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POLLEN CHARACTERS AS TAXONOMIC EVIDENCE IN SOME SPECIES OF DIPSACACEAE FROM IRAN

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Abstract

Pollen morphology of nine species representing four genera: *Cephalaria* Schrad, *Dipsacus* L., *Pterocephalus* Vaill. and *Scabiosa* L. of the family Dipsacaceae in Iran has been investigated by means of scanning electron microscopy (SEM). The results showed that pollen grains were triporate and tricolpate. The pollen type of *Scabiosa rotata* Bieb. (tri- and tetraporate) is the first report in the world. The sizes of pollen grains fall into the classification group magna (pollen grain diameter 50–100 µm). Pollen shapes vary from preoblate to prolate and their polar views were triangulate and lobate. The exine ornamentation varies from gemmate in *S. rotata* to spinulate in the rest studied species. Species of *Scabiosa* have been dispersed in UPGMA tree that this confirmed the previous studies about taxonomic problems and species complexity in this genus. These results show the transfer of the some *Scabisoa* species to *Lomelosia* Raf. based on palynological characters. Pollen morphology of the family is helpful at the generic and specific level.

Introduction

The family Dipsacaceae consists of around 10-13 genera and more or less 300 species (Ehrendorfer, 1965; Verlaque, 1977; Mabberley, 2008) of annual to perennial herbs and shrubs that occur primarily in the Mediterranean Basin, with about 20% distributed in Asia and Africa. In Iran, Dipsacaecae is represented by 54 species belonging to five genera distributed in different regions (Jamzad, 1993). The family has long been regarded as belonging to the Dipsacaceae, whereas according to APG III it is included within the larger family Caprifoliaceae (Reveal and Chase, 2011). Delimitation of taxa within the family has always been subject to argument; accordingly, circumscription of genera and tribes has repeatedly changed over of the overall morphological similarity among the taxa in the family. The family was divided into two tribes by De Candolle (1830), viz. Morineae (including a single genus, *Morina* L.) and Scabioseae (including *Cephalaria*, *Dipsacus*, *Knautia*, *Pterocephalus*, and *Scabiosa*). Verlaque (1984) divided this family into three tribes with nine genera. Caputo and Cozzolino (1994) divided Dipsacaceae into two major clades (based on morphological and palynological characters), one includes *Dipsacus* and *Cephalaria*, the other contain the remaining genera.

The significance of pollen morphology in Plant Systematics has been stressed by various researchers. Stuessy (2009) state that data from pollen grains are known to be useful at all levels of the taxonomic hierarchy (generic, subgeneric, inter-specific and intraspecific levels), and can often be helpful in suggesting a relationship. Some studies (for example, Feng *et al*., 2000; Khalik, 2010; Perveen, 2011) showed that pollen morphological characteristics play a major role in solving taxonomic problems. Palynological characteristics have been able to reposit several disputed genera and interpret problems related to the origin and evolution of many taxa (Nair, 1980) and to derive a classification of angiosperms (Cronquist, 1981).

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The works of Mayer and Ehrendorfer (2000) on the pollen morphology of *Pterocephalus* and Feng *et al*., (2000) on the palynology of the genus *Dipsacus* show that the study of pollen grains provides useful data for the taxonomy of different genera.

There is a modicum of information on the pollen morphology of the family Dipsacaceae, most especially in Iran. This study reports the pollen morphology of some species in the family Dipsacaceae from Iran in order to establish their availability for future taxonomic works.

Materials and Methods

Pollen grains of 9 species, representing 4 genera of Dipsacaceae distributed in Iran were studied by means of scanning electron microscope (SEM). The studied plant samples were collected from natural populations in different regions during spring and summer in 2014-2015. The voucher specimens were deposited in Azarbaijan Shahid Madani University Herbarium (ASMUH). The list of voucher specimens and details of localities are given in Table 1.

Table 1. List of species used in the study along with localities and vouchers.

Genus	Species	Locality	Voucher No.
Cephalaria Schrad	*C. kotschyi* Boiss.	Mazndaran, Chalus	ASMUH95001
	C. procera Fisch.	Ardebil, Khalkhal	ASMUH95002
Pterocephalus Vaill.	*P. plumosus* (L.) Coulter	Mazndaran, Chalus	ASMUH95003
	P. canus Coulter	Tehran, Ab-ali	ASMUH95004
Dipsacus L.	*D. strigosus* Willd	Mazndaran, Chalus	ASMUH95005
Scabiosa L.	*S. caucasica* M. B.	Ardebil, Khalkhal	ASMUH95006
	S. amoena Jacq.	Gilan, Masuleh	ASMUH95007
	S. koelzii Rech. f.	Khorasan, Bojnord	ASMUH95008
	S. rotata Bieb.	Khorasan, Mashhad	ASMUH95009

Pollen grains were separated from anthers by using binocular microscope. For each taxon, three specimens were used, and from each specimen at least five anthers were examined. Pollen grains for scanning electron microscopy were mounted on standard aluminum stubs using double-sided adhesive tape and then photographed using Phenomprox scanning electron microscope at 10 KV voltages.

Palynological characters such as equatorial diameter (E), polar axis length (P), P/E, exine ornamentation etc. were measured (at least 30 pollen grains) by using Image Tools software with high accuracy and confidence degree. For grouping of the studied taxa, data were standardized (mean = 0, variance = 1) and used for the multivariate analyses using unweighted pair-group method with arithmetical mean (UPGMA) based on Euclidean Distances and principal component analysis (PCA) by means of PAST Package (Hammer *et al*., 2001). The terminology used is in accordance with Erdtmann (1952) and Punt *et al*. (2007).

Results

The pollen grains of the studied species revealed some variations. All palynological structures and measurements for the examined species concerning pollen type from polar view, polar (P) and equatorial (E) measurements, P/E ratio, pollen shape, polar view and exine ornamentation were shown in Table 2.

Table 2. Measurements of pollen-morphological character in the studied taxa.

	Pollen type	Polar axis (P)	Equatorial axis (E)	(P/E)	Pollen shape	Polar view	Spine length	Exin ornamentation
C. kotschyi	Triporate	57.52 ± 1.36	86.90 ± 0.65	0.66	Oblate	Triangulate	1.60 ± 0.11	Spinulate-Spinuloid
C. procera	Triporate	55.73 ± 1.71	70.05 ± 0.56	0.79	Suboblate	Triangulate	1.72 ± 0.15	Spinulate-Spinuloid
P. plumosus	Tricolpate	78.51 ± 0.27	54.93 ± 0.81	1.42	Prolate	Lobate	1.37 ± 0.09	Spinulate-Spinuloid
P. canus	Tricolpate	75.52 ± 0.17	60.36 ± 1.76	1.25	Subprolate	Lobate	2.40 ± 0.08	Spinulate-Spinuloid
D. strigosus	Triporate	90.43 ± 1.81	74.59 ± 0.28	1.21	Subprolate	Triangulate	1.14 ± 0.09	Spinulate
S. caucasica	Triporate	50.97 ± 1.67	92.47 ± 1.01	0.55	Oblate	Triangulate	1.83 ± 0.14	Spinulate-Spinuloid
S. amoena	Tricolpate	92.1 ± 0.14	61.70 ± 0.19	1.49	Prolate	Triangulate	1.30 ± 0.11	Spinulate-Spinuloid
S. koelzii	Tricolpate	91.53 ± 0.26	65.57 ± 0.2	1.39	Prolate	Lobate	1.55 ± 0.15	Spinulate-Spinuloid
S. rotata	Tri and tetraporate	28.97 ± 0.59	75.05 ± 1.47	0.38	Preoblate	Triangulate	-	Gemmate-Spinuloid

Generally, there are two major types of pollen grain apertures, varying from triporate to tricolporate among studied species (Fig 1). The pollen grains of *S. rotata* are triporate and tetraporate (Fig 1. I, J).

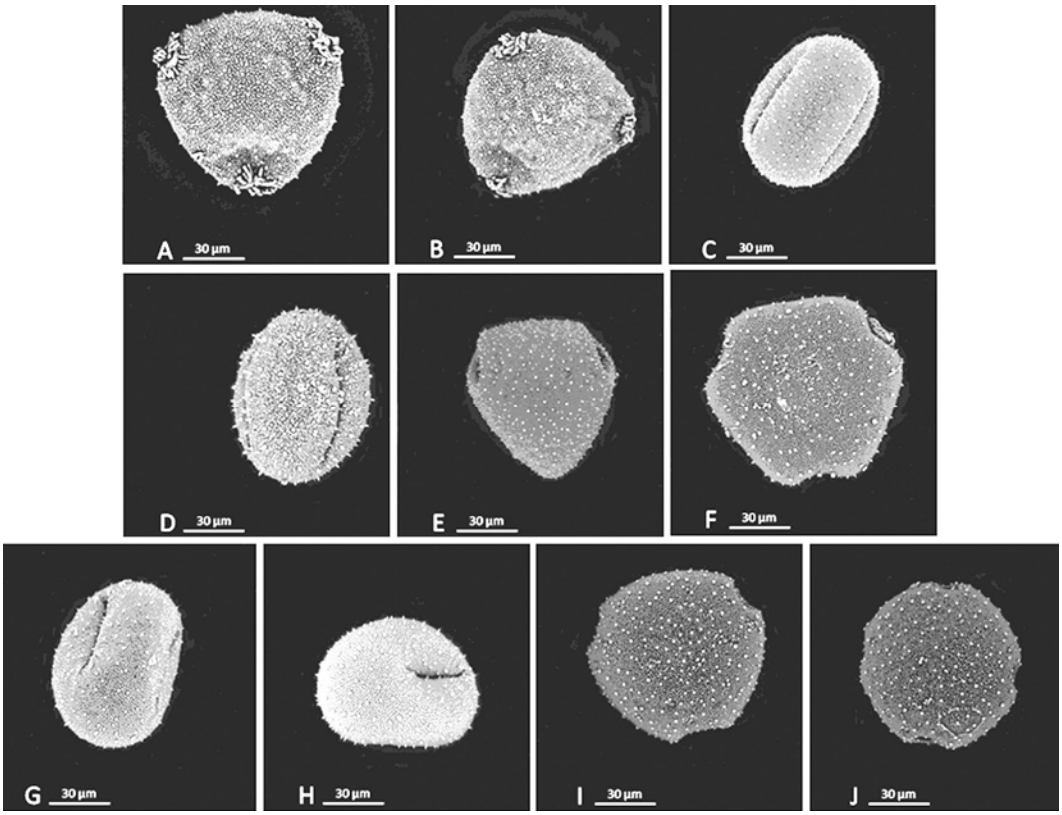

Fig. 1. Scanning electron microscope photographs of pollen grains. A: *C. kotschyi*, B: *C. procera*, C: *P. plumosus*, D: *P. canus*, E: *D. strigosus*, F: *S. caucasica*, G: *S. amoena*, H: *S. koelzii*, I: *S. rotata* (triporate), J: *S. rotata* (tetraporate)

Polar axis (P) length of pollen grains showed large variation, ranging from the smallest size for *S. rotata* (28.97 µm) to the largest size for *S. amoena* (92.1 µm). Equatorial axis (E) length of pollen grains ranged from the smallest size in *P. plumosus* (54.93 µm) to the largest size in *S. caucasica* (92.47µm). The shape classes are based on the ratio between the length of polar axis (P) and equatorial diameter (E). The P/E ratio ranged from 0.38 µm to 1.49 µm, therefore the pollen shape is subprolate to prolate in *Seabiosa amoena*, *S. koelzii*, *Pterocephalus plumosus*, *P. canus* and *D. strigosus* but preoblate to oblate in the rest studied species. However, the pollen shape in polar view varies from lobate in *P. plumosus*, *P. canus* and *S. koelzii* to triangulate in the rest of the species (Fig. 1).

The exine sculpturing showed a complex structure. The SEM showed that the outer surface of the tectum is a solid layer which is covered by numerous similar small conical spinuloid (Fig. 2). The exine surface with gemmate (wart-like pegs) was found in *S. rotata* but spinulate (more than 3 µm long) in the rest studied species.

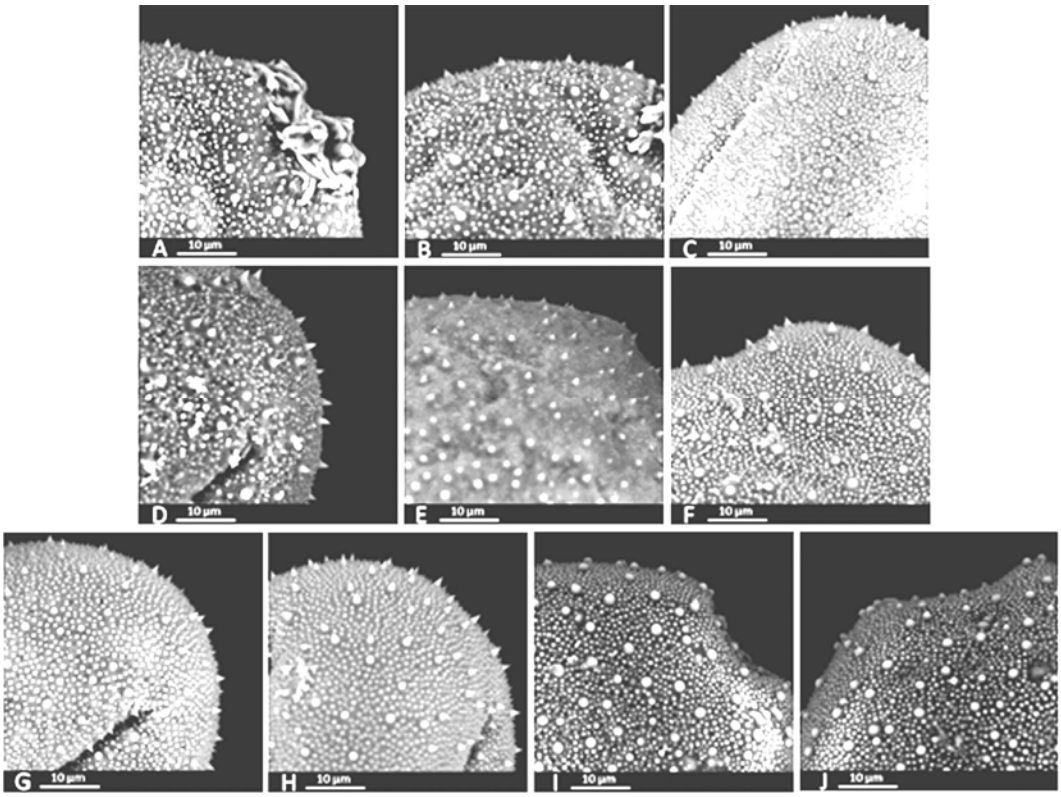

Fig. 2. Scanning electron microscope photographs of pollen surface ornamentation. A: *C. kotschyi*, B: *C. procera*, C: *P. plumosus*, D: *P. canus*, E: *D. strigosus*, F: *S. caucasica*, G: *S. amoena*, H: *S. koelzii*, I: *S. rotata* (triporate), J: *S. rotata* (tetraporate)

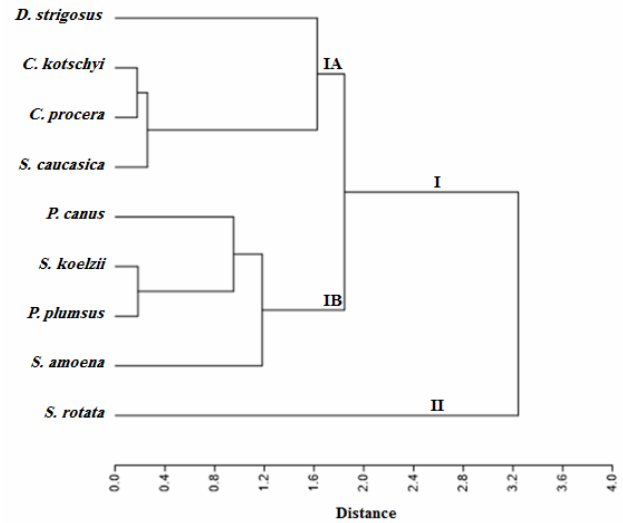

Fig. 3. UPGMA dendrogram showing the relationship among studied taxa based on pollen characters.

The studied taxa were separated from each other in a UPGMA tree based on palynological characters. Cluster analysis showed that the studied species placed in two majorclusters (Fig. 3). One contained *S. rotata* (cluster II) and remainders were clustered in other major branch (cluster I) of two sub-clusters assigned as IA and IB. Sub-cluster IA included *D. strigosus*, *C. kotschyi*, *C. procera* and *S. caucasica*. *Pterocephalus canus*, *P. plumsus*, *S. koelzii* and *S. amoena* grouped together within the sub-cluster IB (Fig. 3).

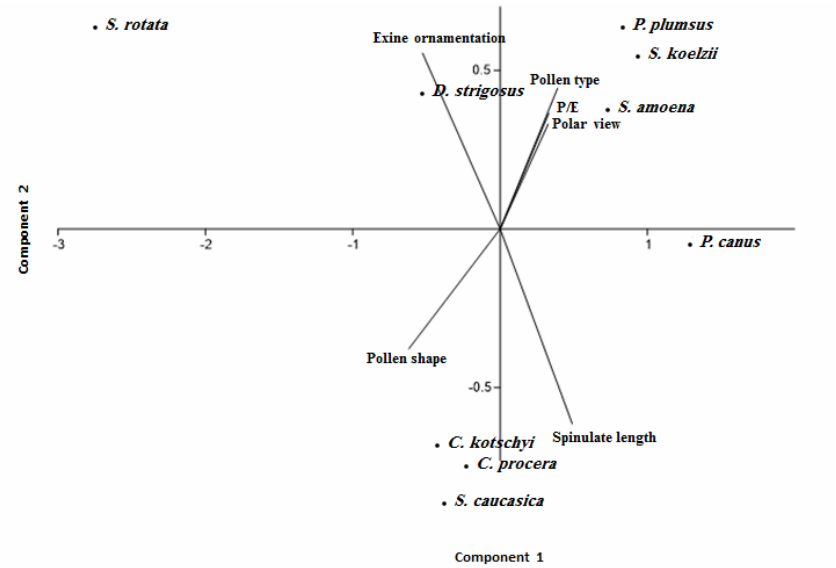

Fig. 4. Principal Component Analysis (PCA) among studied taxa based on pollen characters.

Principal component analysis (PCA) showed that the pollen type, pollen view, P/E and exine ornamentation have main role in grouping of species belonging to the clade IA. While the pollen shape and spine length are importance characters in grouping of species belonging to the clade IB (Fig. 4). According to the exine ornamentation, *S. rotata* was separated from the remaining species.

Discussion

The present study shows a palynological polymorphism within the family Dipsacaceae. Generally, the interspecific differences within a genus are often trivial, but there are remarkable differences among the various genera (Khalik, 2010).Various palynological investigations on different species of Dipsacaceae confirmed importance of pollen traits for distinguishing taxa. For instance, Khalik (2010) studied pollen characteristics in nine species belonging to four genera of Dipsacaceae in Egypt and showed that pollen character can be used to delimit the species.

Statistical analysis showed that most of the qualitative characters were useful in classification of studied species and had taxonomic value. Aperture types, polar view and pollen shape can be used as diagnostic evidence in palynological studies.

In this study, two types of pollen apertures (porate and colpate) were found in this family. In addition, we distinguished the pollen with tetraporate aperture in *S. rotata* for the first time. Erdtmann (1952) and Clarke and Jones (1981) studied the pollen morphology of the Dipsacaceae, distinguished two types of pollen apertures: porate and colpate.

Considering the exine ornamentation, it is obvious that this palynological character cannot be used to delimit studied species, because all of them were spinulate except *S. rotata* that was gemmate. Interestingly, spine length varied between studied species and it could be suitable as a diagnostic character. Based on spine length, *P. canus* can be distinguished from the other taxa. In our study, *S. rotata* has specific palynological character such as gemmate of exine ornamentation and tri-tetraporate pollen that helps to distinguish it.

The findings of the present study on the size of pollen are in agreement with previous study (Ebadi-Nahari and Nikzat-Siahkolaee, 2016). Pollen grains have been classified into groups according to their sizes by Erdtman (1952) as perminuta (diameter less than 10 µm), minuta (diameter 10-25 µm), media (diameter 25-50 µm), magna (diameter 50- 100 µm), permagna (diameter 100-200 µm) and giganta (diameter greater than 200µm). Based on this classification, the pollen grains of the species studied belong to group magna (diameter 50-100µm). Khalik (2010) reported nine species belonging to four genera of the Dipsacaceae in the group size magna (diameter 50-100 µm). The pollen grain size reported for this family supports the fact that the flowers in the genera are more insect-and-bird pollinated than by wind.

Ehrendorfer (1965) first studied phylogeny of Dipsacaceae at generic and infrageneric levels. The morphology and anatomy of flowers and the phylogeny, palynology, and karyology of Dipsacaceae were studied by Verlaque (1977, 1986). The work of Verlaque demonstrated that the evolution within the Dipsacaceae followed complex paths and that several genera were polyphyletic. There has been much discussion about infrageneric taxonomy of the genus *Scabiosa* (Bobrov, 1957; Jasiewicz, 1976), and in spite of the fact that the genus has been the subject of many taxonomic studies.

In this survey, *Scabiosa* s. l Rechinger and Lack (1991) is considered. On the basis of Greuter and Raus (1985) studies, Iranian species of *Scabiosa* divided into two genera: *Lomelosia* (= *Scabiosa* sec. *Astrocephalus* and sec. *Olivoerinae*) and *Scabiosa* s.s (*Scabiosa*s. l sec. *Scabiosa*) that this classification was not accepted in Flora Iranica (Rechinger and Lack, 1991) and Flora of Iran (Jamzad, 1993). Existence of eight pits on the epicalyx tube is a distinguishing character of *Lomeloisa* genus that separated this genus from other related genera (de Castro and Caputo, 1999). Regarding to this classification, *S. rotata* and *S. caucasica* grouped in *Lomeloisa* and *S. koelzii* and *S. amoena* maintained in *Scabiosa* s. s. Considering this point of view, studied species of *Scabisa* (*S. koelzii* and *S. amonea*) were closely related to *Pterocephalus* that supported Verlaque (1986) regarding *Scabiosa* s.s and *Ptercephalus* have closely related than *Lomelosia*, *Sixalix* and *Pycnocomon*.

There is high degree of homoplasy at generic level in Dipsacaceae (de Castro and Caputo, 1999). As shown in this study and previous study (Ebadi-Nahari and Nikzat-Siahkolaee, 2016), all studied *Scabiosa* species with 8 groove on the epicalyx (*S. columbaria*, *S. koelzii*, *S. amoena*) have colpate pollen apertures and all *Scabiosa* species with 8 pits on the epicalyx (*S. micrantha*, *S. persica*, *S. calocephala*, *S. olivieri*, *S. flavida*, *S. rotata*, *S. caucasica*) have porate pollen apertures. These results show the transfer of the some *Scabisoa* species to *Lomelosia* based on palynological characters. However, there is parallelism in the genus *Lomelosia* with related genera (*Sixalix*, *Scabiosa*) (de Castro and Caputo, 1999) that confusing relationships within and between specimens.

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