaran: Kojur; Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1997.	33155 (TUH) 20598 (TUH)
3. *A. citrina* Fröhner	Guilan: Deylaman; Shahe shahidan; Chaichi, Faghir and Shahi; 6.2012.	4876 (GUH)
4. *A. erythropoda* Juz.	Mazandaran: Kojur; Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1997.	20595 (TUH)
5. *A. condensa* Fröhner	Guilan: Masal; Chaichi; 2012. Guilan: Deylama, Larikhani, 1500 m; Saeidi; 20.5.1993.	4871 (GUH) 18845 (TUH)
6. *A. farinosa* Fröhner	Ardebil: Almas pass; Chaichi, Faghir and Shahi; 8.2012. Mazandaran: Ramsar; between Lapasar and Pishgah, 2600-3200 m; Maasumi.	4870 (GUH) 55188/9 (TARI)
7. *A. fluminea* Fröhner	Guilan: Deylama, Larikhani, 1530 m; Ghahreman and Attar.	18844 (TUH)
8. *A. gigantodus* Fröhner	Mazandaran: Kojur: Keikuh Mountain, 2000-2300 m; Khatamsaz and Gholoizadeh.	57149 (TARI)
9. *A. hessii* Rothm.	Mazandaran: Kojur: Firozabad Village, 1700 m; Ghahreman and Attar; 19.6. 1997. Mazandaran: Kandovan; Ghahreman, Aghostin and Sheikholeslami; 6.1974.	20600/1 (TUH) 19418 (TUH)
10. *A. hyrcana* (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)
11. *A. kurdica* Rothm in Bornm.	Guilan: Masal; Khashkhami; Chaichi, Faghir and Shahi; 6.2012.	4875 (GUH)
12. *A. melancholica* Fröhner	Guilan: Espili; Larikhani; 1530 m; Saeidi; 1993.	18841 (TUH)
13. *A. microscopica* Fröhner	Ardebil: Almas pass, 2200m; Khatamsaz and Salehnia; 6.1984.	56694 (TARI)
14. *A. persica* Rothm.	Mazandaran: Tonokabon, Jannat rudbar, 1600 m; Ghahreman, Attar and Khatamsaz; 20.6.1997. Azarbijan: Arasbaran, After three way to Veighan; Makidan; 1400 m; Ghahreman, Attar and Hamzehei; 2006.	20603 (TUH) 35575 (TUH)
15. *A. pectinoloba* Fröhner	Guilan: Deylama; Larikhani; 1530 m; Saeidi; 5.1993.	18837 (TUH)
16. *A. plicatissima* Fröhner	Ardebil: Almas pass; Chaichi, Faghir and Shahi; 8.2012.	4869 (GUH)
17. *A. pseudocartalinica* Juz.	Mazandaran: Kojur; Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1997.	20602 (TUH)
18. *A. rechingeri* Rothm.	Mazandaran: Kojur; Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1997.	20601 (TUH)
19. *A. retinervis* Busser	Mazandaran: Kojur: Firozabad Village, 1700 m; Ghahreman and Attar; 19.6. 1997.	20599 (TUH)
20. *A. sedelmeyeriana* Juz.	Mazandaran: Kojur: Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1997.	20593 (TUH)
21. *A. sericata* Reichen.	Azarbaijan: Kaleibar to Makidi; 1510; Ghahreman, Mozaffarian and Sheikholeslami; 5.1993.	17540 (TUH)
22. *A. surculosa* Fröhner	Guilan: Masal; Chaichi; 2012.	4874 (GUH)
23. *A. rigida* Buser	Guilan: Espili; Larikhani; 1510 m; Saeidi; 5.1993. Mazandaran: Kojur; Firozabad Village; 1700 m; Ghahreman and Attar; 19.6. 1996.	18842 (TUH) 20598 (TUH)
24. *A. valdehirsuta* Buser	Mazandaran: Kojur; Firozabad Village; 2200 m; Khatamsaz and Gholizadeh; 3.7.1989.	57160 (TARI)

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