Pollen morphology of some species of genus *Astragalus* L. (Fabaceae) in Northern region of Saudi Arabia

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Abstract: Pollen morphology of 13 species of *Astragalus* distributed in Northern region of Saudi Arabia was studied with light and electron microscopes. The most important diagnostic characters are Pollen is generally 3-zonocolporate, perprolate, prolate, subprolate or prolate-spheroidal. Polar axis ranges from 12.80 to 21.73 µm, while the equatorial axis varies between 24.52 to 37.22 µm. Pollen is trilobulate or triangular in polar outline, elliptic or compressed ovate in equatorial outline. Sculpturing is micro-reticulate, reticulate or rarely perforate in equatorial view (with irregular muri), and psilate, perforate or seldom scabrate (with irregular or circular perforations) in polar view. Six pollen types were recognized viz *Astragalus asterias*, *A. schimperi*, *A. palaestinus*, *A. spinosus*, *A. corrugatus* and *A. sieberi* pollen types. Description of each type, a key to investigated species as well as SEM micrographs of pollen types is provided. On the other hand, analysis based on UPGMA clustering, factor analysis and factor loading to pollen data has led to recognize two major clades. The first major clade comprises only one species (*A. schimperi*) and the second major clade separated into two branches: the first branch comprises three species (*A. dactylocarpus*, *A. palaestinus* and *A. sieberi*) and the second branch includes two subclades, the first one has six species (*A. collenettiae*, *A. corrugatus*, *A. tribuloides*, *A.hauarensis*, *A.kahiricus* and *A.spinosus*) while the second one possesses three species (*A. asterias*, *A. caparinus* and *A. bombycinus*). Discussion of the pollen morphology result with the *Astragalus* sections to determine the extent of congruence and differences between them. [Ahmed Osman, Faraj Al-Ghamdi and Arbi Guetat. **Pollen morphology of some species of genus *Astragalus* L. (Fabaceae) in Northern region of Saudi Arabia.** *Life Sci J* 2014;11(11):1006-1019]. (ISSN:1097-8135). http://www.lifesciencesite.com. 176

Key words: Pollen morphology, UPGMA clustering analysis, Fabaceae, *Astragalus*.

1. Introduction

Astragalus L., generally considered to be the largest genus in the Angiosperms, encompasses more than 2500 annual and perennial species (Podlech, 1986; Maassoumi, 1986-2005; Lock and Simpson, 1991; Zarre and Podlech, 1997; Maassoumi, 1998; Podlech, 1999; Podlech *et al*., 2001 and Lock & Schrire, 2005). The majority of species are found in the temperate, semiarid and arid continental regions of south-western and central Asia, western North America, and along the Andes and Patagonia in South America (Sanderson and Wojciechowski, 1996 and Zarre-Mobarakeh, 2000). Moreover, many *Astragalus* species are distributed in the Mediterranean climatic regions along the Pacific coasts of North and South America and in southern Europe and northern Africa (Lock and Simpson, 1991; Yakovlev *et al*., 1996 and Maassoumi, 1998).

In Saudi Arabia, about 29 species of *Astragalus* (http://plantdiversityofsaudiarabia.info/) have been recorded (Collenette, 1999; Chaudhary, 1999-2001 and Al-Hassan, 2006). The northern region of the kingdom with its 25000 sq. km shares alone 13 species. Although there are many systematic studies on the *Astragalus* species (Sanderson and Doyle 1993; Podlech, 1998; Osaloo *et al*., 2003; Pirani *et al*., 2006 and Scherson *et al*., 2008), some taxonomic problems concerning this genus have not been resolved yet (Wojciechowski *et al*., 1999; Karamali *et al*., 2007 and Khodaei *et al*., 2007). The pollen characters of *Astragalus* have yet to be studied in detail (Ekici *et al*., 2005; Dane *et al.,* 2007 and Pinar *et al*., 2009).

The main objective is to examine the pollen morphology of the genus in the Northern region and to test its taxonomic value. A cladistic analysis (UPGMA clustering, factor analysis and factor loading) based on pollen characters data will help understand better the relationships between species within the genus and to determine the extent of congruence and difference between this result and the genus sections based on morphological characters.

To the best of our knowledge, this is the first comprehensive pollen study of this section. The pollen grains of 13 *Astragalus* species are examined in details. Then, adequate keys according to pollen characters are established for an easy discrimination of the investigated species.

2. Material and methods

2.1. Pollen morphology

Thirteen species of the genus *Astragalus* are the subject of the present study. The studied species are arranged alphabetically to facilitate consultation (Table 1). For each species, the valid scientific name is given followed by the citation of the authority.

The investigated species are arranged also according to Podlech, (1990) and Thomas *et al*., (2013) sections to determine the extent of congruence and difference between the sections based on pollen morphology data and these sections (Table 2).

The plant material was collected from the field between March and June 2013. The prospected area covers the Northern region with focusing on wadi Arar. The *Astragalus's* collection of Herbarium of Range and Animal Development Research Center – Al Jouf has been used as a collection reference.

Specimens from the studied species are deposited in the Herbarium of the College of Science, Northern Border University, Arar, Kingdom of Saudi Arabia for conservation purposes.

Pollen material was removed from dry flowers. The studied species are identified according to Megahid, 1996; Collenette, 1999 and Chaudhary, 1999-2001. Pollen slides were prepared using the technique of Wodehouse, 1935. Prepared samples were then examined with Wolfe Digital TM CVM light microscope. The measurements were based, at least, on 20 pollen grains for each species. The values of the measurements are presented as a mean value with the maximum and the minimum put between parentheses. For SEM investigations, dry pollen grains were mounted on stubs and coated with gold according to Büyükkartal *et al*., (2012) and Bona, (2013). Pollen grains were examined with a JSM T200 of Electron Microscopy Unit, Assuit University, Assuit, Egypt. The terminology is based on Punt *et al*., (1994, 2007) and Hesse *et al*., (2009). The class of pollen shape are subdivided according to the value of the ratio between Polar axis and Equatorial diameter (P/E) as described by Erdtman's system (Erdtman, 1969) (Tables 4 & 5).

2.2. Statistical analysis

A total of 19 characters were measured for each species, comprising 12 morphometrical (quantitative) and 7 morphological (qualitative) characters. Some character's observations were omitted and hence they were coded as missing data (−0.999). The 13 species were clustered based on phenotypic traits the scales portray a dissimilarity index calculated using the Euclidean distance coefficient, and the dendrogram was developed using UPGMA clustering procedures "according to Sokal and Michener, 1958". Factor analysis and factor loading were also applied. All calculations were made using the STATISTICA software (STATISTICA 5.0).

3. Results

A careful examination of the available pollen material of the 13 species studied belonging to the genus *Astragalus* revealed the presence of six pollen types (Table 3), which can be distinguished through the following key:

3.1. Keys to the pollen types

1.a. Pollen sculpture is reticulate and Muri with the same diameter on all pollen surfaces ……....*A. spinosus* type

b. Pollen sculpture is otherwise …….. 2

2.a. Pollen sculpture is reticulate to psilate ……..…..……..*A. schimperi* type

b. Pollen sculpture is otherwise …….. 3

3.a. Pollen sculpture is reticulate to scabrate …..…..…….. *A. sieberi* type

b. Pollen sculpture is otherwise …….. 4

4.a. Pollen sculpture is microreticulate to psilate……..... *A. asterias* type

b. Pollen sculpture is otherwise…….. 5

5.a. Pollen sculpture is microreticulate to perforate……..…..….. *A. palastinus* type

b. Pollen sculpture is perforate to psilate……..……..…..…….. *A. corrugatys* type

The following pollen types are recorded among the species of genus' *Astragalus* in the flora of Northern region of Saudi Arabia (Table 3).

3.2. Main characters of pollen types

1. *A. asterias* pollen type, SEM (Plate 1: 1-2, 5-6; Plate 3: 3-4; Plate 5: 1-2; Tables 4, 5).

Pollen grains are 3-zonocolporate, perprolate (P/E = 2.03–2.56), 13.20 (11.99-13.83) – 15.66 (14.09-17.13) x 28.80 (27.16-30.58) – 37.22 (35.14-39.30) µm, trilobulate in polar outline, with total area 169.54 (154.31-178.58) – 225.61 (202.35-249.35) µm, elliptic in equatorial outline, with an area of 370.32 (353.74-386.90) – 637.95 (587.78-658.49) µm. Apocolpium diameter is 7.37 (6.84-7.88) – 9.70 (9.22-10.32) µm. Colpus is 24.40 (23.27-25.15) – 32.00 (31.09-33.11) µm long, 0.88 (0.42-1.36) – 1.38 (0.86-1.89) µm wide, narrow or slender at the equator, needle-like, acute or blunt towards the ends. Mesocolpium is 8.40 (6.84-10.59) – 11.88 (10.59-13.12) µm wide. Ora is lolongate with elliptic shaped, 5.45 (4.32-6.37) – 9.58 (3.19-12.61) µm in diameter and 8.01 (7.86-9.93) – 20.61 (14.1-36.03) µm in area. Exine is microreticulate to psilate; Muri decrease towards both the two pollen poles to form psilate texture and towards the pollen apertures to form perforate pattern, Muri decrease only towards the two pollen poles to form psilate to perforate texture or Muri decrease towards the two pollen poles to form psilate sculpture, 0.86 (0.61-1.25) – 0.98 (0.86-1.25) µm in diameter.

The following species belong to this type (Table 3):

1. *A. asterias* Steven, Bull. Soc. Nat. Moscou 4:267 (1832).

2. *A. caparinus* L., Sp. Pl., ed. 2, 1071 (1763).

3. *A. kahiricus* DC., Prodr. 2: 292 (1825).

4. *A. tribuloides* Delile, Fl. Orient. 2: 225 (1872).

Key to species of *A. asterias* pollen type

1.a. Colpus length is 24.40 (23.27-25.15) µm..…….....……...…...….. *A. kahiricus*

b. Mean of colpus length is 28.19-32.00 (27.58-33.11) µm ....……....…….. 2

2.a. Colpus width is 0.88 (0.42-1.36) µm ....…..……..…….... *A. asterias*

b. Mean of colpus width is 1.26-1.38 (1.00-1.89) µm ……...….....…… 3

3.a. Mesocolpium diameter is 11.88 (10.59-13.12) µm ……..... *A. caparinus*

b. Mesocolpium diameter is 9.87 (8.64-9.75) µm ……...……..…... *A. tribuloides*

2. *A. schimperi* pollen type, SEM (Plate 1: 3-4; Plate 4: 1-2; Tables 4, 5).

Pollen grains are 3-zonocolporate, prolate orperprolate (P/E = 1.49 –2.21), 13.88 (12.57-15.28) – 17.40 (16.09-18.77) x 25.99 (24.73-27.22) – 30.66 (28.43-32.89) µm, trilobulate in polar outline, with an area of 150.49 (135.45-170.49) – 228.50 (207.75-243.41) µm, elliptic in equatorial outline, with an area of 356.37 (342.50-370.14) –457.44 (448.08-466.98) µm. Apocolpium diameter is 8.29 (6.29-9.70) – 10.24 (9.82-11.47) µm. Colpus is 19.79 (18.05-21.98) – 25.68 (24.48-27.47) µm long, 0.74 (0.56-1.24) – 1.90 (0.91-2.88) µm wide, slightly wide or slender at the equator, acute or needle-like towards the ends. Mesocolpium is 11.09 (10.38-12.69) – 12.12 (10.64-13.54) µm wide. Ora is lolongate or lalongate with elliptic shaped, 4.93 (3.83-5.59) – 5.64 (4.31-6.70) µm in diameter and 8.30 (6.71-9.89) – 33.60 (30.50-37.76) µm in area. Exine is reticulate to psilate. Muri decrease towards both the two pollen poles to form psilate-perforate sculpture and towards the pollen apertures to form microreticulate style or Muri decrease towards the two pollen poles and apertures to form psilate-perforate texture, 1.26 (0.72-1.71) – 1.37 (0.94-1.92) µm in diameter.

The following species belong to this type (Table 3):

1. *A. bombycinus* Boiss., Diagn. Pl. Orient., ser. 1, 2: 50 (1843).

2. *A. schimperi* Boiss., Diagn. Pl. Orient., ser. 1, 2: 53 (1843).

Key to species of *A. schimperi* pollen type

1.a. Polar view long axis 13.88 (12.57-15.28) µm.…..……..…..…… *A. bombycinus*

b. Polar view long axis 17.40 (16.09-18.77) µm ……...…...…..…….…… *A. schimperi*

3. *A. palaestinus* pollen type, SEM (Plate 2: 1-2, 5-6; Plate 4: 5-6; Tables 4, 5).

Pollen grains are 3-zonocolporate, prolate, subprolate or perprolate (P/E = 1.29–2.61), 12.80 (11.98-13.54) – 20.63 (19.50-21.46) x 26.59 (25.68-28.30) – 33.41 (31.43-35.39) µm, trilobulate to triangular in polar outline, with an area of 136.04 (123.83-146.06) – 273.87 (258.57-287.83) µm, ovate in equatorial outline, with an area of 438.41 (398.47-478.36) – 495.82 (486.60-505.03) µm. Apocolpium diameter is 6.53 (4.35-8.71) –10.63 (8.15-12.91) µm. Colpus is 21.46 (16.17-25.04) – 28.94 (27.22-30.66) µm long, 1.72 (0.81-2.89) – 3.42 (2.18-5.22) µm wide, wide or slightly wide at the equator, acute towards the ends, with scabrate membrane only in *A. dactylocarpus* ssp. *acinaciferus*. Mesocolpium is 12.84 (10.66-15.12) – 16.45 (14.34-18.64) µm wide. Ora is lalongate with ovate shaped, 6.07 (4.65-7.22) – 8.04 (6.97-9.21) µm in diameter and 12.05 (8.05-17.97) – 24.91 (21.29-28.50) µm in area. Exine is microreticulate to perforate. Muri decrease towards the two pollen poles and apertures to form perforate form, decrease towards the two pollen poles only to from perforate texture or decrease towards apertures margins to form psilate pattern, 0.87 (0.51-1.35) – 0.97 (0.56-1.24) µm in diameter.

The following species belong to this type (Table 3):

1. *A. collenettiae* Hedge and Podl., in Bot. Jahrb. Syst. 108, 2/3: 269 (1987).

2. *A. dactylocarpus* Boiss., Phanerog. Monogr. 9: 62 (1978).

3. *A. palaestinus* Eig, J. Bot. 72: 124 (1934).

Key to species of *A. palaestinus* pollen type

1.a. The ratio of P/E is 1.29……*A. dactylocarpus*

b. The ratio of P/E is 1.99-2.61……...…… 2

2.a. Polar view long axis is 12.80(11.98-13.54) µm ……..……... *A. collenettiae*

b. Polar view long axis is 15.08(14.32-16.69) µm..…….…...……..….. *A. palaestinus*

4. *A. spinosus* pollen type, SEM (Plate 3: 1-2; Plate 4: 5-6; Tables 4, 5).

Pollen grains are 3-zonocolporate, perprolate (P/E = 2.16–2.27), 13.60 (12.29-14.66) – 15.94 (14.64-17.16) x 30.95 (30.86-31.04) – 34.42 (32.17-36.68) µm, trilobulate to triangular in polar outline, with an area of 151.62 (143.53-163.71) – 199.25 (174.31-209.79) µm, elliptic in equatorial outline, with an area of 418.57 (414.68-432.46) –472.66 (453.60-487.53) µm. Apocolpium diameter is 5.44 (4.18-6.94) – 8.51(7.32-8.70) µm. Colpus is 25.68 (24.88-26.08) – 31.44 (30.92-32.05) µm long, 1.42 (1.08-1,71) – 1.68 (1.45-1.84) µm wide, narrow or slightly wide at the equator, acute or acuminate towards the ends. Mesocolpium is 10.19 (8.37-11.03) – 10.37 (9.09-

11.96) µm wide. Ora is lolongate with elliptic shaped, 5.91 (4.31-5.73) – 8.19 (7.14-9.24) µm in diameter and 10.40 (8.96-11.85) – 13.93 (11.71-14.96) µm in area. Exine is reticulate. Muri diameter is the same on all of the pollen surface or their diameter is the same at two pollen poles only and decrease towards pollen apertures to form perforate texture, 1.34 (0.99-1.87) – 1.44 (0.83-1.98) µm in diameter.

The following species belong to this type (Table 3):

1. *A. hauarensis* Boiss., Diagn. Pl. Orient., ser. 1, 9: 63 (1849).

2. *A. spinosus* (Forssk.) Muschl., Verh. Bot. Vereins Prov. Brandenb. 49: 98 (1907).

Key to species of *A. spinosus* pollen type

1.a. Colpus length is 25.68 (24.88-26.08) µm ……..……..……..…….. *A. hauarensis*

b. Colpus length is 31.44 (30.92-32.05) µm .....……..…….*A. spinosus*

5. *A. corrugatus* pollen type, SEM (Plate 2: 3-4; Tables 4, 5).

Pollen grains are 3-zonocolporate, perprolate (P/E = 2.34), 13.40 (11.10-14.89) x 13.40 (11.10-14.89) µm, triangular in polar outline, with an area of 154.17 (146.38-163.04) µm, ovate in equatorial outline, with an area of 461.21 (458.75-464.66) µm. Apocolpium diameter is 9.18 (8.42-9.67) µm. Colpus is 25.61 (24.94-25.99) µm long, 1.91 (1.39-2.65) µm wide, it is slightly wide at the equator and blunt towards the ends. Mesocolpium is 11.06 (10.54-11.55) µm wide. Ora is lolongate with elliptic shaped, 6.61 (5.38-7.76) µm in diameter and 9.92 (4.61-13.77) µm in area. Exine is perforate to psilate. Muri decrease towards the two pollen poles to form psilate sculpture, 0.38 (0.19-0.54) µm in diameter.

The following taxon belongs to this type (Table 3):

1. *A. corrugatus* Bertol., Rar. Ital. Pl. Dec. 3: 33 (1810).

6. *A. sieberi* pollen type, SEM (Plate 4: 3-4; Tables 4, 5).

Table 1: A list of the investigated species with their localities.

No.	Species	Locality
1	*A. asterias*	Nr.: Wadi Mayaala, 30.53 N, 41.02 E; 29 March, 1988.
2	*A. bombycinus*	Nr.: Wadi Mayaala, 30.50 N, 41.00 E; 25 May, 1982.
3	*A. caparinus*	Nr.: Al-Shama, 31.24857 N, 38.02740 E; 25 March, 2004.
4	*A. collenettiae*	Nr.: Kunfa, 28.39 N, 39.06 E; 3 May, 1993; -247.
5	*A. corrugates*	Nr.: Tamriat, 30.30 N, 40.23 E; 18 April, 1982.
6	*A. dactylocarpus*	Nr.: Tamriat, 30.30 N, 40.23 E; 29 April, 1988.
7	*A. hauarensis*	Nr.: Tamriat, 30.30 N, 40.23 E; 18 April, 1982.
8	*A. kahiricus*	Nr.: Sandy dunes, 28.39 N, 38.55 E; 2 May, 1993.
9	*A. palaestinus*	Nr.: Tamriat, 30.30 N, 40.23 E; 21 February, 1988.
10	*A. schimperi*	Nr.: Quneitra, 10km of Sakaka, 30.00 N, 40.04 E; 11 March, 1984.
11	*A. sieberi*	Nr.: Al-Haugaa, 28.59055 N, 38.26172 E; 29 March, 2004.
12	*A. spinosus*	Nr.: Wadi Mareer, 30.04 N, 39.54 E; 8 March, 1983.
13	*A. tribuloides*	Nr.: Quneitra, 10km NW of Sakaka, 30.00 N, 40.08 E; 12 March, 1984.

Table 2: Astragalus species with their sections.

No.	Species	Section
1	*A. collenettiae*	Ammodendron
2	*A. caparinus*	Caprini
3	*A. dactylocarpus*	Chronopus
4	*A. sieberi*	
5	*A. kahiricus*	Eremophysa
6	*A. spinosus*	Falcinellus (Sec. Poterion)
7	*A. corrugates*	Harpilobus
8	*A. hauarensis*	
9	*A. asterias*	Oxyglottis
10	*A. schimperi*	
11	*A. tribuloides*	
12	*A. bombycinus*	Platyglottis
13	*A. palaestinus*	

Table 3: Species of *Astragalus* and their representative pollen types. No. of species = 13.

Species	Sculpture Type	No. of Species	Pollen Type
A. asterias	microreticulate to psilate	4	***A. asterias***
A. caparinus			
A. kahiricus			
A. tribuloides			
A. bombycinus	reticulate to psilate	2	***A. schimperi***
A. schimperi			
A. collenettiae	microreticulate to perforate	3	***A. palaestinus***
A. dactylocarpus			
A. palaestinus			
A.hauarensis	reticulate	2	***A. spinosus***
A. spinosus			
A. corrugates	perforate to psilate	1	***A. corrugatus***
A. sieberi	reticulate to scabrate	1	***A. sieberi***

Table 4: Tabular summary showing the description of LM and SEM samples.

No	Species	Pollen Shape	Sculp. Type	Sculp. State	Col. wid. at eq.	Colpi Ends	Colpi membrane	Ora Shape
1	*A. asterias*	Perprolate	**1**	I	slender	Needle-like	-	lolongate, elleptic
2	*A. bombycinus*	Perprolate	**2**	II	s. wide	acute	granulate	lalongate, elleptic
3	*A. caparinus*	Perprolate	**1**	III	narrow	obtuse	-	lolongate, elleptic
4	*A. collenettiae*	Perprolate	**3**	IV	wide	acute	-	lalongate, ovate
5	*A. corrugates*	Perprolate	**4**	V	s. wide	obtuse	-	lolongate, elleptic
6	*A. dactylocarpus*	Subprolate	**3**	VI	wide	acute	scabrate	lalongate, ovate
7	*A.hauarensis*	Perprolate	**5**	VII	s. wide	acuminate	-	lolongate, elleptic
8	*A. kahiricus*	Perprolate	**1**	III	narrow	acute	-	lolongate, elleptic
9	*A. palaestinus*	prolate	**3**	IV	s. wide	acute	-	lalongate, ovate
10	*A. schimperi*	prolate	**2**	VIII	slender	Needle-like	-	lolongate, elleptic
11	*A. sieberi*	Prolate-spheroidal	**6**	IX	wide	acute	granulate	Circular
12	*A. spinosus*	Perprolate	**5**	X	narrow	acute	-	lolongate, elleptic
13	*A. tribuloides*	Perprolate	**1**	V	narrow	obtuse	-	lolongate, elleptic

Col. wid. at eq. = Colpi width at equator,1 = microreticulate to psilate, 2 = reticulate to psilate, 3 = microreticulate to perforate, 4 = perforate to psilate, 5 = reticulate, 6 = reticulate to scabrate, I = Muri decrease towards the two pollen poles to form psilate texture and decrease towards pollen apertures to form perforate pattern, II = Muri decrease towards the two pollen poles to form psilate to perforate sculpture and decrease towards pollen apertures to form microreticulate style, III = Muri decrease only towards the two pollen poles to form psilate to perforate texture, IV = Muri decrease towards the two pollen poles and apertures to form perforate form, V = Muri decrease towards the two pollen poles to form psilate sculpture, VI = Muri decrease towards the two pollen poles to from perforate texture and decrease towards apertures margins to form psilate pattern, VII = Muri size the same at all pollen surface, VIII = Muri decrease towards the two pollen poles and apertures to form psilate to perforate texture, IX = Muri decrease towards the two pollen poles and apertures to form scabrate pattern, X = Muri size the same at two pollen poles and decrease at pollen apertures to form perforate texture.

Table 5: Tabular summary showing the pollen grains dimensions (µm).

Species	P.	E.	P/E	Colp. Len.	Colp. wid.	Ora.		Apo. diam.	Meso. diam.	Muri Diam	Total Area	
						Diam.	Area				P.	E.
A. asterias	13.42(12.75-14.74)	34.33(33.59-35.08	2.56	30.39(28.58-31.48	0.88(0.42-1.36)	9.58(3.19-12.61)	20.61(14.1-36.03)	7.37(6.84-7.88)	8.40(6.84-10.59)	0.86(0.61-1.25)	225.61(202.35-249.35)	496.54(488.46-504.62)
A. bombycinus	13.88(12.57-15.28)	30.66(28.43-32.89)	2.21	25.68(24.48-27.47)	1.90(0.91-2.88)	5.64(4.31-6.70)	33.60(30.50-37.76)	8.29(6.29-9.70)	12.12(10.64-13.54)	1.26(0.72-1.71)	150.49(135.45-170.49)	457.44(448.08-466.98)
A. caparinus	15.66(14.09-17.13)	37.22(35.14-39.30)	2.38	32.00(31.09-33.11)	1.38(0.86-1.89)	8.44(6.16-9.72)	16.50(14.93-17.95)	9.70(9.22-10.32)	11.88(10.59-13.12)	0.89(0.62-1.14)	192.37(175.32-207.73)	637.95(587.78-658.49)
A. collenettiae	12.80(11.98-13.54)	33.41(31.43-35.39)	2.61	28.94(27.22-30.66)	3.38(2.74-5.54)	7.74(7.37-8.12)	12.05(8.05-17.97)	6.53(4.35-8.71)	12.84(10.66-15.12)	0.91(0.77-1.31)	136.04(123.83-146.06)	477.73(456.18-489.28)
A. corrugates	13.40(11.10-14.89)	31.33(30.39-32.68)	2.34	25.61(24.94-25.99)	1.91(1.39-2.65)	6.61(5.38-7.76)	9.92(4.61-13.77)	9.18(8.42-9.67)	11.06(10.54-11.55)	0.38(0.19-0.54)	154.17(146.38-163.04)	461.21(458.75-464.66)
A. dactylocarpus	20.63(19.50-21.46)	26.59(25.68-28.30)	1.29	21.46(16.17-25.04)	3.42(2.18-5.22)	8.04(6.97-9.21)	24.91(21.29-28.50)	10.63(8.15-12.91)	16.45(14.34-18.64)	0.97(0.56-1.24)	327.19(301.23-354.71)	438.41(398.47-478.36)
A.hauarensis	13.60(12.29-14.66)	30.95(30.86-31.04)	2.27	25.68(24.88-26.08)	1.68(1.45-1.84)	5.91(4.31-5.73)	13.93(11.71-14.96)	8.51(7.32-8.70)	10.37(9.09-11.96)	1.34(0.99-1.87)	151.62(143.53-163.71)	418.57(414.68-432.46)
A. kahiricus	14.20(13.42-15.13)	28.80(27.16-30.58)	2.03	24.40(23.27-25.15)	1.26(1.00-2.02)	5.45(4.32-6.37)	8.01(7.86-9.93)	7.73(7.52-8.11)	8.77(7.52-9.68)	0.98(0.86-1.25)	169.54(154.31-178.58)	370.32(353.74-386.90)
A. palaestinus	15.08(14.32-16.69)	30.01(28.66-31.19)	1.99	23.45(20.31-25.51)	1.72(0.81-2.89)	6.07(4.65-7.22)	17.54(13.88-20.73)	8.85(8.38-9.34)	13.28(12.11-14.15)	0.87(0.51-1.35)	273.87(258.57-287.83)	495.82(486.60-505.03)
A. schimperi	17.40(16.09-18.77)	25.99(24.73-27.22)	1.49	19.79(18.05-21.98)	0.74(0.56-1.24)	4.93(3.85-5.59)	8.30(6.71-9.89)	10.24(9.82-11.47)	11.09(10.38-12.69)	1.37(0.94-1.92)	228.50(207.75-243.41)	356.37(342.50-370.14)
A. sieberi	21.73(20.84-22.64)	24.52(23.69-25.27)	1.13	23.24(21.69-24.06)	3.61(2.59-4.11)	7.17(6.85-7.60)	26.37(18.77-36.38)	7.32(6.26-8.43)	17.36(16.49-18.11)	1.27(0.92-1.80)	334.08(317.41-351.76)	401.16(388.16-414.15)
A. spinosus	15.94(14.64-17.16)	34.42(32.17-36.68)	2.16	31.44(30.92-32.05)	1.42(1.08-1,71)	8.19(7.14-9.24)	10.40(8.96-11.85)	5.44(4.18-6.94)	10.19(8.37-11.03)	1.44(0.83-1.98)	199.25(174.31-209.79)	472.66(453.60-487.53)
A. tribuloides	13.20(11.99-13.83)	31.72(28.50-33.99)	2.40	28.19(27.58-29.19)	1.35(1.09-1.76)	7.29(5.86-8.36)	11.82(9.66-14.51)	7.41(6.54-8.34)	9.87(8.64-9.75)	0.93(0.89-1.34)	189.26(176-48-197.25)	406.05(395.70-416.40)

P. = Polar axis, E. = Equatorial diameter, P/E = The ratio of the length of the polar axis (P) to the equatorial diameter (E), Colp. Len. = Colpus length, Colp. wid. = Colpus width, Ora. diam. = Ora diameter, Apo. diam. = Apocolpium diameter, Meso. diam. = Mesocolpium diameter, µm = micrometer.

Table 6: Factor loadings showed the most intrinsic characters enhanced separations of the studied species.

Characters		Factor Loadings (Unrotated)		
		Extraction: Principal components		
		(Marked loadings are > .700000)		
		Factor 1	Factor 2	Factor 3
1	Pollen shape	0.863822891	-0.14429963	0.054578492
2	Sculpture type	-0.114383542	0.603287314	-0.633086045
3	Sculpture state	0.508965505	-0.418011953	0.386613617
4	Colpus width at equator	0.457819198	0.644242773	0.165782989
5	Colpi ends	-0.361160241	0.29343653	-0.022908543
6	Colpi membrane	0.473536645	0.451888444	-0.182508828
7	Ora shape	0.11127357	-0.513586076	0.264974758
8	Polar view	0.820622875	-0.101790875	0.196226978
9	Equatorial diameter	-0.854841993	0.244110165	0.275845828
10	P/E	-0.933884476	-0.089399486	-0.150113808
11	Colpus length	-0.791072812	0.148272939	0.514302334
12	Colpus width	0.457819198	0.644242773	0.165782989
13	Ora diameter	-0.172055913	0.433761422	0.52501357
14	Ora area	0.346392916	0.54493472	0.377636797
15	Apocolpium diameter	0.367675803	0.152372022	-0.617963895
16	Mesocolpium diameter	0.762218251	0.474410955	0.181833374
17	Muri diameter	0.337148099	-0.518338588	0.49008714
18	Polar view total area	0.739891591	0.010094286	-0.008137344
19	Equatorial view total area	-0.450185278	0.605974363	0.42005691
	Percentage per PCA	6.436213568	3.412984091	2.363788708
Percentage for total variation for the three factors extracted 36.84 %				

Pollen grains are 3-zonocolporate, prolate-spheroidal (P/E = 1.13), 21.73 (20.84-22.64) x 24.52 (23.69-25.27) µm, triangular in polar outline, with an area of 334.08 (317.41-351.76) µm, ovate in equatorial outline, with an area of 401.16 (388.16-414.15) µm. Apocolpium diameter is about 7.32 (6.26-8.43) µm. Colpus is 23.24 (21.69-24.06) µm long, 3.61 (2.59-4.11) µm wide, wide at the equator, acute towards the ends, with granulate membrane. Mesocolpium is 17.36 (16.49-18.11) µm wide. Ora are circular in shaped, 7.17 (6.85-7.60) µm in diameter and 26.37 (18.77-36.38) µm in area. Exine is reticulate to scabrate. Muri decrease towards the two pollen poles and apertures to form scabrate pattern, 1.27 (0.92-1.80) µm in diameter.

The following taxon belongs to this type (Table 3):

1. *A. sieberi* DC., Astragalogia 186 (1802).

On the other hand, Figure (1) shows the UPGMA cladistic tree of the 13 species of *Astragalus* depending on 19 main pollen grains characters which discriminate these species into two major clades at 18 dissimilarity distance. The first major clade at 17 dissimilarity distance comprised only one species of the total number: *Astragalus schimperi*. The second major clade at 16.5 dissimilarity distance comprised the remaining 12 species and separated into two branches. The first branch comprised three species: *A. dactylocarpus*, *A. palaestinus* and *A. sieberi* at 10.75 dissimilarity distance. The second branch included two subclades: 1) A subclad at 13 dissimilarity distance with six species: *A. collenettiae*, *A. corrugatus*, *A. tribuloides*, *A.hauarensis*, *A.kahiricus* and *A.spinosus*. 2) A subclad at 12.5 dissimilarity distance with three species: *A. asterias*, *A. caparinus* and *A. bombycinus*. Factor analysis using Principal Component Analysis (PCA) showed that the most intrinsic characters enhanced separation of the total species which are pollen shape only of the morphological characters, polar view, equatorial diameter, P/E, colpus length, mesocolpium diameter and polar view total area of the morphometrical characters (Table 6). The characters of separation are of high factor loadings ≥ (± 0.7). They are represented by a percentage of the total variation equalling 36.84% for the first factor. However, both the second and the third factors were excluded because there were no characters scored on them (Figure 2).

4. Discussion

In this palynological investigation, an additional perspective on the relations between the different studied species of *Astragalus* was provided. The pollen grains are usually 3-zonocolporate and distinguished according to nature of exine sculpture into six pollen types as the following: The *Astragalus asterias* pollen type, in which the pollen grains have microreticulate to psilate sculpture, is characteristic of *A. asterias*, *A. caparinus*, *A. kahiricus* and *A. tribuloides*. The *Astragalus schimperi* pollen type, in which the pollen grains have reticulate to psilate sculpture, is characteristic of both *A. bombycinus* and *A. schimperi* species. The *A. palaestinus* pollen type, in which the pollen grains have microreticulate to perforate sculpture, is characteristic of *A. collenettiae*, *A. dactylocarpus* and *A. palaestinus* species. The *A. spinosus* pollen type, in which the pollen grains have reticulate sculpture, is characteristic of both *A. hauarensis* and A. *spinosus* species. The *A. corrugatus* pollen type, in which the pollen grains have perforate to psilate sculpture, is characteristic of *A. corrugatus* species only. Finally, *A. sieberi* pollen type in which the pollen grains have reticulate to scabrate sculpture, is characteristic of *A. sieberi* species only (Tables 3, 4).

Pollen size of the studied species ranged between 23–37 µm. The smallest pollen grains are those of *A. sieberi* 24.52(23.69-25.27) µm and the largest one are those of *A. caparinus* 37.22(35.14-39.30) µm. Pollen size of other species ranged between 25–35 µm (Table 5). Pollen grains which are more or less similar in shape being perprolate, prolate, subprolate or prolate-spheroidal. Colpi are narrow in *A. caparinus*, *A. kahiricus*, *A. spinosus* and *A. tribuloides*, wide in *A. collenettiae*, *A. dactylocarpus* and *A. sieberi*, slender in both *A. asterias* and *A. schimperi* and slightly wide in the remaining studied species. Furthermore, colpi ends contribute to differentiate between the species of *A. caparinus*, *A. corrugatus*, and *A. tribuloides* which are characterized by blunt ends. However, both *A. asterias* and *A. schimperi* exhibit Needle-like ends. Only *A. hauarensis* has acuminate ends and the remnants of investigated species have acute ends (Table 4). The granulation of pollen apertures membranes is showed in both *A. bombycinus* and *A. sieberi*, scabrate pollen apertures membranes is appeared in *A. dactylocarpus* ssp. *acinaciferus* only, while the aperture's membranes of other species were not cleared.

Moreover, the sculpture at pollen pole could also help in differentiation of *A. sieberi*, which is characterized by scabrate texture, *A. collenettiae*, *A. dactylocarpus* and *A. palaestinus* which are characterized by perforate texture, *A. hauarensis* and *A. spinosus* which are characterized by reticulate texture from all other remaining species, which possess psilate texture (Table 4). Additionally, the diameter of muri also contributed to differentiating *A. bombycinus, A.hauarensis, A. schimperi, A. sieberi* and *A. spinosus* species, which were characterized by large muri with diameter medium 1.26-1.44 µm, from the other remaining investigated species that exhibited small muri with diameter medium 0.38-0.98 µm (Table 4).

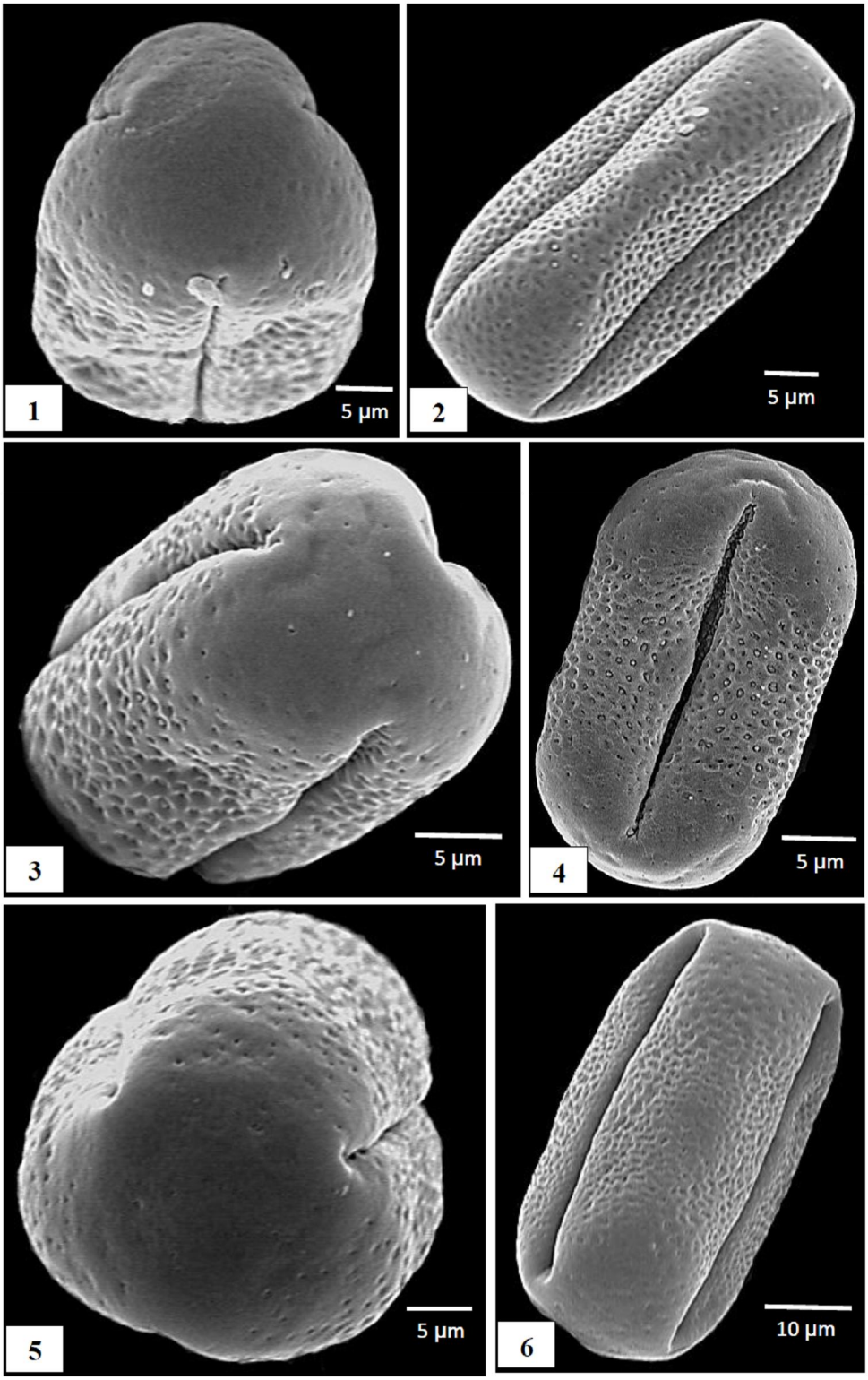

Plate 1. SEM micrographs of *A. asterias* 1-2, (1) polar view, (2) equatorial view; *A. bombycinus* 3-4, (3) polar view, (4) equatorial view and *A. caparinus* 5-6, (5) polar view, (6) equatorial view.

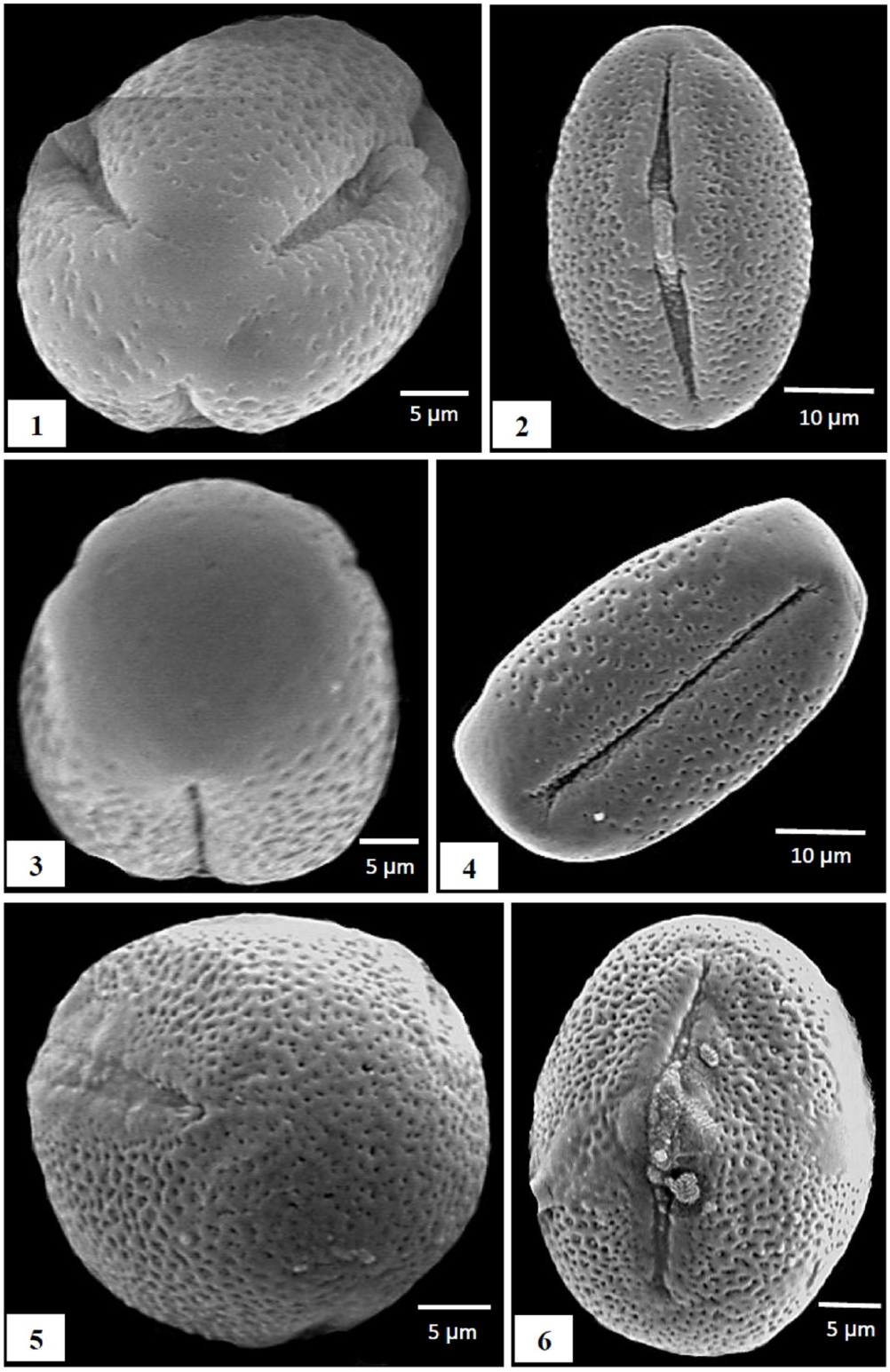

Plate 2. SEM micrographs of *A. collenettiae* 1-2, (1) polar view, (2) equatorial view; *A. corrugatus* 3-4, (3) polar view, (4) equatorial view and *A. dactylocarpus* 5-6, (5) polar view, (6) equatorial view.

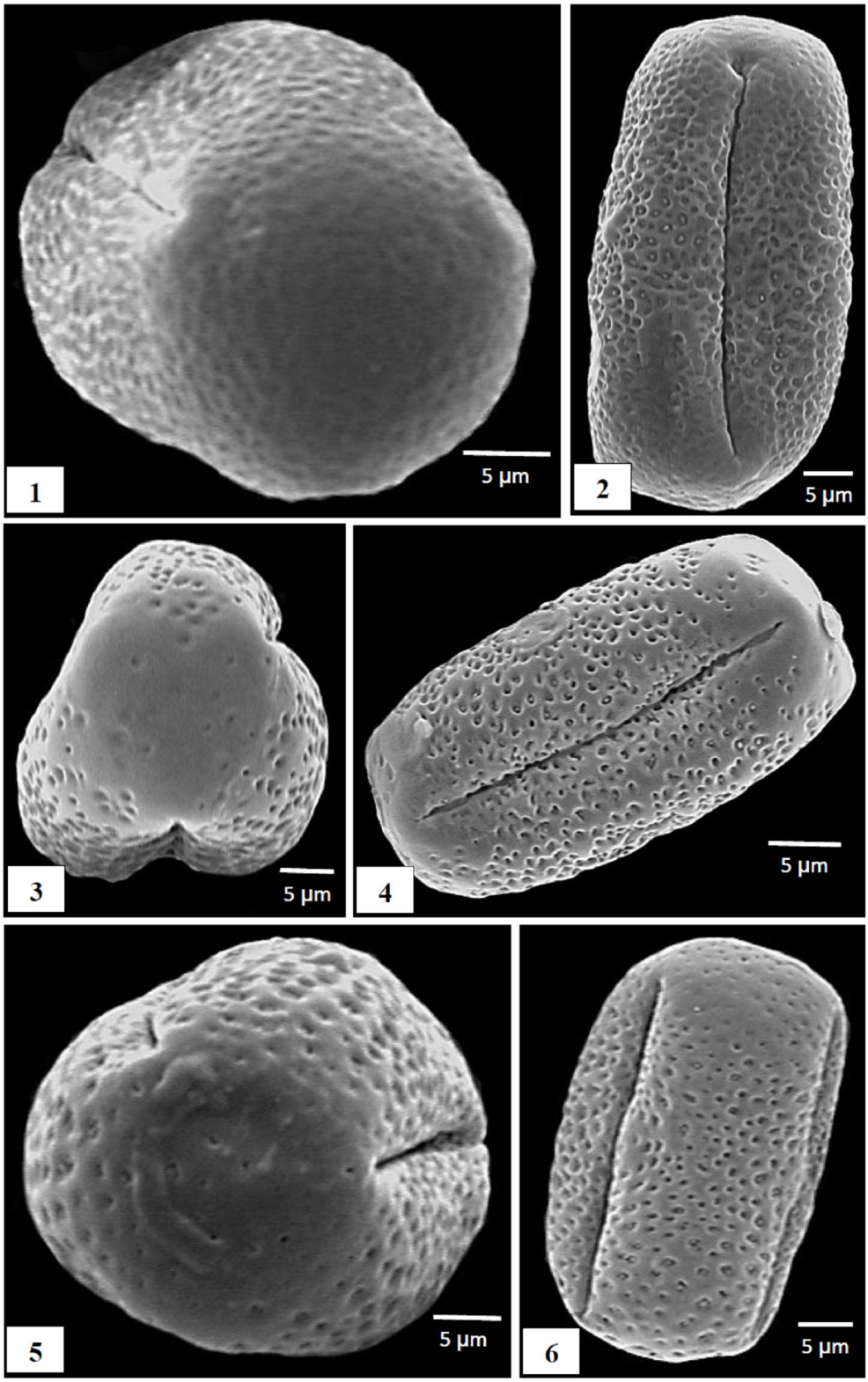

Plate 3. SEM micrographs of *A. hauarensis* 1-2, (1) polar view, (2) equatorial view; *A. kahiricus* 3-4, (3) polar view, (4) equatorial view and *A. palaestinus* 5-6, (5) polar view, (6) equatorial view.

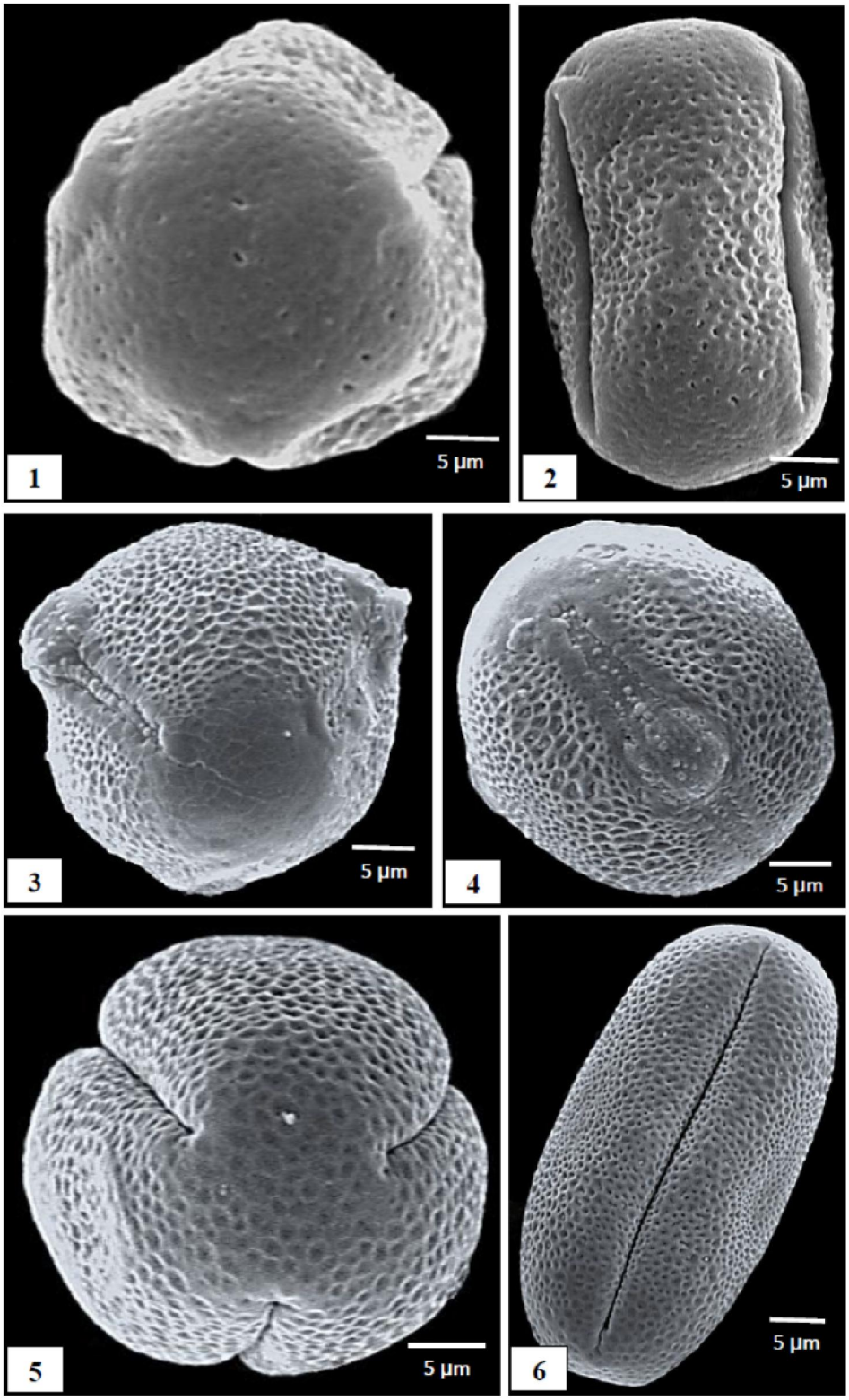

Plate 4. SEM micrographs of *A. schimperi* 1-2, (1) polar view, (2) equatorial view; *A. sieberi* 3-4, (3) polar view, (4) equatorial view and *A. spinosus* 5-6, (5) polar view, (6) equatorial view.

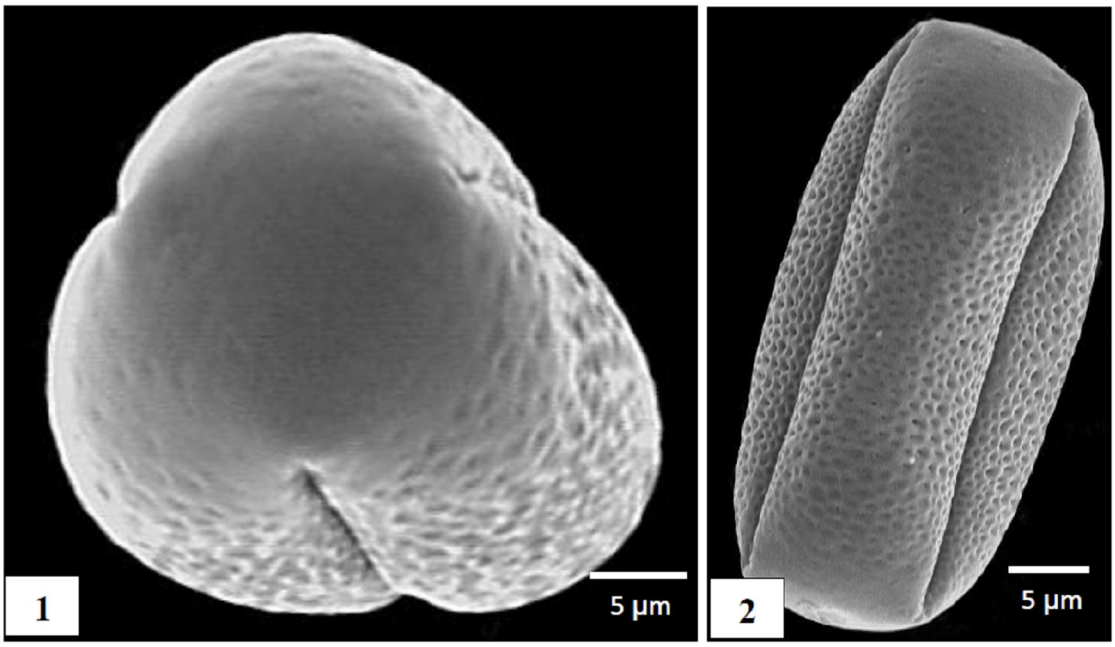

Plate 5. SEM micrographs of *A. tribuloides* 1-2, (1) polar view, (2) equatorial view.

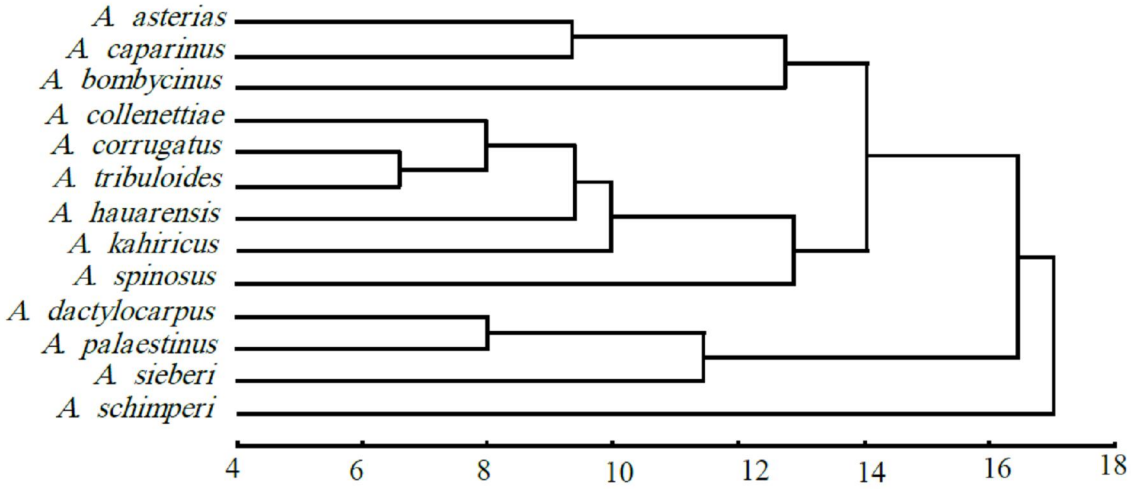

Figure 1. Phenogram of the 13 studied *Astragalus* species, clustering with the UPGMA method.

On the other hand, a large number of pollen morphology characters were scored, and numerical methods (UPGMA and PCA) were applied to study the relationships between thirteen *Astragalus* species and estimate the level of variation within and between these species and also determine their conformity with on the basis of morphology sections. UPGMA gives insight into the degree of similarity between the studied species and whether they form groups (clusters) and give an indication of the level of variation within and between species. PCA reflects which characters are important on the axes, and indicates the significant characters on the bases of the highest factor score (Table 6). Therefore, it becomes clear which characters cause the separation between groups and can be useful to distinguish species. Pollen grains showed the most powerful significant characters. Generally, our results arose congruence between the UPGMA clustering and PCA analysis in suggesting two main branches and three subgroups, which included the distribution of thirteen species studied (Figure 1). The UPGMA results showed that the *A. schimperi* is separated in the initial major clade of the cladistic tree, and all the remaining 12 species are found in the other major clade. The alternate major clade is separated into two branches. The primary branch contains three species

(*A. dactylocarpus*, *A. palaestinus* and *A. sieberi*). The second branch comprises two subclades. The first subclade includes six species (*A. collenettiae*, *A. corrugatus*, *A. tribuloides*, *A. hauarensis*, *A. kahiricus* and *A.spinosus*), while the second subclade comprises three species (*A. asterias*, *A. caparinus* and *A. bombycinus*) (Figure 1). Then, the applied methods of UPGMA and PCA can be used to study the variation among the species in the genus' *Astragalus* to determine the relationship between different species. Our results revealed there is a clear separation between *A. schimperi* and all another investigated species. Moreover, species *A. asterias*, *A. bombycinus* and *A. caparinus* showed a much closer relationship species in the same clade. In addition to species of *A. dactylocarpus*, *A. palaestinus* and *A. sieberi* arises also a much closer relationships being existed in the same sub-branch (Figure 2). According to Podlech, (1990) and Thomas *et al*., (2013), the studied species falls under eight sections: Ammodendron (*A. collenettiae*), Caprini (*A. caparinus*), Chronopus (*A. dactylocarpus* and *A. sieberi*), Eremophysa (*A. kahiricus*), Falcinellus " Poterion" (*A. spinosus*), Harpilobus (*A. corrugates* and *A. hauarensis*), Oxyglottis (*A. asterias*, *A. schimperi* and *A. tribuloides*) and Platyglottis (*A. bombycinus* and *A. palaestinus*) (Table 2). This is not consistent with the results of the research, which was divided the studied species into four groups (Figure 1)

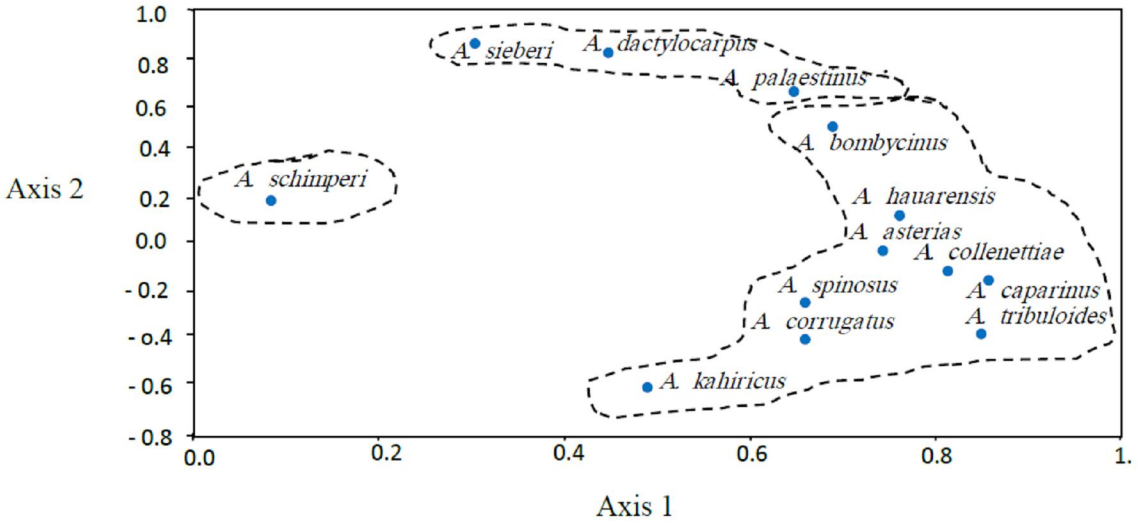

Figure 2. Scatter-plot of 13 studied species plotted against the first factor by the second factor.

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