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Pollen morphology of *Convolvulus* L. populations in Markazi Province, Iran

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Abstract

Pollen morphology has been extremely useful in clarifying systematic relationships within the plant taxa. Moreover, palynology of the species and subspecies could have taxonomic value as the supporting evidence to the morphological and phylogenetic studies. Palynological studies of 12 collected *Convolvulus* populations from Markazi Province of Iran were done using light and scanning electron microscopy methods. PCA of obtained data was done using the SPSS. Results showed that there are wide variations in size of pollen grains, (min. in *C. lineatus* L. with *P*=38.40µm, *E*=37.80µm, *P/E*=1.01 and max. in *C. commutatus* Boiss. with *P*=54.00µm, *E*=56.70µm, *P/E*=0.95). The pollen shape, pollen type, exine ornamentation, length of the polar Axis (*P*) and length of the equatorial diameter (*E*), *P/E*, granule diameter and pore diameter are the most representative characters for separation of some *Convolvulus* taxa. Furthermore, *P* and *E* are more valuable in comparison with other pollen characters. Two exine ornamentation types and two pollen shapes were recognized confirming that the studied *Convolvulus* taxa are stenopalynous. The pollen grains are symmetrical, isopolar, monad, trizonocolpate and medium to large in size. The shape of the grains is oblate-spheroidal with the exception of *C. lineatus* (prolate- spheroidal). The exine ornamentation of grains is scabrate-punctate with the exception of *C. lineatus* (scabrate-punctate /fossulate). Since *C. lineatus* differs from other populations in size, *P/E*, shape and exine ornamentation, then it could be separated from the others. Finally identification key was prepared based on the studied palynological characters.

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Introduction

The Convolvulaceae (Morning Glory family) is a beautiful family which is widely cultivated as ornamentals (Rajurkar *et al*, 2011). *Convolvulus* L. from *Convolvulae* (Convolvulaceae) has about 250 species worldwide (Perveen *et al*, 1989) and 60 species in Iran (Ghahreman, 1994). The family is widely distributed in cold regions, temperate, subtropical and tropical areas all over the world. There have been some attempts to use pollen features in the delimitation of the genera in the family Convolvulaceae (Saensouk, 2007; Wills, 1973 and Mabberey, 1987).).Tellera and Daners (2003) studied of 75 species pollen grain, and described three main pollen types. Osman and Abdel Khalik (2005) studied the pollen morphology of Convolvulaceae in Egypt and recognized three main pollen types. Palynological studies of form and function are far from complete despite more than a century and a half of light microscopy investigations and nearly one-half century of electron microscopic research (Stuessy *et al*, 2003). The pollen morphology of some species of Convolvulaceae was first carried out by Hallier (1893) who recognized *Psiloconiea* and *Echinoconiea* as two main groups. Gamble (1933) divided the family into two groups based on spinulose or non-spinulose pollen grains, with further division on the basis of apertural types. Erthman (1953) distinguished the pollen of Convolvulaceae into two distinct types, viz. *Ipomoea* (*Echinoconiea*) and *Convolvulus* (*Psiloconiea*) type. O'Donell (1955) separated the genera *Convolvulus* and *Calystegia* R.BR. based on Hallier's aperture descriptions. Lewis and Oliver (1965) described the pollen grains of *Convolvulus* as prolate to more frequently subspheroidal. Sengupta (1972) in his comprehensive palynological survey of *Convolvulus* divided the family into four main groups based on the number and distribution of apertures and placed the genus *Convolvulus* in tricolpate pollen group. Pollen morphology studies of 22 species of the genus *Convolvulus* from south west Asia and Arabian peninsula was carried out by Perveen and his coworkers (1989). They found that the studied pollen grains are mostly trizonocolpate, occasionally tetra or hexazonocolpate, prolate-spheroidal to oblate-spheroidal rarely prolate and tectum punctate-scabrate. Menemen and Jury (2002) studied pollen grains of nine species of *Convolvulus* from Morocco and reported that all pollen grains were trizonocolpate, suboblate, oblate-spheroidal, prolate-spheroidal, prolate and the nexine is thinner than the sexine (Menemen and Jury 2002). In this study, palynological data of the 12 collected *Convolvulus* populations from Markazi province of Iran was reported using light and scanning electron microscopy. Finally, a key was prepared based on the *Convolvulus* L. pollen characters.

Materials and methods

Collection and determination of plants

Twelve populations of four *Convolvulus* species were collected from Markazi Province, Iran (Table 2). Plants were identified using available references (IBPGR 1988, Ghahreman, 1979-2008, Rechinger, 1754). Voucher specimens were deposited at Arak university herbarium.

Pollen papering, LM & SEM

Anthers of all obtained populations were dissected from the mature flowers of the sampled plants. The separated anthers were cleaned and then air dried on drying papers, Anthers of each samples was then cloven by a thin needle in order to free the pollen grains. Some pollens of each sample moved to labeled vials for scanning electronic microscopy. Pollen grain samples for light microscopy (LM) were processed by the standard acetolysis method of Erdtman (1952, 1960) with slight modification. In this method, equal amounts of 10% acetic acid was added to all test tubes containing pollen grain samples and then centrifuged in 1000-1800 rpm for 10 minutes. After that, the supernatant was discarded and acetolysis solution (9 volume of acetic anhydride and 1 volume of sulfuric acid) was added to the test tubes. Because of extreme heat caused after adding acetolysis solution to pollens, test tubes were left on room temperature to cool down for a few minutes. Afterwards, the test tubes were put in hot bath (100°C) for 10 minutes and then were

centrifuged. The supernatant was discarded and the first step of experiment (adding 10% acetic acid and centrifuging for 10 minutes) was repeated. In the next stage, equal amounts of distilled water were added to pollens in each test tube and then centrifuged in order to clean pollens completely from chemical contaminants. The pollens were then mounted in glycerol jelly and then investigated and photographed under a Leica Galen III photomicroscope.

Scanning electronic microscope (SEM) was made by a KYKY, model EM3200. Pollens were mounted onto specimen stubs and then coated with a thin layer of gold using sputter Argon Gas Bombarding, made by KYKY, model SBC 12. Gold layer thickness was 100 Angstrom and does not affect the microstructures of pollens. Electron micrographs were taken of each sample.

Pollen Terminology

The pollen terminology in general was followed by that recommended by Punt *et al.* (2007), and Moor *et al.* (1991). Eight quantitative and qualitative palynological characters for each population studied with combined LM (Fig. 1) and SEM (Fig. 2). Measurements were carried out at 32 pollen grains. These features are listed in Table 1 and are scored qualitatively. Palynological characters for statistical analysis were based on Punt *et al.* (2007) as well as our own observations.

Data analysis

Three qualitative and five quantitative palynological characters were coded and measured as multistate characters (Table 3). Data were analyzed using the SPSS (1997) for windows release 22.0 statistical package for social scientists and Minitab software by principal component analysis (PCA) test (Tables 5 and 6). Cluster analysis using Ward, Average Linkage (between groups) and Median methods were then performed on standardised pollen grain characters data. Fitting of the clusters to the original data was checked using cophenetic correlation (Fig. 3). Finally, a key based on the *Convolvulus* L. pollen characters was prepared (Table 7).

Results

Quantitative Characters

Table 2 shows collection information of the 12 *Convolvulus* populations from markazi province of Iran. Palynological data of the studied populations are indicated in table 3. Results show that there are wide variations in dimensions of pollen grain in investigated *Convolvulus* populations. Variation range of polar axis length was from min. 32.50µm in CBB_{10}, *C. pilosellafolius* to max. 60 µm in CBB_8, *C. commutatus*. Equatorial diameter was from min. 30µm in CBB_{13}, *C.lineatus* to max. 66.25µm in CBB_8, *C. commutatus*.

Table 1. Listing of studied palynological encoded quantitative and qualitative characters for multivariate analysis in 12 *Convolvulus* populations in Markazi Province, Iran based on Punt *et al* (1994).

Characters	Abbreviations Signs
Polar Axis Length (µm)	*P*
Equatorial diameter (µm)	*E*
Polar axis length / Equatorial diameter	*P/E*
Granule Diameter	GD
Pore Diameter	PD
Pollen Shape: 1. Prolate – Spheroidal (PS), 2. Oblate – pheroidal (OS)	PS
Exine Ornamentation: 1. Scabrate – Punctat/ Fossulate (SPF) 2. Scabrate - Punctate(SP)	EO
Pollen Type: Trizonocolpate (TZ)	PT

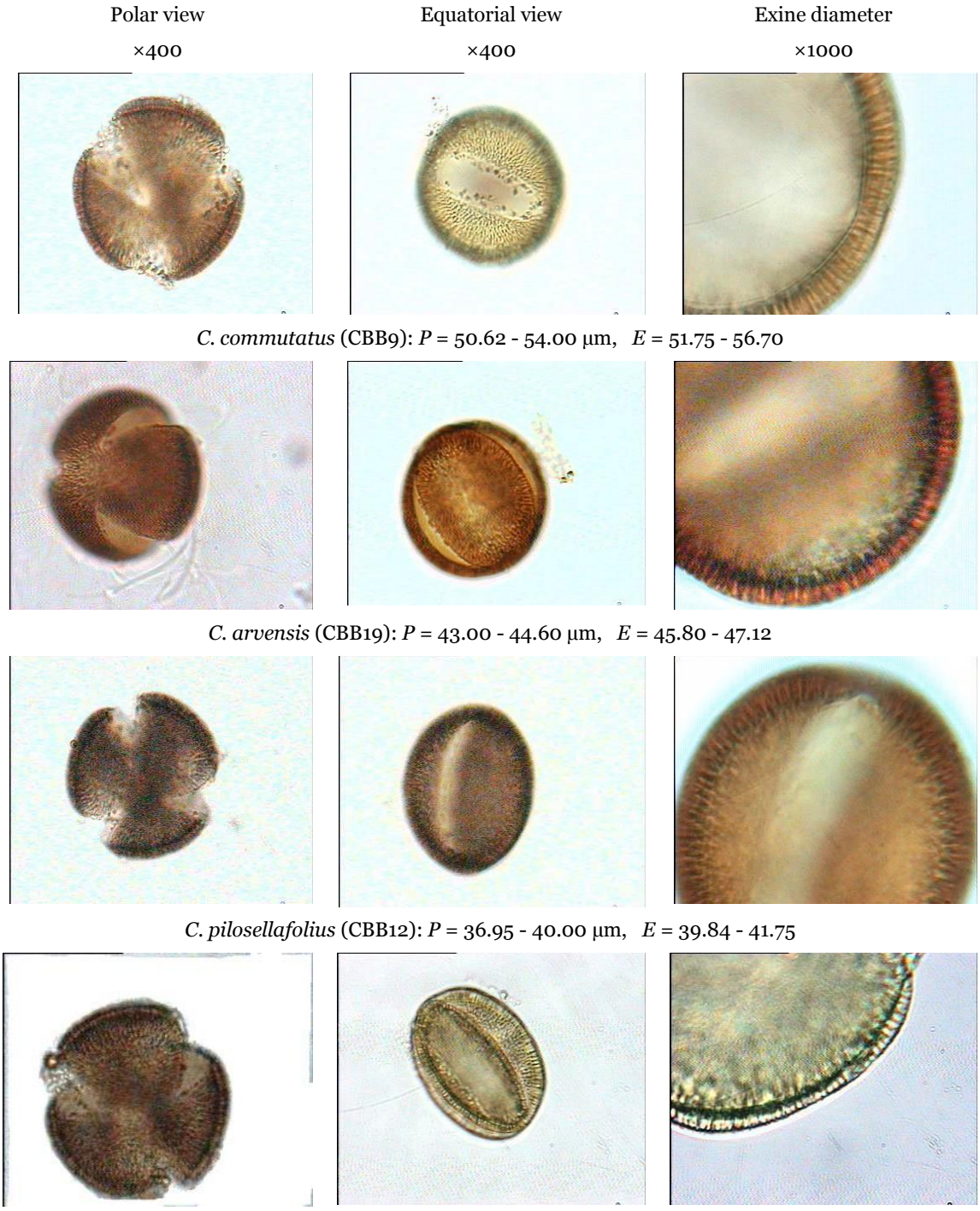
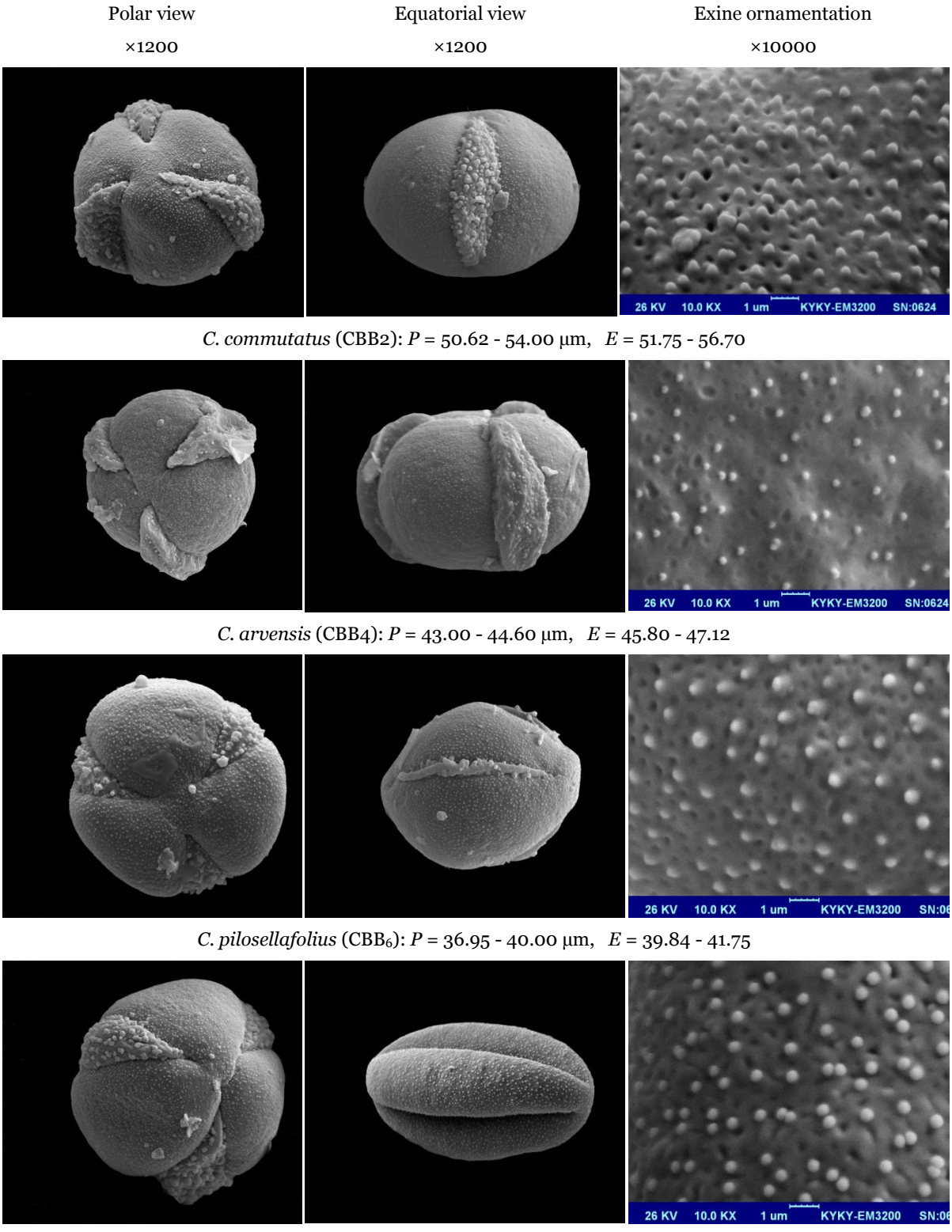


Fig. 1. Studied *Convolvulus* species from Markazi Province, Iran pollen grain using light microscopy, left: polar view (×400), Middle: equatorial view (×400), Right: exine diameter (×1000).

The smallest mean polar axis length (*P*) was belonged to CBB_{10} (*C. pilosellafolius, P*=36.95 µm) and the largest one to the CBB_8, (*C. commutatus*, *P*=54µm). Furthermore, the smallest *E* was seen in CBB_{13}, (*C.lineatus E*=37.80µm) and the largest one in CBB_8, (*C.commutatus*, *E*=56.70µm).



C. commutatus (CBB2): *P* = 50.62 - 54.00 µm, *E* = 51.75 - 56.70

C. arvensis (CBB4): *P* = 43.00 - 44.60 µm, *E* = 45.80 - 47.12

C. pilosellafolius (CBB$_6$): *P* = 36.95 - 40.00 µm, *E* = 39.84 - 41.75

C. lineatus (CBB$_{13}$): *P* = *38.40* µm, *E* = 37.80

Fig. 2. Scaning electron mirographs of four *Convolvulus* species pollen grains; left. Polar view, middle. equatorial view, right. exine ornamentation. Scale bars for all polar and equatorial pollen grains view 10 µm, Scale bars for all exine ornamentations 1 µm.

Table 2. Collection information of 12 studied *Convolvulus* populations from Markazi Province, Iran.

Code	Species	Date	Sampeling locality	Latitude N	Longitude E	Altitude (m)
CBB_2	*C. commutatus*	17.05.2013	Arak - Sardasht	34° 04′	49° 37′	1870
CBB_8	*C. commutatus*	27.05.2013	Arak - Nazmabad	34° 03′	49° 44′	1884
CBB_9	*C. commutatus*	30.05.2013	Arak - Gerdo	34° 02′	49° 41′	1886
CBB_4	*C. arvensis*	24.05.2013	Arak - Shahsavaran	34° 09′	49° 59′	1680
CBB_5	*C. arvensis*	24.05.2013	Arak - Shahsavaran	34° 09′	49° 59′	1680
CBB_7	*C. arvensis*	24.05.2013	Tafresh	34° 41′	50° 01′	1948
CBB_{19}	*C. arvensis*	13.06.2013	Delijan - Naragh	34° 00′	50° 50′	1848
CBB_6	*C. Pilosellafolius*	24.05.2013	Saveh - Saft	34° 37′	50° 23′	1350
CBB_{10}	*C. Pilosellafolius*	04.06.2013	Delijan - Hajiabad	34° 15′	50° 32′	1332
CBB_{11}	*C. Pilosellafolius*	04.06.2013	Delijan - Dodahak	34° 07′	50° 35′	1377
CBB_{12}	*C. Pilosellafolius*	04.06.2013	Delijan -15 Khordad Park	34° 02′	50° 40′	1507
CBB_{13}	*C. lineatus*	04.06.2013	Arak - Zaloo	33° 51′	49° 56′	2026

CBB: Batoul Bahrami collection numbers.

Table 3. Palynological data of 12 studied *Convolvulus* populations from Markazi Province, Iran area.

		P (µm)			*E* (µm)			*P*/*E*	GD (µm)		PD (µm)			S	T	EO
Code	Species	Min.	M±SD	Max.	Min.	M±SD	Max.	Min.	M±SD	Max.	Min.	M±SD	Max.			
CBB_2	*C. commutatus*	48.75	[51.12±2.33]	55.00	48.75	[51.75±2.45]	56.25	0.98	0.36	[0.40±0.03]	0.50	0.23	[0.30±0.04]	0.39	OS	TZ	SP
CBB_8	*C. commutatus*	50.00	[54.00±2.64]	60.00	50.00	[56.70±3.86]	66.25	0.95	0.30	[0.36±0.03]	0.40	0.15	[0.20±0.05]	0.31	OS	TZ	SP
CBB_9	*C. commutatus*	42.50	[50.62±4.32]	56.25	43.75	[51.78±4.70]	57.50	0.97	0.25	[0.31±0.06]	0.42	0.10	[0.16±0.04]	0.23	OS	TZ	SP
CBB_4	*C. arvensis*	38.75	[44.24±3.60]	52.50	41.25	[45.80±2.30]	51.25	0.96	0.22	[0.27±0.03]	0.39	0.23	[0.29±0.04]	0.38	OS	TZ	SP
CBB_5	*C. arvensis*	37.50	[44.60±3.07]	52.50	37.50	[46.90±4.00]	52.50	0.95	0.30	[0.33±0.03]	0.40	0.12	[0.17±0.04]	0.23	OS	TZ	SP
CBB_7	*C. arvensis*	40.00	[43.83±2.84]	51.25	35.00	[47.12±3.53]	52.50	0.93	0.25	[0.31±0.04]	0.46	0.15	[0.19±.0.03]	0.26	OS	TZ	SP
CBB_{19}	*C. arvensis*	36.25	[43.00±2.92]	48.75	37.50	[46.20±3.60]	55.00	0.93	0.25	[0.31±0.02]	0.35	0.12	[0.17±0.03]	0.23	OS	TZ	SP
CBB_6	*C. pilosellafolius*	35.00	[38.40±2.46]	43.75	32.50	[40.00±3.22]	43.75	0.96	0.30	[0.42±0.05]	0.52	0.15	[0.28±0.09]	0.46	OS	TZ	SP
CBB_{10}	*C. pilosellafolius*	32.50	[36.95±2.05]	41.25	32.50	[39.84±2.70]	43.75	0.92	0.33	[0.39±0.05]	0.58	0.15	[0.20±0.04]	0.27	OS	TZ	SP
CBB_{11}	*C. pilosellafolius*	36.25	[38.92±1.60]	42.50	33.75	[41.75±2.80]	46.25	0.93	0.29	[0.35±0.05]	0.50	0.12	[0.20±0.06]	0.31	OS	TZ	SP
CBB_{12}	*C. pilosellafolius*	36.25	[40.00±1.55]	42.50	35.00	[40.12±3.90]	47.50	0.99	0.33	[0.43±0.13]	1.00	0.15	[0.24±0.07]	0.39	OS	TZ	SP
CBB_{13}	*C. lineatus*	35.00	[38.40±1.75]	42.50	30.00	[37.80±3.90]	43.75	1.01	0.32	[0.42±0.04]	0.50	0.19	[0.34±0.19]	1.00	PS	TZ	SPF

CBB: Batoul Bahrami collection numbers.

There were variations in size of pollen grain in studied taxa (min. in CBB_{13}, *C. lineatus* with *P*=38.40±1.75µm, *E*=37.80±3.90µm and max. in CBB_8, *C. commutatus* with *P*=54±2.64µm, *E*=56.70±3.86µm). It is suggested for obtaining size of pollen grain; its volume was calculated based on dimensions (*P*&*E*) and the following formulas:

$V_{Prolate}=4/3\pi a2b$, a(*P*/2)&b(*E*/2)

$V_{Oblate}=4/3\pi a2b$, a(*E*/2)&b(*P*/2)

V_{CBB13}, *C. lineatus* was nearly equal to 29170 µm³ and V_{CBB10}, *C. pilosellafolius* was nearly equal to 30692 µm³ indicating that the smallest size of pollen grain was belonged to CBB13, *C. lineatus*.

The smallest proportion *P*/*E* was belonged to CBB_{10}, *C. pilosellafolius* (0.92) and the largest one to CBB_{13}, *C. lineatus* (1.01).

Variation range of granule diameter was from 0.22µm in CBB_4, *C. arvensis* to 1.00µm in CBB_{12}, *C. pilosellafolius* and of pore diameter was from 0.1µm in CBB_9, *C. commutatus* to 1.00µm in CBB_{13}, *C. lineatus*. CBB_4, *C. arvensis* had the smallest mean of granule diameter with 0.27µm and the largest one was belonged to CBB_{12}, *C. pilosellafolius* with 0.43µm. The smallest mean of pore diameter was seen in CBB_9, *C. commutus* with 0.16µm and the largest one in CBB_{13}, *C. lineatus* with 0.34µm (Table3).

Our observations indicated that there are certain proportions between flower and pollen size. It seems that the largest flower has the largest pollen as well (Table4).

Table 4. A comparison of flower size and pollen size in 4 studied *Convolvulus* species from Markazi Province, Iran area.

D	*C. commutatus*	*C.*arvensis	*C. pilosellafolius*	*C.lineatus*
FL(mm)	19.75 – [24.66 ± 3.5] – 27.40	18.17 – [19.32 ± 1.04] – 21.00	11.30 – [12.45 ± 0.74] – 13.24	16.37
P(µm)	50.53 – [51.91 ± 1.5] – 51.20	43.00 – [43.92 ± 0.60] – 44.24	36.95 – [38.57 ± 1.08] – 40.00	38.40
E(µm)	51.75 – [53.41 ±2.33] – 56.70	45.80 – [46.50 ± 0.53] – 47.12	39.84 – [40.43 ± 0.77] – 41.75	37.50

Qualitative Characters

The results indicated that pollen morphology of all studied populations were trizonocolpate (fig. 1 and 2, left), occacionally tetra and rarely hexazonocolpate, ectaperture long with tapering or acute ends, colpal membrane condensely granulated, exin thick, sexine thicker than nexine, distinctly stratified in LM (Fig.1, right) (Perveen *et al*, 1989). The pollen grains were symmetrical, isopolar and monad. Pollen grains were oblate-spheroidal in all of studied *Convolvulus* taxa with the exception of CBB_{13}, *C. lineatus* (prolate - spheroidal). Two exine ornamentation types were recognized: scabrate – punctate / fossulate, in CBB_{13}, *C. lineatus* and scabrate - punctate in other populations (Fig.2, right, Table3).

Table 5. Total Variance explained for principal component analysis for pollen characters of studied *Convolvulus taxa* in Markazi Province, Iran.

Comp-onent	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumu-lative %	Total	Cumu-lative %	% of Variance	Total	Cumu-lative %	% of Variance
1	3.805	54.357	54.357	3.805	46.782	**46.782**	3.275	54.357	54.357
2	1.703	24.324	78.681	1.703	78.681	**31.899**	2.233	**78.681**	24.324
3	.836	11.946	90.627						
4	.425	6.067	96.694						
5	.231	3.297	99.991						
6	.001	.009	100.000						
7	1.748E-16	2.497E-15	100.000						

Analyzing Data

Table 5 shows total variance explained for principal component analysis for studied pollen morphology characters of 12 studied *Convolvulus* populations. This table shows two component PCA test and correlating pollen morphology characters of the studied *Convolvulus* populations (P<0.05).

Table 6. Two components of PCA test and correlating pollen characters of studied *Convolvulus* populations in Markazi Province, Iran. Bold values are positive significant *P<0.05*.

Rotated Component Matrix.

	Component	
	1	**2**
Pollen Axis Length/ Equatoria Diameter	**.891**	
Exine Ornamentation	**-.863**	
Pollen Shape	**-.863**	
Pore Diameter	**.804**	
Granule Diameter	.537	
Pollen Axis Length		**.994**
Equatorial Diameter		**.959**

Table 7. A key to pollen of the 12 *Convolvulus* populations in Markazi Province, Iran examined on the base of the palynological characters.

1.	1a	Exine ornamentation = Scabrate-Punctate/Fossulate, *P*/*E* = 1.1, Pollen Shape = Prolate-Spheroidal	*C. lineatus*
	1b	Exine ornamentation = Scabrate-Punctate, *P*/*E*<1, Pollen Shape = Oblate-Spheroidal	2
2.	2a	*P* = 50.62 - 54.00 µm, *E* = 51.75 - 56.70	*C. commutatus*
	2b	*P* = 43.00 - 44.60 µm, *E* = 45.80 - 47.12	*C. arvensis*
	2c	*P* = 36.95 - 40.00 µm, *E* = 39.84 - 41.75	*C. pilosellafolius*

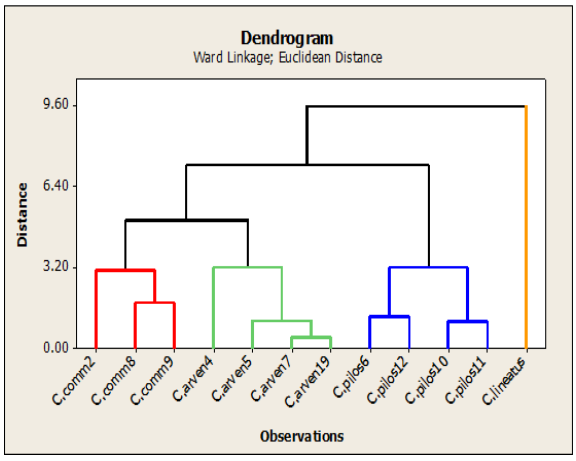


Fig. 3. Cluster analysis (Minitab) of pollen morphology data using cophenetic correlation from 12 studied *Convolvulus* populations in Markazi Province, Iran. Scored characters for cluster analysis have been shown in Table 1.

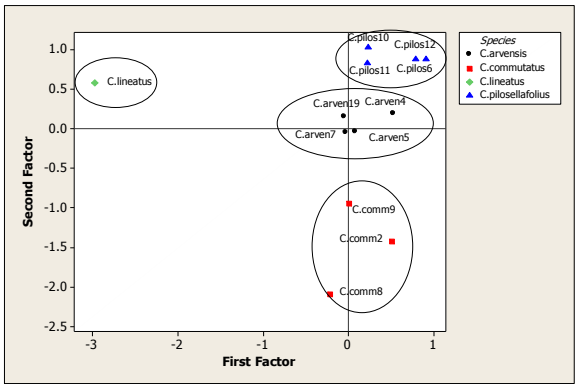


Fig. 4. PCO histogram of studied palynological characters of 12 *Convolvolus* populations in Markazi Province, Iran.

Analyzing palynological qualitative and quantitative characters using three different cluster analysis methods indicated that the Ward method provides

the best data (Fig. 3). Table 6 shows two components of PCA test and correlating pollen characters of studied *Convolvulus* taxa. Data analysis using six different cluster analyzing methods indicated that ward Linkage and Minitab Euclidean Distance (16) were the best (Fig. 3). Fig. 4 show grouping of collected 12 *Convolvulus* populations in Markazi Province, Iran using Minitab software. Table 7 shows an identification key based on the studied *Convolvulus* characters.

Discussion

Studies on pollen grains morphology is considered as the basic necessity for palynology due to its fundamental value in the recognition and identification of grains found in various conditions (Arora and Modi, 2008).

Convolvulaceae is a eurypalynous family in which the pollen morphology has been quite useful in resolving several taxonomical problems, particularly at subfamily and generic level (Perveen *et al*, 1989). Telleria and Daners (2003), found that the pollen morphology of Convolvulaceae is known to be highly divers and has taxonomic importance. There are both spinulose and non-spinulose types of grains in the family (Hallier, 1893; Lewis & Oliver, 1965). The number, position and shape of apertures are also quite variable in Convolvulaceae. However, the pollen of genus *Convolvulus* seem to be fairly uniform and the species do not depict much variation. Then genus *Convolvulus* is stenopalynous (Perveen *et al*, 1989). Sengupta (1972) recognized two different pollen types on the basis of exine pattern in *Convolvulus* L.; *C. arvensis* and *C. clavertii* with distinctly punctate exine were placed in one group while the remaining species with finely punctate exine were placed in the second group. Furthermore, Perveen *et al.* (1989) reported four different pollen types on the basis of exine pattern in their survey, which could be further subdivided on the basis of the shape of the grains. However, palynological studies have significant taxonomic importance on determining relationships between subfamilies, species, tribes and populations and their features. Among pollen grain features, present of aperture and tectum ornamentation and stratification of pollen wall have preference to characters such as shape, size, and tectum ornamentation that are variable within tribes and genera (Noori, 2002).

Our results indicated that although the genus *Convolvulus* is a stenopalynous taxon, but the species showed significant diversity in their quantitative and qualitative features. As Table 3 shows, all of studied pollen grains are trizonocolpate, medium to large in size, oblate–spheroidal in shape (Fig.1 and 2, left) and have scabrate-punctate exine ornamentation (Fig.2, right) with the exception of CBB_{13} that is prolate-spheroidal and has scabrate-punctate/Fossulate. However, five types of pollen shape were observed in all of the studied taxa including oblate, oblate-spheroidal, spheroidal, prolate-spheroidal and prolate. In addition to trizonocolpate pollen grains, there were tetrazonocolpate grains in CBB_9 and CBB_{13}. Hexazonocolpate grains were also observed in CBB_{13}, but their occurrence was infrequent.

Our results are in consistency with Perveen *et al.* (1989) data. They reported that *Convolvulus* species pollens are trizonocolpate and prolate-spheroidal to oblate-spheroidal rarely prolate. They found that *C. pilosellafolius* pollen shape is oblate-spherodal and *C. lineatus* prolate-spheroidal and have scabrate-punctate tectum exine ornamentation. Menemen and Jury (2002) showed that Morroco *Convolvulus* species pollen grains are suboblate, oblate-spheroidal, prolate-spheroidal and prolate. Cronk and Clark (1981) reported hexazonocolpate pollen grains in *C. lineatus* from north European species, while others had normal trizonocolpate grains. Although they did not observe any significant variation among *Convolvulus* species, but they separated *C. lineatus* from other species due to the presence of irregularly shaped luminae formed by fusion of tectal punctate. These results confirm our observations (Fig. 2). Exine ornamentation of the studied pollens had sculptures and pori. Sculptures are micro-echinates or spines

with obtuse and blunt apices that have been irregularly distributed (Menemen and Jury, 2002). Sculpturing elements are smaller than 1µm, the same width as height with the exception of CBB_{13} (*C. lineatus*) that are constricted at their bases. Pori are rounded or elongate tectal perforations, less than 1µm in length or diameter (Punt *et al*., 1994) in all of the studied populations with the exception of *C. lineatus* species that is separated from others by this character.

Pollen grain biometry of the samples showed that there are wide variations only in size, not in shape, exine ornamentation and type (Table 3). These data confirmed that all of the studied *Convolvulus* populations in Markazi Province are stenopalynous.

Factor analysis results of palynological characters (Tables 5 and 6) showed that seven factors (*P*/*E*, exine ornamentation, pollen shape, porus diameter, granule diameter, polar axis length and equatorial axis length) describe about 79% of total variance. The first component with 47% total variance was found positively correlated with *P*/*E* and granule diameter and negatively correlated with exine ornamentation and pollen shape. The second component with 32% total variance was positively and significantly correlated with polar axis length and equatorial diameter ($P<0.05$).

In Fig. 3, cluster analysis of palynology data using cophenetic correlation showed two main clades. The first clade consisted of just CBB_{13} (*C. lineatus*) with 38.40µm for *P* and 37.80µm for *E* that has been separated from others by the reason having different shape and exine ornamentation and the second clade consisted of the others. This clade has two subclades that the first one consisted of two groups. The first group is including CBB_2, CBB_8 and CCB_9 populations (*C. commutatus*) and the second group CBB_4, CBB_5, CBB_7 and CBB_{19} (*C. arvensis*). The second subclade from the main clade consisted of CBB6, CBB12, CBB10 and CBB11 (*C. pilosellafolius*). These two subclades have been separated from each other by having different pollen grain sizes.

Our results indicated that there is a certain proportion between flower and pollen sizes. It seems that the largest flowers have the largest pollens (*C. commutatus* in Table 4). Others with medium pollen grains have the medium flowers in size. Similar relations were not found between other quantitative features with the flower size. Karamian *et al*., (2010) found a similar conclusion about perennial species of *Heliobrychis* and *Onobrychis* genus members.

Fig. 4 has been concluded on the basis of analysis of palynology data using minitab software and PCA indicating four main groups. In this fig., populations have been segregated based on species which completely confirms cluster analysis of palynology data using cophenetic correlation in Fig. 3.

According to the Table 2, geographical latitude and longitude ranges of the collected samples sites were from 33° 41' to 34° 41'N and from 49° 37' to 50° 50' E, respectively. Altitude ranges of the sites were from 1332m (for CBB_{10}=*C. pilosellafolius*) to 2026 m (for CBB_{13}=*C. lineatus*). Our results suggest that these two populations have the smallest size of the pollen among all of the studied samples. It seems that there is not any certain proportion between altitudes with pollen size, although it may be between altitude with shape and exine ornamentation. An identification key based on obtained data is presented in Table 7.

Recommendations

It is suggested that for more subtle results, studying other biosystematics characters is required. Moreover, measuring pollen volume average based on dimensions and geometrical shape of the pollens is necessary for reaching to better conclusion in palynology. Application of the molecular markers along with the current research strategies could be useful.

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