Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 12 (5): 176-189, 2013
ISSN 1682-3974 / DOI: 10.3923/ajps.2013.176.189

Contributions to the Pollen Morphology of Genus *Astragalus* L. (Fabaceae) and its Taxonomic Implications

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Abstract: Pollen morphology of 13 species of genus' *Astragalus* distributed in Saudi Arabia was studied with light and electron microscopes. Pollen is generally 3-zonocolporate, perprolate, prolate, subprolate or prolate-spheroidal. Polar axis is ranged 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: *Astragalus asterias* pollen type, *A. schimperi* pollen type, *A. palaestinus* pollen type, *A. spinosus* pollen type, *A. corrugatus* pollen type and *A. sieberi* pollen type. Description of each type, a key to investigated species as well as SEM micrographs of pollen types is provided. On the other hand, numerical analysis based on UPGMA clustering, factor analysis and factor loading to pollen data has led to recognize two major clads. The second major clad separated into two branches: the first branch includes two subclads and the second branch comprises three species.

Key words: Pollen morphology, UPGMA clustering analysis, Fabaceae, *Astragalus* L., Taxonomy, Saudi Arabia

INTRODUCTION

Astragalus L., generally considered to be the largest genus in the *Angiosperms*, encompasses more than 2500 annual and perennial species (Podlech, 1986; Maassoumi, 2005; Lock *et al*., 1991; Zarre and Podlech, 1997; Maassoumi, 1998; Podlech, 1999, 2010; Podlech *et al*., 2001; Lock and Schrire, 2005; Zarre *et al*., 2008). 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; 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 *et al*., 1991; Yakovlev *et al*., 1996; Maassoumi, 1998).

In Saudi Arabia, the genus' *Astragalus* is one of the most important genera represented in the flora of the country. About 29 species have been recorded (Collenette, 1999; Chaudhary, 2001). The northern regions of the kingdom with its 25000 km^2 shares alone 13 species. Although, there are many systematic studies on the *Astragalus* species (Osaloo *et al*., 2003; Pirani *et al*., 2006; Podlech, 1998; Sanderson and Doyle, 1993; Scherson *et al*., 2008), some taxonomic problems concerning this genus have not been resolved yet (Wojciechowski *et al*., 1999; Karamali *et al*., 2007; Khodaei *et al*., 2007). The pollen characters of *Astragalus* have yet to be studied in detail (Tewari and Nair, 1979; Perveen and Qaisar, 1998; Simons and Chinnappa, 2004; Akan *et al*., 2005; Ekici *et al*., 2005; Dane *et al*., 2007; Oskouian *et al*., 2007; Pinar *et al*., 2009; Ceter *et al*., 2013). In this study, the 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. To the best of our knowledge, this is the first comprehensive pollen study of this section. The pollen grains of 13 Saudi *Astragalus* species are examined in details. Then, adequate keys according to pollen characters are established for an easy discrimination of the investigated species. Finally, the specimens from the different studied species of the *Astragalus* genus are deposited in the herbarium of Arar Science College at the Northern Borders University for conservation purposes.

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MATERIALS AND METHODS

Pollen morphology: Thirteen species out from 29 members of the genus' *Astragalus* in Saudi Arabia 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 and the date of publication. Synonymy is at a minimum to avoid complications. For full synonymy of the species in Collenette (1999), Cope (1985), Migahid (1996) and Chaudhary (2001).

The planting materials (hermaphrodite flowers) are collected from the wild between March and June 2013. The prospected area covers the northern border 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, with the amiably cooperation of Al-Hassan (2006).

Specimens of different species are deposited in the herbarium of the college of science, Arar (Northern Border University, Arar, Kingdom of Saudi Arabia).

Pollen materials (buds or anthers) were removed from dry flowers. The studied species are identified according to Collenette (1999), Cope (1985), Migahid (1996) and Chaudhary (2001). Pollen slides were prepared using the technique of Woodehouse method (Wodehouse, 1935). Prepared samples were then examined with Wolfe Digital TM CVM 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 Scanning Electron Microscopy (SEM), dry pollen grains were mounted on stubs and coated with gold (Buyukkartal *et al*., 2013; Bona, 2013). Pollen grains were examined with a JSM T200 of Electron Microscopy Unit, Assuit University, Assuit, Egypt. The terminology and main morphological concepts are based on Faegri and Iversen (1989), Punt *et al.* (1994, 2007), Guner *et al.* (2011) and Akyalcin *et al.* (2011). 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) (Table 3, 4).

Numerical 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 (unweighted pair group method using arithmetic averages) clustering procedures "according to Seberg *et al.* (1991) and Sokal and Michener (1958). Factor analysis and factor loading were also applied. All calculations were made using the STATISTICA software (STATISTICA 5.0).

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 2), which can be distinguished through the following key:

Keys to the pollen types:

- Pollen sculpture is reticulate and Muri with the same diameter on all pollen surfaces (*A. spinosus* type)
- Pollen sculpture is otherwise (2)
- Pollen sculpture is reticulate to psilate (*A. schimperi* type)
- Pollen sculpture is otherwise (3)
- Pollen sculpture is reticulate to scabrate (*A. sieberi* type)
- Pollen sculpture is otherwise (4)
- Pollen sculpture is microreticulate to psilate (*A. asterias* type)

Table 1: A list of the investigated species with their origin and collectors

Species	Locality
A. asterias	Nr.: Wadi Mayaala, 30.53 N, 41.02 E; 29 March, 1988
A. bombycinus	Nr.: Wadi Mayaala, 30.50 N, 41.00 E; 25 May, 1982
A. caparinus spp. *caparinus*	Nr.: Al-Shama, 31.24857 N, 38.02740 E; 25 March, 2004
A. collenettiae	Nr.: Kunfa, 28.39 N, 39.06 E; 3 May, 1993; -247
A. corrugates	Nr.: Tamriat, 30.30 N, 40.23 E; 18 April, 1982
A. dactylocarpus spp. *acinaciferus*	Nr.: Tamriat, 30.30 N, 40.23 E; 29 April, 1988
A.hauarensis	Nr.: Tamriat, 30.30 N, 40.23 E; 18 April, 1982
A. kahiricus	Nr.: Sandy dunes, 28.39 N, 38.55 E; 2 May, 1993
A. palaestinus	Nr.: Tamriat, 30.30 N, 40.23 E; 21 February, 1988
A. schimperi	Nr.: Quneitra, 10 km of Sakaka, 30.00 N, 40.04 E; 11 March, 1984
A. sieberi	Nr.: Al-Haugaa, 28.59055 N, 38.26172 E; 29 March, 2004
A. spinosus	Nr.: Wadi Mareer, 30.04 N, 39.54 E; 8 March, 1983
A. tribuloides var. *minutus*	Nr.: Quneitra, 10 km NW of Sakaka, 30.00 N, 40.08 E; 12 March, 1984

Table 2: Taxa 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 spp. *Caparinus*			
A. kahiricus			
A. tribuloides var. *minutes*			
A. bombycinus	Reticulate to psilate	2	*A. schimperi*
A. schimperi			
A. collenettiae	Microreticulate to perforate	3	*A. palaestinus*
A. dactylocarpus spp. *Acinaciferus*			
A. palaestinus			
A. hauarensis	Reticulate	2	*A. spinosus*
A. spinosus			
A. corrugates	Perforate to psilate	1	*A. corrugates*
A. sieberi	Reticulate to scabrate	1	*A. sieberi*

Table 3: Tabular summary showing the description of LM and SEM samples

Species	Pollen shape	Sculpture type	Sculpture state	Col. wid. at eq.	Colpi ends	Colpi membrane	Ora shape
A. asterias	Perprolate	1	I	Slender	Needle-like	-	Lolongate, Elleptic
A. bombycinus	Perprolate	2	II	S. wide	Acute	Granulate	Lalongate, Elleptic
A. caparinus Spp. *Caparinus*	Perprolate	1	III	Narrow	Obtuse	-	Lolongate, Elleptic
A. collenettiae	Perprolate	3	IV	Wide	Acute	-	Lalongate, Ovate
A. corrugates	Perprolate	4	V	S. wide	Obtuse	-	Lolongate, Elleptic
A. dactylocarpus Spp. *acinaciferus*	Subprolate	3	VI	Wide	Acute	Scabrate	Lalongate, Ovate
A.hauarensis	Perprolate	5	VII	S. wide	Acuminate	-	Lolongate, Elleptic
A. kahiricus	Perprolate	1	III	Narrow	Acute	-	Lolongate, Elleptic
A. palaestinus	prolate	3	IV	S. wide	Acute	-	Lalongate, Ovate
A. schimperi	prolate	2	VIII	Slender	Needle-like	-	Lolongate, Elleptic
A. sieberi	Prolate-spheroidal	6	IX	Wide	Acute	Granulate	Circular
A. spinosus	Perprolate	5	X	Narrow	Acute	-	Lolongate, Elleptic
A. tribuloides var. *minutus*	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

- Pollen sculpture is otherwise (5)
- Pollen sculpture is microreticulate to perforate (*A. palastinus* type)
- 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 Saudi Arabia (Table 2).

Main characters of pollen types:

- *A. asterias* pollen type, SEM (Fig. 1a-c, g-i; Fig. 3d-f; Fig. 5a-c; Table 3, 4)

Pollen grains are 3-zonocolporate, perprolate (P/E = 2.03-2.56), 13.20 (11.99-13.83)-15.66 (14.09-17.13)×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 2):

- *A. asterias* Steven, Bull. Soc. Nat. Moscou 4:267 (1832)
- Syn. *A. cruciatus*sensu Zohary, Fl. Palaest. 2: 59 (1972)

Table 4: Tabular summary showing the pollen grains dimensions (µm)

Species	P.	E.	P/E	Colp. Len.	Colp. wid.	Ora. Diam.	Ora. Area
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)
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)
A. caparinus ssp. 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)
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)
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)
A. dactylocarpus ssp. acinaciferus	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)
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)
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)
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)
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 (3.83-5.59)	8.30 (6.71-9.89)
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)
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)
A. tribuloides var. minutus	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)

Species	Apo. diam.	Meso. diam.	Muri diam	Total area P.	Total area E.
A. asterias	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	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 ssp. caparinus	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	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	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 ssp. acinaciferus	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	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	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	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	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	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	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 var. minutus	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

- *A. caparinus* L. Spp. *caparinus* L., Sp. Pl., ed. 2, 1071 (1763)
- Syn. *A. alexandrines* Boiss., Diagn. Pl. Orient., ser. 1, 9: 75 (1849)
- *A. lanigerus* Desf., Fl. Atlant. 2: 181 (1799)
- *A. kahiricus* DC., Prodr. 2: 292 (1825).
- Syn. *A. longiflorus* Delile, Descr. Egypte, Hist. Nat. 356 (1814), non Pallas, Sp. Astragal. 73 (1800-1802), nom illeg
- *A. tribuloides* Delile var. *minutus* (Boiss.) Boiss., Fl. Orient. 2: 225 (1872)
- Syn. *A. minutus* Boiss., Diagn. Pl. Orient., ser. 1, 9: 58 (1849)

Key to species of *A. asterias* pollen type:

- Colpus length is 24.40 (23.27-25.15) μm (*A. kahiricus*)
- Mean of colpus length is 28.19-32.00 (27.58-33.11) μm (2)
- Colpus width is 0.88 (0.42-1.36) μm (*A. asterias*)
- Mean of colpus width is 1.26-1.38 (1.00-1.89) μm (3)
- Mesocolpium diameter is 11.88 (10.59-13.12) μm (*A. caparinus* Spp. caparinus)
- Mesocolpium diameter is 9.87 (8.64-9.75) μm (*A. tribuloides* var. *minutus*)
- *A. schimperi* pollen type, SEM (Fig. 1d-f; Fig. 4a-c; Table 3, 4)

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)×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 he 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 2):

- *A. bombycinus* Boiss., Diagn. Pl. Orient., ser. 1, 2: 50 (1843)
- *A. schimperi* Boiss., Diagn. Pl. Orient., ser. 1, 2: 53 (1843)

Key to species of *A. schimperi* pollen type:

- Polar view long axis 13.88 (12.57-15.28) μm (*A. bombycinus*)
- Polar view long axis 17.40 (16.09-18.77) μm (*A. schimperi*)
- *A. palaestinus* pollen type, SEM (Fig. 2a-c, g-i; Fig. 3g-i; Table 3, 4)

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* Spp. *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 2):

- *A. collenettiae* Hedge and Podl., in Bot. Jahrb. Syst. 108, 2/3: 269 (1987)
- *A. dactylocarpus*Spp. *acinaciferus*(Boiss.) Eug. Ott, Phanerog. Monogr. 9: 62 (1978). Syn. *A. acinaciferus* Boiss., Diagn. Pl. Orient., ser. 1, 9: 93 (1849)
- *A. palaestinus* Eig, J. Bot. 72: 124 (1934)

Key to species of *A. palaestinus* pollen type:

- The ratio of P/E is 1.29 (*A. dactylocarpus* spp. *acinaciferus*)
- The mean ratio of P/E is 1.99-2.61 (2)
- Polar view long axis is 12.80(11.98-13.54) μm (*A. collenettiae*)
- Polar view long axis is 15.08(14.32-16.69) μm (*A. palaestinus*)
- *A. spinosus* pollen type, SEM (Fig. 2a-c, g-i; Fig. 3g-i; Table 3, 4)

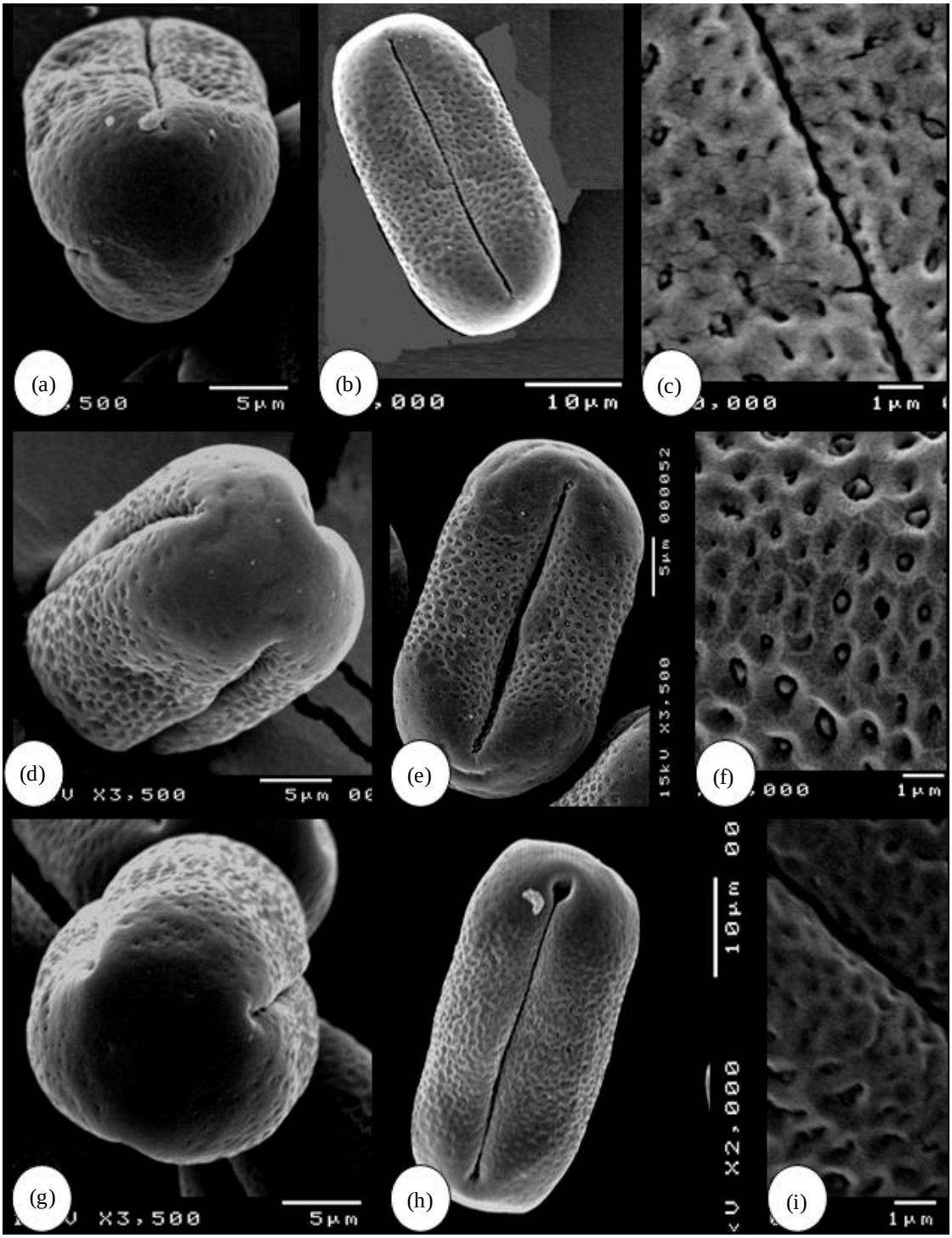

Fig. 1(a-i): SEM observations of non acetolyzed pollen grains (SEM×2000-10,000). (a-c) *A. asterias*: (a) Polar view, (b) Equatorial view, (c) Magnified part of exine. (d-f) *A. bombycinus*: (d) Obliquepolar view, (e) Equatorial view, (f) Magnified part of exine. (g-i) *A. caparinus* ssp. *caparinus*: (g) Polar view, (h) Equatorial view and (i) Magnified part of exine

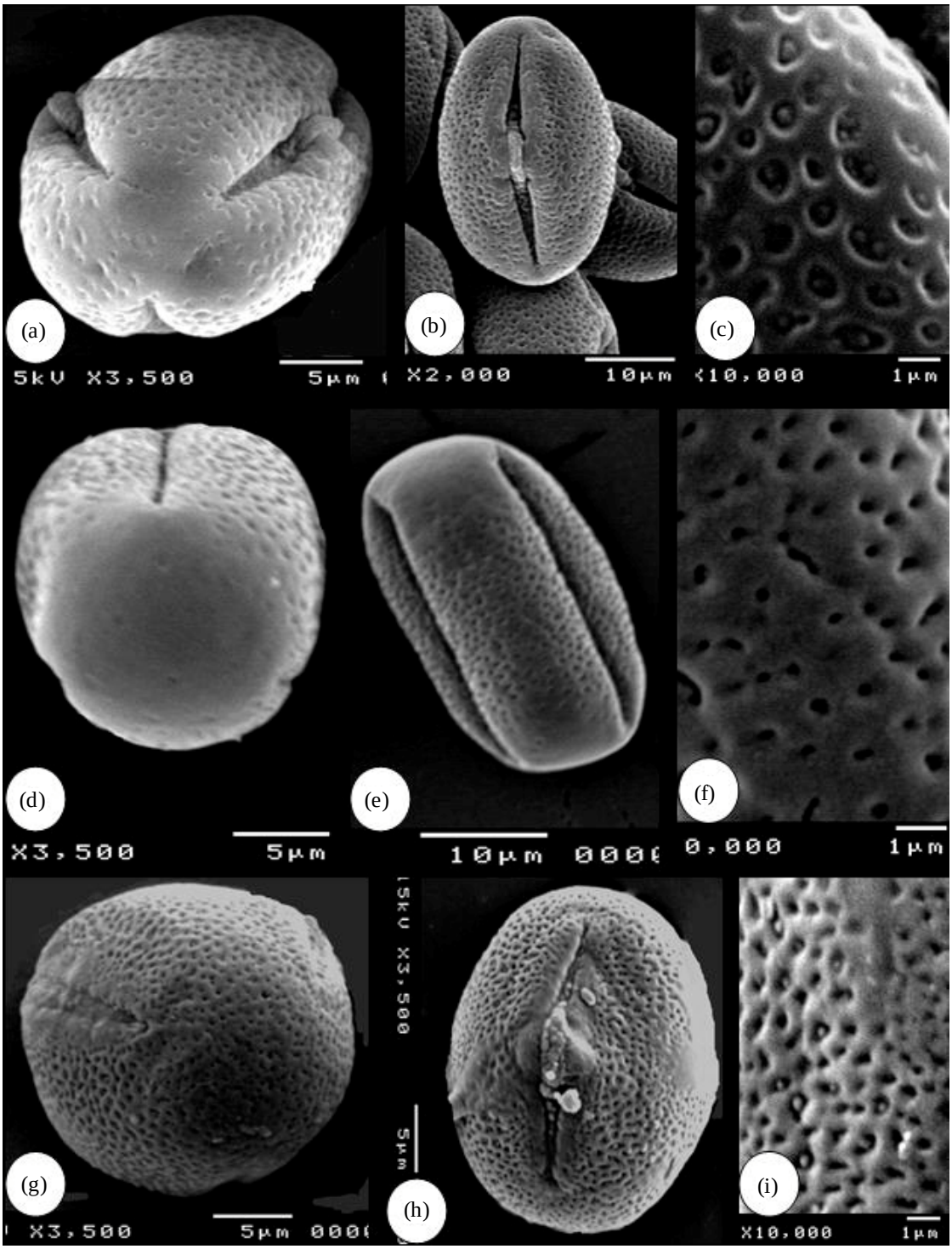

Fig. 2(a-i): SEM observations of non acetolyzed pollen grains (SEM×2000-10,000). (a-c) *A. collenettiae*: (a) Polar view, (b) Equatorial view, (c) Magnified part of exine. (d-f) *A. corrugatus*: (d) Polar view, (e) Equatorial view, (f) Magnified part of exine. (g-i) *A. dactylocarpus* ssp. *acinaciferus*: (g) Oblique polar view, (h) Equatorial view and (i) Magnified part of exine

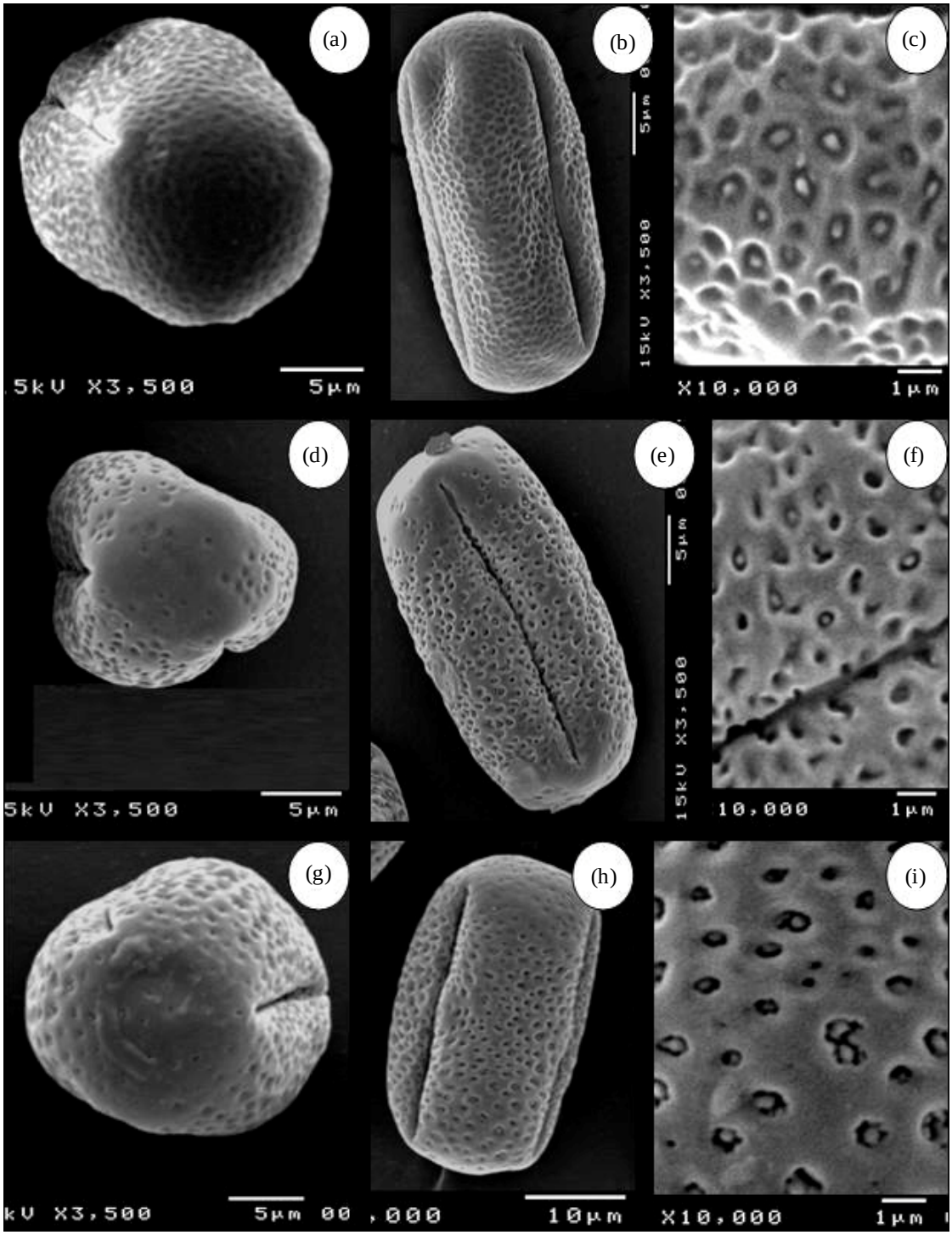

Fig. 3(a-i): SEM observations of non acetolyzed pollen grains (SEM×2000-10,000). (a-c) *A. hauarensis*: (a) Oblique polar view, (b) Equatorial view, (c) Magnified part of exine. (d-f) *A. kahiricus*: (d) Polar view, (e) Equatorial view, (f) Magnified part of exine. (g-i) *A. palaestinus*: (g) Oblique polar view, (h) Equatorial view and (i) Magnified part of exine

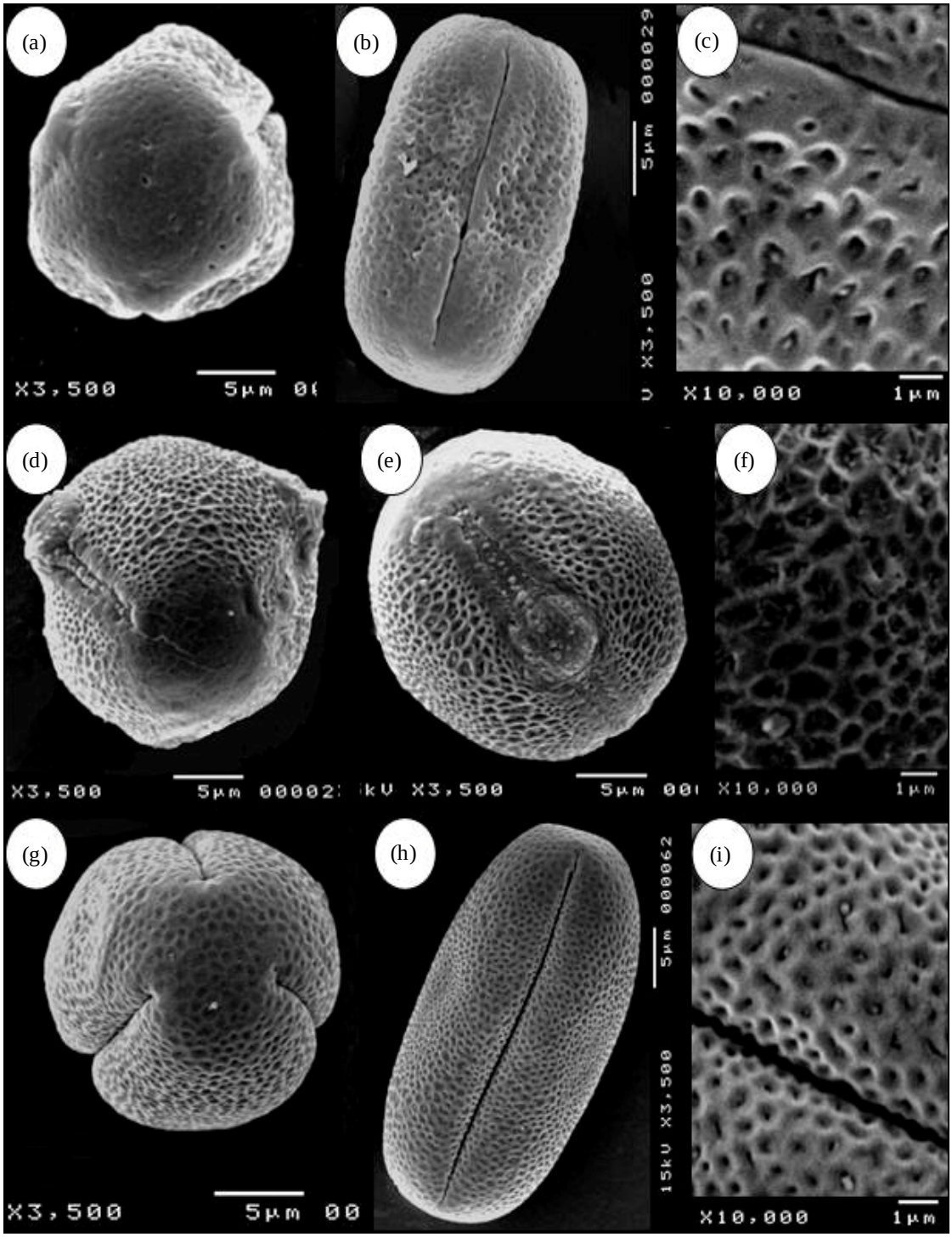

Fig. 4(a-i): SEM observations of non acetolyzed pollen grains (SEM×2000-10,000). (a-c) *A. schimperi*: (a) Polar view, (b) Equatorial view, (c) Magnified part of exine. (d-f) *A. sieberi*: (d) Oblique polar view, (e) Oblique equatorial view, (f) Magnified part of exine. (g-i) *A. spinosus*: (g) Oblique polar view, (h) Equatorial view and (i) Magnified part of exine

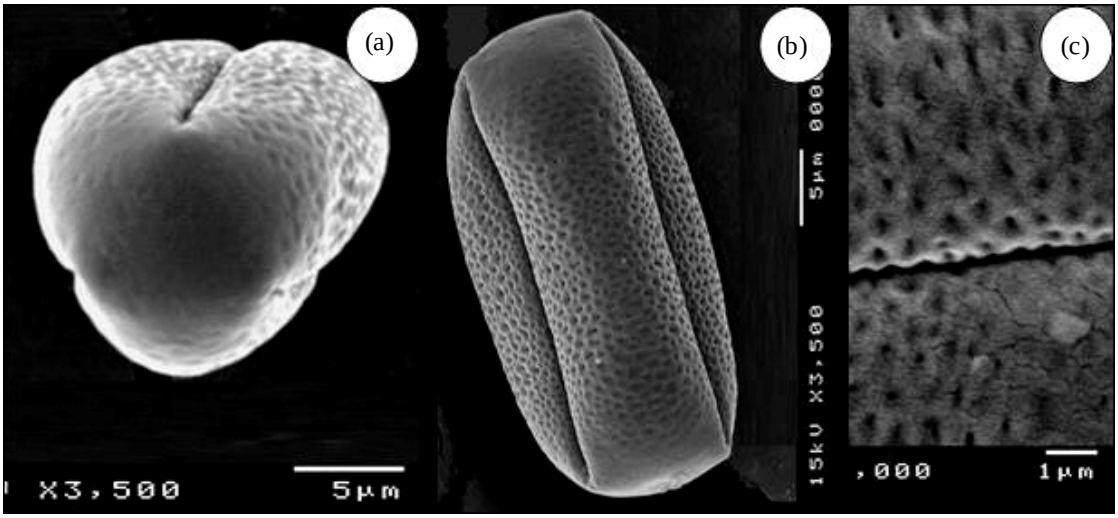

Fig. 5(a-c): SEM observations of non acetolyzed pollen grains (SEM×2000-10,000). (a-c) *A. tribuloides* var. *minutus*: (a) Oblique polar view, (b) Equatorial view and (c) Magnified part of exine

Pollen grains are 3-zonocolporate, perprolate (P/E = 2.16-2.27), 13.60 (12.29-14.66)-15.94 (14.64-17.16)×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 2):

- *A. hauarensis*Boiss., Diagn. Pl. Orient., ser. 1, 9: 63 (1849)
- Syn. *A. gyzensis* Buhge, Mém. Acad. Sci. Pétersb. 11 (16): 14 (1868)
- *A. spinosus* (Forssk.) Muschl., Verh. Bot. Vereins Prov. Brandenb. 49: 98 (1907)
- Syn. *Colutea spinosa* Forssk., Fl. Aegypt.-Arab. 131 (1775)
- *A. forsskaolii* Boiss., Diagn Pl. Orient., ser. 1, 9: 101 (1849), nom. illeg

Key to species of *A. spinosus* pollen type:

- Colpus length is 25.68 (24.88-26.08) µm (*A. hauarensis*)
- Colpus length is 31.44 (30.92-32.05) µm (*A. spinosus*)
- *A. corrugatus* pollen type, SEM (Fig. 2d-f; Table 3, 4)

Pollen grains are 3-zonocolporate, perprolate (P/E = 2.34), 13.40 (11.10-14.89)×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 2):

- *A. corrugatus* Bertol., Rar. Ital. Pl. Dec. 3: 33 (1810)
- yn. *A. cruciatus* Link, Enum. Hort. Berol. Alt. 2: 256 (1822)
- *A. sieberi* pollen type, SEM (Fig. 2d-f; Table 3, 4)

Pollen grains are 3-zonocolporate, prolate-spheroidal (P/E = 1.13), 21.73 (20.84-22.64)×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 is 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 2):

- *A. sieberi* DC., Astragalogia 186 (1802)
- Syn. *A. leucacanthus* Boiss., Diagn. Pl. Orient., ser. 1, 9: 93 (1849)

Table 5: Factor loadings showed the most intrinsic characters enhanced separations of the studied species

	Factor loadings (Unrotated) Extraction: Principal components (Marked loadings are >0.700000)		
Characters	Factor 1	Factor 2	Factor 3
Pollen shape	0.863822891	-0.14429963	0.054578492
Sculpture type	-0.114383542	0.603287314	-0.633086045
Sculpture state	0.508965505	-0.418011953	0.386613617
Colpus width at equator	0.457819198	0.644242773	0.165782989
Colpi ends	-0.361160241	0.29343653	-0.022908543
Colpi membrane	0.473536645	0.451888444	-0.182508828
Ora shape	0.11127357	-0.513586076	0.264974758
Polar view	0.820622875	-0.101790875	0.196226978
Equatorial diameter	-0.854841993	0.244110165	0.275845828
P/E	-0.933884476	-0.089399486	-0.150113808
Colpus length	-0.791072812	0.148272939	0.514302334
Colpus width	0.457819198	0.644242773	0.165782989
Ora diameter	-0.172055913	0.433761422	0.52501357
Ora area	0.346392916	0.54493472	0.377636797
Apocolpium diameter	0.367675803	0.152372022	-0.617963895
Mesocolpium diameter	0.762218251	0.474410955	0.181833374
Muri diameter	0.337148099	-0.518338588	0.49008714
Polar view total area	0.739891591	0.010094286	-0.008137344
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%			

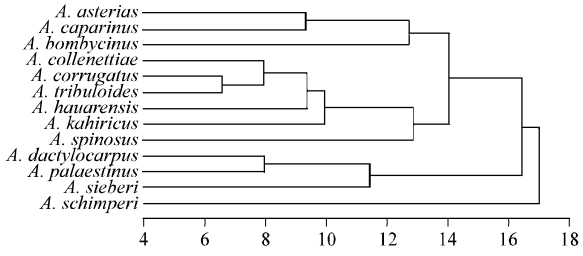

Fig. 6: Phenogram of the 13 studied *Astragalus* species, clustering with the UPGMA method

On the other hand, Fig. 6 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 clads at 18 dissimilarity distance. The first major clad at 17 dissimilarity distance, comprised only one species of the total number: *Astragalus schimperi*. The second major clad at 16.5 dissimilarity distance comprised the remaining 12 species. The second major clad separated into two branches. The first branch includes two subclads: (1) A subclad at 13 dissimilarity distance with six species: *A. collenettiae*, *A. corrugatus*, *A. tribuloides* var. *minutus*, *A. hauarensis*, *A. kahiricus* and *A. spinosus*, (2) A subclad at 12.5 dissimilarity distance with *A. asterias*, *A. caparinus* spp. *caparinus* and *A. bombycinus*. The second branch of the second major clad comprises three species: *A. dactylocarpus* spp. *acinaciferus*, *A. palaestinus* and *A. sieberi* at 10.75 dissimilarity distance. 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 5). 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 (Fig. 7).

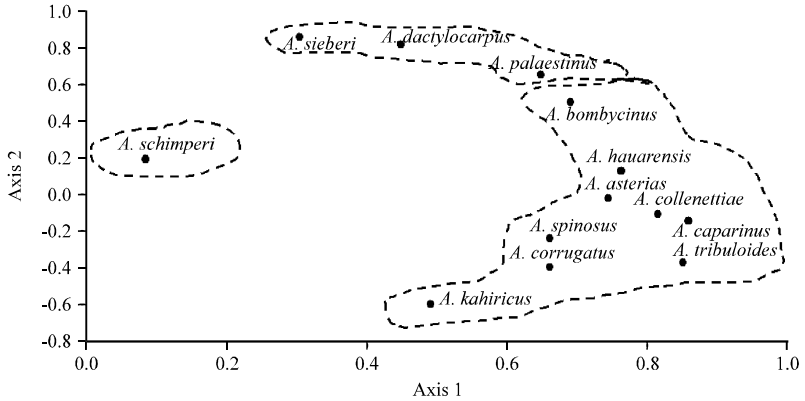

Fig. 7: Scatter-plot of 13 studied taxa plotted against the first factor by the second factor

DISCUSSION

In this palynological investigation, an additional perspective on the relations between the different *Astragalus* species studied 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* Spp. *caparinus*, *A. kahiricus* and *A. tribuloides* var. *minutus*. 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* Spp. *acinaciferus* 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 (Table 2, 3). Ceter *et al.* (2013) investigated 15 taxa belonging to the section *Hololeuce* Bunge of genus *Astragalus* and demonstrated that, Pollen grains show reticulate, perforate polar sections and perforate, reticulate, microreticulate, perforate-granulate, microreticulate-perforate, microrugulate-perforate, microrugulate-microreticulate, granulate-perforate, microreticulate-granulate at meridional sections. Akan and Aytaç (2014) studied the section *Alopecuroidei* DC. belonging to genus *Astragalus* and observed that, the pollen type in the members of this section is subprolate or prolate-spheroid. The pollen is tricolporate and the ornamentation is reticulate. The amb type is semiangular (Akan *et al*., 2005). Oskouian *et al.* (2007) examined pollen morphology of 37 *Astragalus* section *Malacothrix* and its allies. He observed that, the most common exine surface sculpturing is reticulate, rarely verrucate (only in *A. laristanicus* of subsect. *Bornmuelleriana* of sect. *Malacothrix*). Pollen shape ranges from spheroidal in *A. sphaeranthus* (sect. *Stereothrix*) to prolate in the remaining studied species. The current palynological data is of less taxonomic value to distinguish the studied taxa at both species and sectional level.

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* Spp. *caparinus* 37.22(35.14-39.30) μm. Pollen size of other species ranged between 25-35 μm (Table 4). Pollen grains which are more or less similar in shape being perprolate, prolate, subprolate or prolate-spheroidal. Colpi are narrow in *A. caparinus* Spp. *caparinus*, *A. kahiricus*, *A. spinosus* and *A. tribuloides* var. *minutus*, wide in *A. collenettiae*, *A. dactylocarpus* Spp. *acinaciferus* 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* Spp. *caparinus*, *A. corrugatus* and *A. tribuloides* var. *minutus* 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 remnant of investigated species have acute ends (Table 3). The granulation of pollen apertures membranes is showed in both *A. bombycinus* and *A. sieberi*, scabrate pollen apertures membranes is appeared in *A. dactylocarpus* Spp. *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* Spp. *acinaciferus* 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 3). 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 3).

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. 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 5). 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 (Fig. 6). Our UPGMA results showed that the *A. schimperi* is separated in the initial major clad of the cladistic tree and all the remaining 12 species are found in the other major clad. The alternate major clad is separated into two branches. The primary branch includes two subclads. The first subclad includes six species (*A. collenettiae*, *A. corrugatus*, *A. tribuloides* var. *minutus*, *A. hauarensis*, *A. kahiricus* and *A. spinosus*), while the second subclad comprises three species (*A. asterias*, *A. caparinus* spp. *caparinus* and *A. bombycinus*). The second branch of the second major clad contains three species (*A. dactylocarpus* spp. *acinaciferus*, *A. palaestinus* and *A. sieberi*) (Fig. 6). 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* spp. *caparinus* showed a much closer relationship speciesin the same clad. In addition to species of *A. dactylocarpus* spp. *acinaciferus*, *A. palaestinus* and *A. sieberi* arises also a much closer relationships being existed in the same sub-branch (Fig. 7).

ACKNOWLEDGMENTS

The authors are grateful to Deanship of Scientific Research, Northern Border University, for its great interest and financial support, which lead to the final successful completion of this research.

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