PALYNOLOGICAL STUDY OF THE GENUS CARDUUS L. (ASTERACEAE) IN IRAN

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Carduus L. (*Asteraceae*) comprised about 90 species of which 8 of them are distributed in Iran. From these only one species is endemic. The pollen morphology of eight species of *Carduus* was investigated using light and scanning electron microscope (SEM). The pollen type was sub-oblate to oblate-spheroidal, with echinate sculpturing. The grains sizes varies from 35.76-50.46 µm in polar view and from 37.38-59.63 µm in equatorial view. Palynological data are useful characters to separate studied species. The out-group species belong to the genus *Cirsium* Mill. and are located in a distinct cluster. The obtained species relationships are in agreement with the morphological results and previous taxonomic treatment of the genus.

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Key words. Carduus, Asteraceae, SEM , LM, Palynology.

مطالعات گرده‌شناسی جنس Carduus (Asteraceae) در ایران

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جنس *Carduus* L. (*Asteraceae*) شامل حدود 90 گونه است که 8 گونه آن در ایران پراکنش دارد و تنها یک گونه آن انحصاری ایران می‌باشد. ریخت‌شناسی دانه‌های گرده 8 گونه از جنس با استفاده از میکروسکوپ نوری و میکروسکوپ الکترونی نگاره مورد بررسی قرار گرفت. نوع دانه گرده sub- oblate تا oblate- spheroidal با تزئینات خاردار است. اندازه دانه‌های گرده در دید قطبی از 50/46 µm – 35/76 و در دید استوایی از 37/38–59/63 µm متغیر هستند. داده‌های گرده‌شناسی صفات خوبی برای جدایی گونه‌های مطالعه شده می‌باشد. گونه‌های برون‌گروه متعلق به جنس *Cirsium* Mill. در یک خوشه جدا قرار گرفته اند. روابط گونه‌ای مطابق با نتایج مورفولوژیکی و رفتارهای تاکسونومیکی قبلی این جنس می‌باشد.

Introduction

Tribe *Cardueae* of the subfamily Cichorioideae, (*Asteraceae*) has about 2500 species distributed in 83 genera (Bremer 1994; Susanna 2006; Funk & al. 2009) which grow mainly in the northern hemisphere of the Old World. The genus *Carduus* L. (subtribe *Carduinae*) contains about 90 species and is native to Europe, Asia and Africa.

The pollen characters are useful patterns in the context of recent molecular hypotheses of relationship, and could be used to support the species relationships, or provide diagnostic characters for groups at a variety of levels particularly in *Asteraceae* (Wortley & al. 2007). Palynology provides a whole new set of characters for studying the species relationships and taxonomy, compared to the conventional macro-morphological study and also provides data which may be considered less affected by ecological specialization of the taxa, the use of additional pollen characters possibly avoid such limitations and improve the results (Scotland & al. 2003).

Asteraceae taxa have the 3-celled pollen grains which might be an apomorphy for them, while spinulate (or smooth) pollen grains are shared between

Table 1. *Carduus* species examined in palynological study and out group species.

Species	Voucher no.	Locality
Carduus hamulosus Ehrh. subsp. *hystrix* Ehrh.	H S B U 8700100	West Azerbaijan : Bazargan, near Kalisakandy,1412 m, Azizi
C. thoermeri Weinm subsp. *armenus* Weinm	H S B U 8700129	East Azerbaijan: Kaleibar,Ghaleh Babak,1139 m,Nouroozi
C. onopordioides Fisch.	H S B U 8700119	West Azarbaijan: Ghotour, Razi, 1935 m, Azizi
C. pycnocephalus L. subsp. *pycnocephalus*	H S B U 8700136	Tehran: Niavaran, 1672m, Azizi
C. transcaspicus Gandog subsp. *macrocephalus* Kazmi	H S B U 8700112	Tehran: Road of Karaj to Chaloos, Polezanguleh, 2064 m , Azizi
C. arabicus Jacq. Ex Murray	H S B U 8700138	West Azerbaijan :Oromie lake, Ashk Island,1268 m, Azizi
C. seminudus M. B.	H S B U 870012	Mazandaran: Kelardasht , 1219 m , Azizian & Zehzad
C. getulus Pomel	7752-IRAN	Khoozestan : Aghajari, 200 m , Termeh
Cirsium adancum Fisch. & C. A. Mey. (out group)	H S B U 8600170	Azerbaijan:Meshkinshahr,MazraeJahan,1169 m, Nouroozi & Fathollahi
Cirsium ciliatum (Murray) Moench (out group)	H S B U 8600160	Azerbaijan:Salmas to Khoy, 1400 m, Nouroozi
Cirsium vulgare (Savi) Ten (out group)	H S B U 8600218	Tehran: Road of Karaj to Chaloos, Asara, 2050 m, Nourooz

Barnadesioideae and *Mutisioideae* and also found in *Calyceraceae* and some other families (Hansen 1991; Urtubey & Telleria 1998).

In Flora Iranica, eight *Carduus* species have been reported from Iran (Rechinger 1979), which have not been investigated at the population level and no clear idea is available for their morphological diversity and the species affinities. Therefore, the present study reports palynological characteristics of these species and tries to illustrate their relationships.

Materials and methods

Pollen morphology was studied by light microscopy (LM) and scanning electron microscopy (SEM) in 8 *Carduus* species growing in Iran (table 1). Nnorouzi & al. (2012) investigated pollen characteristics of out-group taxa of the genus *Cirsium* in Iran (table 1). The voucher specimens are deposited in the Shahid Beheshti University herbarium(HSBU) and herbarium of IRAN. The pollen samples were obtained mostly from freshly collected specimens and also from dry herbarium materials. Fully mature anthers were removed from specimens and prepared by the standard acetolysis method (Erdtman 1952, 1969). And then they were mounted in glycerin jelly and seal with paraffin wax for light microscopy. Morphological studies were performed on minimum 30 pollen grains for each taxon by an Olympus Light Microscope Model BH-2.

For scanning electron microscopy, the acetolysed pollen grains were attached to aluminum stubs with double-sided cellophane tape, air-dried at room temperature and coated with gold. The specimens were examined with a Philips XL 20 SEM at 20kV. UTHSCSA Image Tool ver. 3 software was used for pollen measurements, and then data obtained were scored. For multivariate analyses 18 pollen grain characteristics including 5 qualitative and 13 quantitative characters were used (table 2; table 3). For multivariate analyses the mean of quantitative characters were used while qualitative characters were coded as binary/multistate characters (table 4). Standardized data (mean = 0, variance = 1) were used for multivariate statistical analyses. The average taxonomic distance and squared Euclidean distance were used as dissimilarity coefficient in cluster analysis of data (Podani 2000). Principal Components Analysis (PCA) was performed among the species studied to determine palynological characteristics useful for separating the species. In order to group the species, cluster analysis using UPGMA (Unweighted Paired Group with Arithmetic Average) and NJ(Neighbor–joining) methods and PCA ordination plot were used performed using euclidean and taxonomic distances calculated among the species (Podani 2000). Clustering and ordination plot analyses using NTSYS ver.2 (1998).

Table 2. Pollen qualitative characters in *Carduus* and *Cirsium* out group species.

Species	Type of pollen	Shape of spine	Opercule surface	Spine type	Spinule
C. hamulosus	oblate – spheroidal	erect and curved	stripy-granular	acute	absent
C. thoermeri	suboblate	erect and curved	granular	acute	absent
C. onopordioides	suboblate	erect and curved	granular	acute	absent
C. pycnocephalus	oblate- spheroidal	erect	stripy-granular	obtuse	present
C. transcaspicus	oblate - spheroidal	erect and curved	stripy-granular	acute	absent
C. arabicus	oblate - spheroidal	erect	stripy-granular	obtuse	present
C. seminudus	oblate - spheroidal	erect and curved	stripy-granular	acute	absent
C. getulus	oblate - spheroidal	erect and curved	stripy-granular	acute	absent
Cirsium adancum (out group)	oblate- spheroidal	erect	stripy-granular	obtuse	absent
Cirsium ciliatum (out group)	oblate- spheroidal	erect	stripy-granular	acute	absent
Cirsium vulgare (out group)	oblate- spheroidal	erect	stripy-granular	obtuse	absent

Table 3. Pollen quantitative characters in *Carduus* and outgroup *Cirsium* species. (Species abbreviations: h= *Carduus hamulosus*, tr= *C. transcaspicus*, th= *C. thoermeri*, on= *C. onopordioides*, py= *C. pycnocephalus*, ar= *C. arabicus*, se= *C. seminudus*, ge= *C. getulus*, c1= *Cirsium adancum* (out group), c2= *C. ciliatum* (out group) and c3= *C. vulgare* (out group). Character abbreviations: 1. polar length (P) (µm) 2. equatorial length (E) (µm) 3. P/E ratio 4. exine thickness (µm) 5. spine length (µm) 6. colpus length (µm) 7. spine width (µm) 8. distance between two spines (µm) 9. Mesocolpium width (µm) 10. opercule length (µm) 11. opercule width (µm) 12. length to width ratio of opercule 13. number of spines per 10 µm 2.The quantitative data presented in this table are the mean size of at least 30 pollen grains observed.

	1	2	3	4	5	6	7	8	9	10	11	12	13
h	42.99	47.09	.91	5.40	5.36	26.20	7.10	10.20	34.00	13.30	11.30	1.18	4
th	50.46	59.63	.85	6.10	5.39	29.90	8.03	11.73	44.4	14.00	11.50	1.22	3
on	38.87	44.65	.87	5.20	4.79	20.80	7.89	10.64	33.6	12.10	9.16	1.32	2
py	38.33	41.97	.91	5.53	3.37	17.30	6.35	9.47	31.30	7.05	6.31	1.12	2
tr	46.60	52.55	.89	5.90	4.39	21.90	7.63	11.27	39.80	12.90	9.35	1.38	4
ar	39.27	41.07	.96	4.91	3.54	20.20	6.99	10.50	27.80	8.79	5.34	1.65	2
se	40.56	44.51	.91	5.18	4.92	16.40	7.99	10.97	33.9	9.67	6.04	1.60	4
ge	35.76	37.38	.96	4.87	2.93	13.90	5.19	8.27	26.2	7.44	5.59	1.33	4
c1	64.61	68.20	.95	8.88	6.50	30.23	9.22	15.89	53.59	16.54	13.44	1.23	3
c2	77.05	87.25	.89	10.10	8.20	30.11	9.65	20.14	64.20	15.87	13.76	1.15	4
c3	69.58	79.23	.89	9.63	7.28	31.12	8.88	19.34	60.39	16.94	13.32	1.27	3

Results

The pollen grain type ranged from sub-oblate to oblate-spheroid in *Carduus* species studied (Fig. 1; Fig. 2; Fig. 3). The mean size of pollen grains in polar view (P) ranged from 35.76 µm in *C. getulus* to 50.46 µm in *C. thoermeri*, while the mean size of pollen in equatorial view (E) ranged from 37.38 µm in *C. getulus* to 59.63 µm in *C. thoermeri*. The P/E ratio ranged from 0.85 in *C. thoermeri* to 0.96 in *C. arabicus* and *C. getulus*.

The mean pollen wall exine thickness varied from 4.87 µm in *C. getulus* to 6.10 µm in *C. thoermeri* and the mean of spine length varied from 2.93 µm in *C. getulus* to 5.39 µm in *C. thoermeri*. Similarly the mean spine width differed from 5.19 µm in *C. getulus* to 8.03 µm in *C. thoermeri* .The mean colpus length ranged from 13.90 µm in *C. getulus* to 29.90 µm in *C. thoermeri*. The mean distance between two spines ranged from 8.27 µm in *C. getulus* to 11.73 µm in *C. thoermeri*. The mean of colpus length varied from 26.2 µm in *C. getulus* to 44.4 µm in *C. thoermeri*. The mean of operculum length varied from7.05 µm in *C. pycnocephalus* to 14 µm in *C. thoermeri*. The mean of operculum width varied from 5.34 µm in *C. arabicus* to 11.50 µm in *C. thoermeri*. The length to width ratio of operculum ranged from 1.12 in *C. pycnocephalus* to 1.65 in *C. arabicus*. The mean number of spines per 10 μm^2 varied from 2 in *C. onopordioides*, *C. pycnocephalus* and *C. arabicus* to 4 in *C. hamulosus*, *C. transcaspicus*, *C. seminudus* and *C. getulus* . The shape of spine was erect in *C. pycnocephalus* and *C. arabicus* while, it was erect-curved in the other *Carduus* species studied.

UPGMA, NJ, PCA, PCO and parsimony trees of palynological characters produced similar results

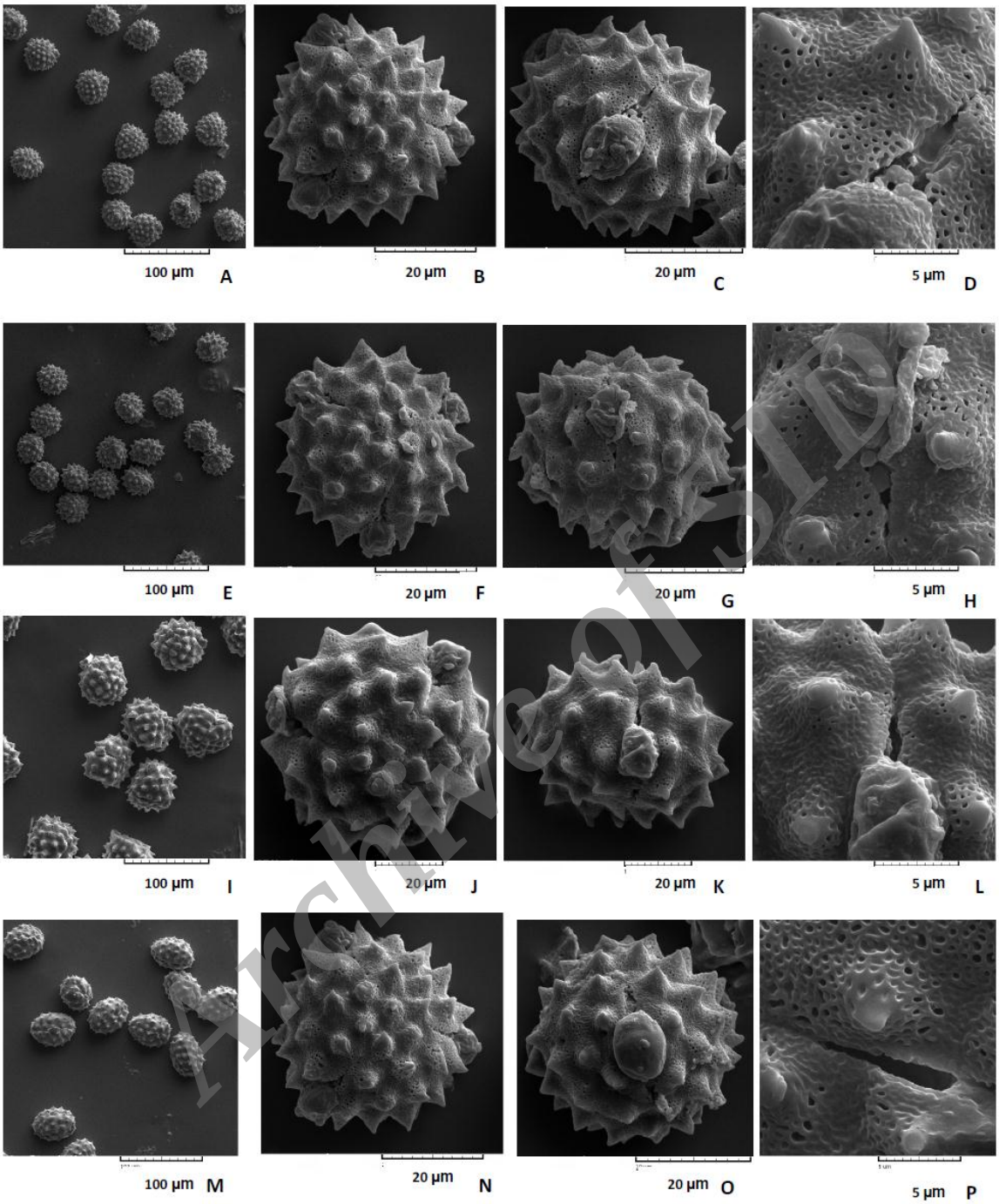

Fig. 1. Representative SEM photographs of pollen grains in *Carduus* species studied. A: *C. transcaspicus* (general view), B: *C. transcaspicus* (polar view), C*: C. transcaspicus* (equatorial view), D: *C. transcaspicus* (exin surface), E: *C. seminudus* (general view), F: *C. seminudus* (polar view), G: *C. seminudus* (equatorial view), H: *C. seminudus* (exin surface), I: *C. getulus* (general view), J: *C. getulus* (polar view), K: *C. getulus* (equatorial view), L: *C. getulus* (exin surface), M: *C. onopordioides* (general view), N: *C. onopordioides* (polar view),O: *C. onopordioides* (equatorial view),P: *C. onopordioides* (exin surface).

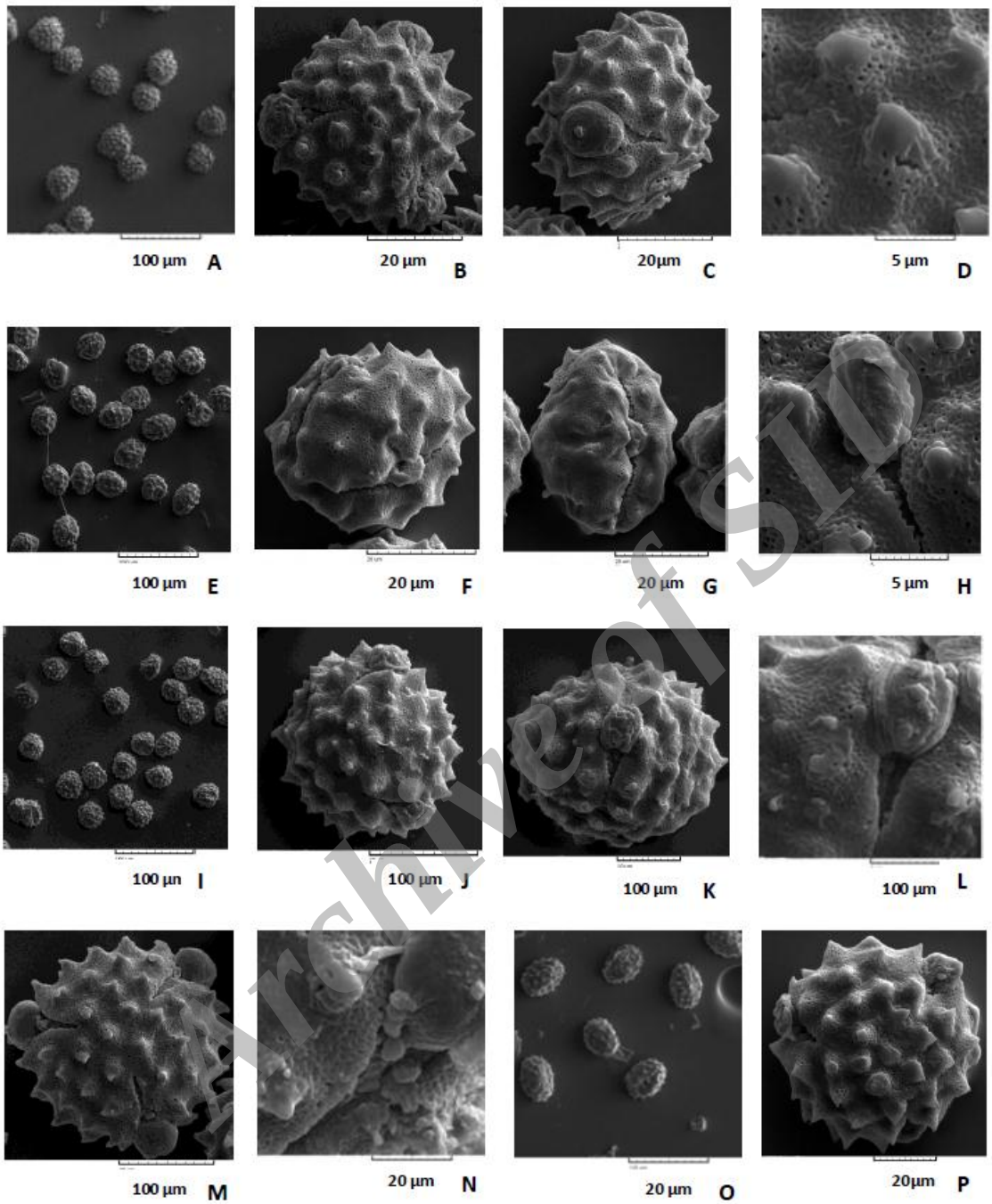

Fig. 2. Representative SEM photographs of pollen grains in *Carduus* species studied and out group species. A: *C. hamulosus* (general view), *B: C. hamulosus* (polar view),*C: C. hamulosus* (equatorial view), D: *C. hamulosus* (exin surface), E: *C. arabicus* (general view), F: *C. arabicus* (polar view), G: *C. arabicus* (equatorial view), H: *C. arabicus* (exin surface), I: *C. pycnocephalus* (general view), J: *C. pycnocephalus* (polar view), K: *C. pycnocephalus* (equatorial view), L: *C. pycnocephalus* (exin surface), M: *Cirsium ciliatum*(out group) (polar view), N: *C. ciliatum* (out group)(exin surface), O: *C. vulgare* (out group)(general view),P: *C. vulgare (out group)* (polar view).

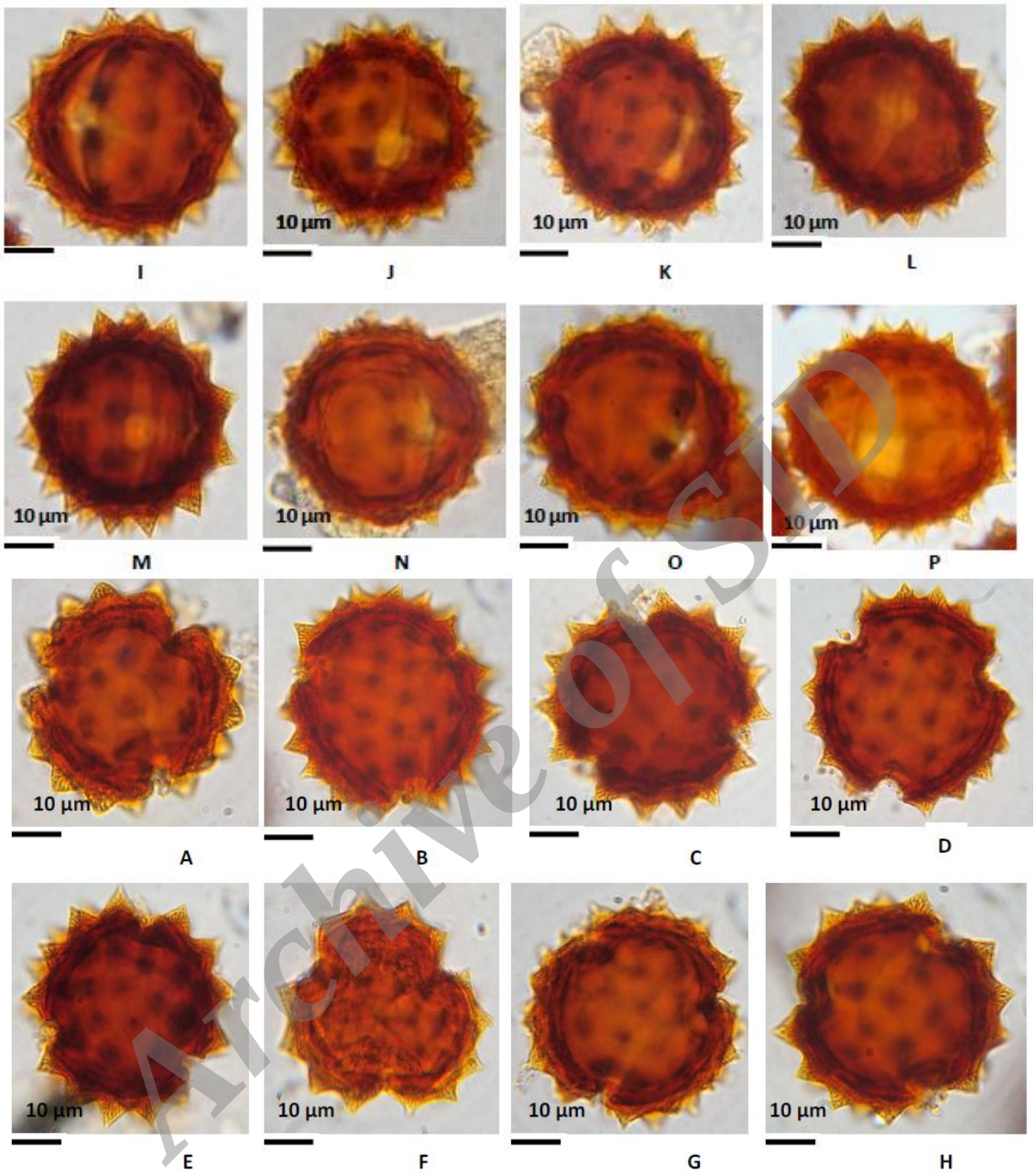

Fig. 3. Representative LM photographs of pollen grains in *Carduus* species studied and out group species. A: *C. thoermeri* (polar view), B: *C. onopordioides* (polar view), C: *C. homulosus* (polar view), D: *C. transcaspicus* (polar view), E: *C. seminudus* (polar view), F: *C. getulus* (polar view), G: *C. pycnocephalus* (polar view), H: *Cirsium adancum* (polar view), , I: *C. thoermeri* (equatorial view), J: *C. onopordioides* (equatorial view) K: *C. homulosus* (equatorial view), L: *C. transcaspicus* (equatorial view), *M: C. seminudus* (equatorial view), *N: C. getulus* (equatorial view), *O: C. pycnocephalus* (equatorial view),P: *Cirsium adancum* (equatorial view).

Table 4: Palynological qualitative and quantitative characters and their coding. (Character abbreviations: 1. type of pollen 2. shape of spine 3. opercule surface 4. spine type 5. spinule 6. polar length (P) (µm) 7. equatorial length (E) (µm) 8. P/E ratio 9. exine thickness (µm) 10. spine length (µm) 11. colpus length (µm) 12. spine width (µm) 13. distance between two spines (µm) 14. Mesocolpium width (µm) 15. opercule length (µm) 16. opercule width (µm) 17. length to width ratio of opercule 18. number of spines per 10µm^2.

Coding Characters	1	2	3	4	5	6
1	oblate – spheroid	suboblate				
2	erect and curved	erect				
3	stripy-granular	granular				
4	acute	obtuse				
5	absent	present				
6	35<x<41	41<x<47	x<47			
7	37<x<45	45<x<53	x<53			
8	x<1	x>1				
9	4<x<5.2	5.2<x<6.2	x<6.2			
10	x<3	3<x<4	4<x<5	5<x<6	x<6	
11	13<x<17	17<x<21	21<x<25	x<25		
12	5<x<6	6<x<7	7<x<8	x<8		
13	8<x<9	9<x<10	10<x<11	11<x<12	x<12	
14	26<x<28	28<x<30	30<x<32	32<x<34	34<x<36	x<36
15	5<x<7	7<x<11	x<11			
16	5<x<7	7<x<9	9<x<11	x<11		
17	x<1	1<x<2	x<2			
18	2<x<4	4<x<6	x<6			

(Fig. 4; Fig. 5; Fig. 6; Fig.7; Fig. 8), separating the out-group taxa of the genus *Cirsium* i.e. *C. adancum*, *C. ciliatum* and *C. vulgare* from *Carduus* species studied. In parsimony analysis, fifty-four most parsimonious trees were obtained which after 100 times bootstrapping the consensus tree obtained had tree length = 60, homoplasy index = 0.25 and consistency index = 0.75. Separation of *Carduus* species from *Cirsium* out-group taxa has 100% bootstrap. The trees obtained show affinity between *C. pycnocephalus*, *C. arabicus*, *C. getulus* and *C. seminudus* is with 89% bootstrapping. In neighbor joining cluster analysis three clusters appeared. In first cluster *C. hamulosus* and *C. transcaspicus* show a close relationship which are in concordant with morphological data. The second cluster composed of three sub-clusters. *C. seminudus* in first sub cluster make a separate group and *C. getulus* in second sub cluster formed the sister group of third sub cluster with *C. pycnocephalus* and *C. arabicus.* The closest relationship was observed between *C. pycnocephalus* and *C. arabicus* which is in concordant with morphological studies. In third cluster which have 3 subclusters the first subcluster with *C. thoermeri* and *C. onopordioides* is separated from two subclusters of *Cirsium* species as out-group.

C. thoermeri and C. *onopordioides* species pair and *C. hamulosus* and *C. transcaspicus* species pair are related to each other due to the UPGMA, NJ, PCO and PCA trees. *C. seminudus* and *C. getulus* are related to each other which are shown in UPGMA, PCO and PCA trees. *C. pycnocephalus* and *C. arabicus* are related and it could be seen in UPGMA, PCO and PCA trees.

Discussion

Two basic patterns of exine ultrastructure are found in the *Asteraceae*, the caveate Helianthoid pattern and the non-caveate Anthemoid pattern. The *Heliantheae*, *Astereae*, *Inuleae*, *Sececioneae*, *Calenduleae* and *Eupatorieae* all have pollen with caveate exines. The *Mutisiseae*, *Vernonieae* and *Cardueae* have predominately Anthemoid pollen. The *Anthemideae*, *Arctoteae* and *Lactuceae* have pollen with exines of both patterns (Bolick 1978). Tormo and Ubera (1995) were studied 339 samples from 200 taxa (28 genera) from the Iberian peninsula belonging to the tribe *Cardueae* Cass. with LM, SM and TEM to determine 20 pollen types. The different types were distinguished on the apertural system and exine structure that *Carduus* species have been classified in the *Carduus* type. Palynological study of the genus *Carduus* is the report of Pereira-Coutinho (1996), who studied pollen characteristics of 8 *Carduus* species in Portugal and due to presence of short axis of colpi considered this type of pollen to be primitive within the tribe and sub-tribe. Pollen grains observed are usullay radially symmetrical isopolar, trizonocolporate, suboblate to oblate- spheroidal with echinate sculpturing.

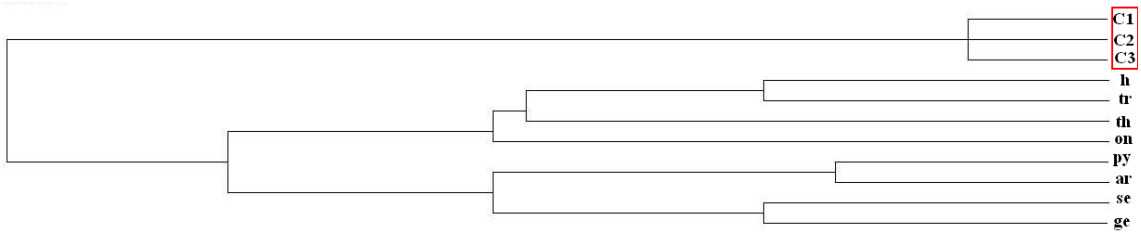

Fig. 4. UPGMA tree of palynological characters. Species abbreviations: C1-C3- *Cirsium adantum, C. ciliatum and C. vulgare* (the out-group taxa), h= *Carduus hamulosus*, tr= *C. transcaspicus*, th= *C. thoermeri*, on= *C. onopordioides*, py= *C. pycnocephalus*, ar= *C. arabicus*, se= *C. seminudus* and ge= *C. getulus*.

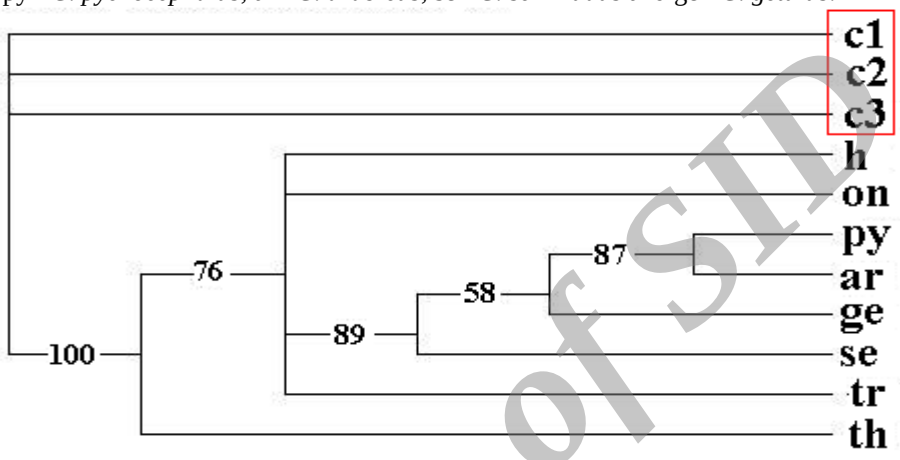

Fig. 5. Parsimony tree of palynological characters. Species abbreviations: C1-C3- *Cirsium adantum, C. ciliatum and C. vulgare* (the out-group taxa), h= *Carduus hamulusus*, tr= *C. transcaspicus*, th= *C. thoermeri*, on= *C. onopordioides*, py= *C. pycnocephalus*, ar= *C. arabicus*, se= *C. seminudus* and ge= *C. getulus*. (values above branches are bootstrap values).

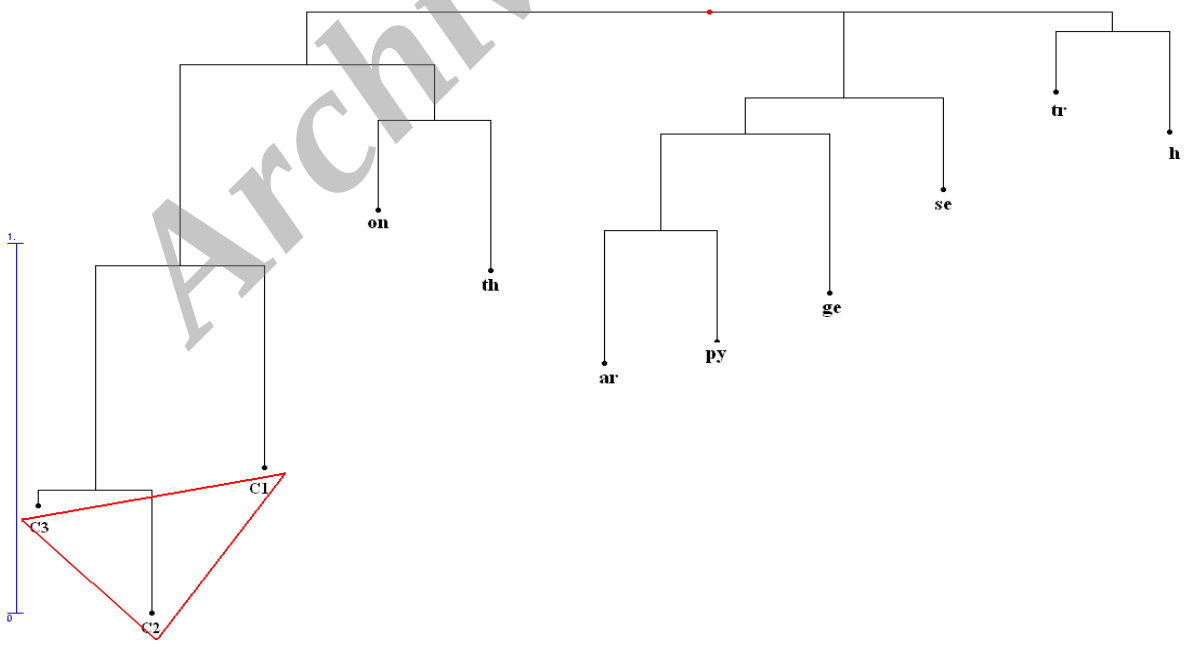

Fig. 6. NJ tree of palynological characters. Species abbreviations: C1-C3- *Cirsium adantum, C. ciliatum and C. vulgare* (the out-group taxa), h= *Carduus hamulosus*, tr= *C. transcaspicus*, th= *C. thoermeri*, on= *C. onopordioides*, py= *C. pycnocephalus*, ar= *C. arabicus*, se= *C. seminudus* and ge= *C. getulus*.

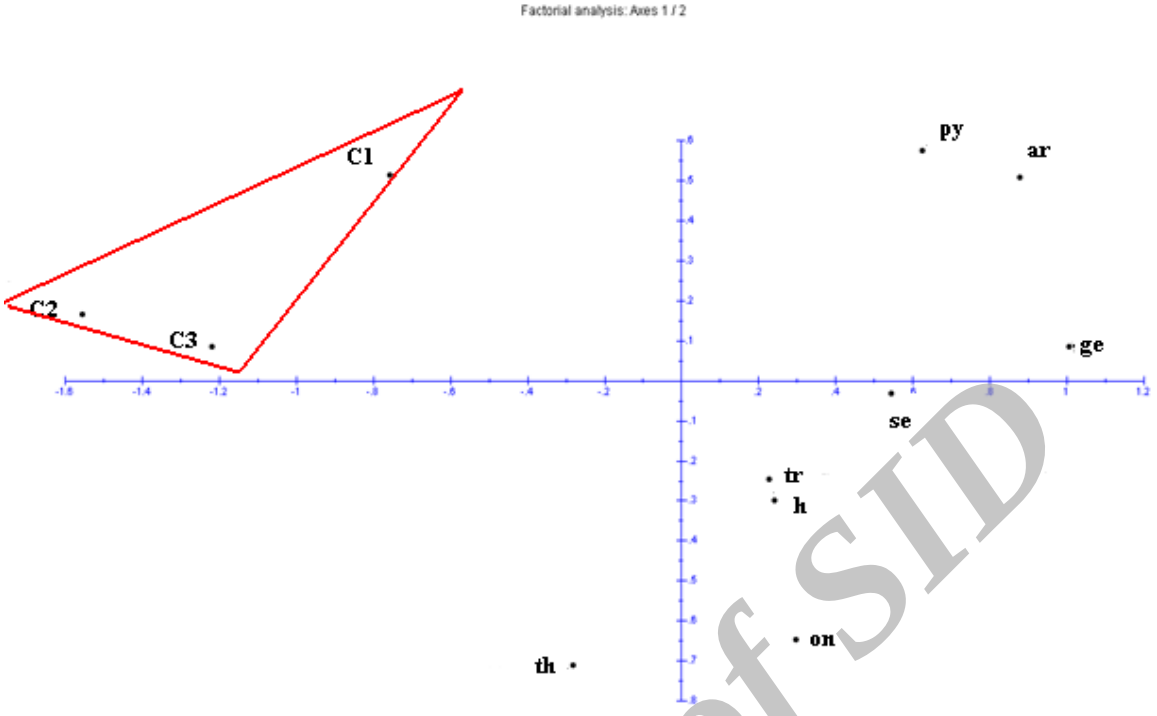

Fig. 7. PCA tree of palynological characters. Species abbreviations: C1-C3- *Cirsium adantum, C. ciliatum and C. vulgare* (the out-group taxa), h= *Carduus hamulosus*, tr= *C. transcaspicus*, th= *C. thoermeri*, on= *C. onopordioides*, py= *C. pycnocephalus*, ar= *C. arabicus*, se= *C. seminudus* and ge= *C. getulus*.

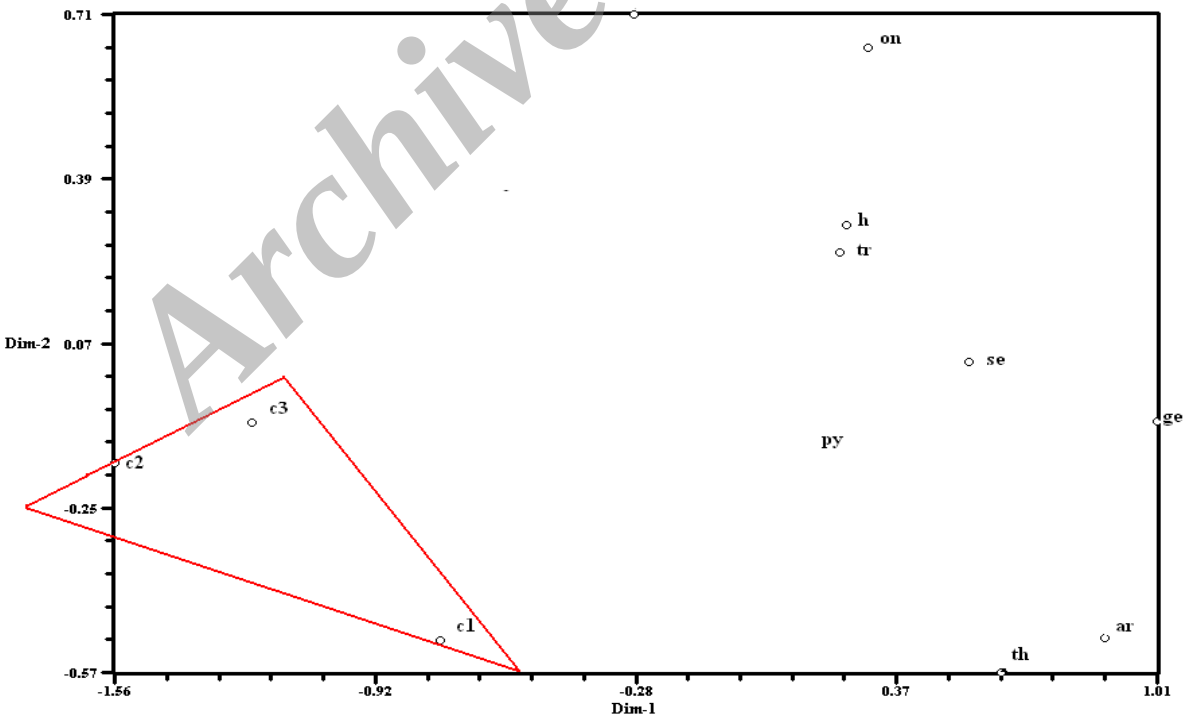

Fig. 8. PCO tree of palynological characters. Species abbreviations: C1-C3- *Cirsium adantum, C. ciliatum and C. vulgare* (the out-group taxa), h= *Carduus hamulosus*, tr= *C. transcaspicus*, th= *C. thoermeri*, on= *C. onopordioides*, py= *C. pycnocephalus*, ar= *C. arabicus*, se= *C. seminudus* and ge= *C. getulus*.

The ANOVA indicated that eight species of Iranian *Carduus* differ significantly in their pollen characteristics. The PCA result which represented the first three factors comprising about 68% of all of the variation, showed that characteristics such as polar length, equatorial length, spine length, distance between two spines and colpus length are the most variable pollen characteristics, the first factor with about 35.67% of the total variation and these characteristics may be used for the species delimitation.

The affinity revealed among four species of *Carduus*, including *C. pycnocephalus*, *C. arabicus*, *C. getulus* and *C. seminudus* is also well supported by 89% bootstrapping, also in agreement with Flora Iranica taxonomic treatment of these species (Rechinger 1979).

Four species namely *Carduus thoermeri*, *C. hamulosus*, *C. transcaspicus* and *C. onopordioides* also show affinity and join each other with some distance, which is in agreement with morphometrical analysis and Flora Iranica taxonomic treatment (Rechinger 1979). Separation of *Carduus* species from *Cirsium* out-group taxa based on palynological data showed 100% bootstrap, indicating that although identification and recognition of *Carduus* and *Cirsium* species seems a little difficult based on morphological characters, but pollen characteristics distinguish between these two genera well enough. The pollen characteristics results indicated that *Carduus* and *Cirsium do* not differ significantly in their qualitative characteristics but *Carduus* and *Cirsium* differs significantly in their quantitative characteristics such as polar length. Equatorial length, exin thickness, spine length and mesocolpium width.

References

Bouck, M. 1978: Taxonomic, evolutionary and functional considerations of Compositae pollen ultrastructure and sculpture. -Pl. Syst. Evol. 130: 209-218.

Bremer, K. 1994: Asteraceae: Cladistics and Classification. -Portland. Oregon: Timber Press.

Erdtman, G. 1952: pollen Morphology and plant Taxonomy. Angiosperm (an introduction to palynology. vol. 1). Pp.539. -Almqvist and Wiksell, Stockholm.

Erdtman, G. 1969: Handbook of Palynology. -Hafner publishing compony, New York. Funk, VA., Susanna, A., Stuessy, TF. & Robinson, H. 2009: Classification of Compositae In: Systematics International Association for Plant Taxonomy (IAPT), pp.171-189.. -Vienna.

Häffner, E. & Hellwig, FH. 1999: Phylogeny of the tribe Cardueae (Compositae) with emphasis on the subtribe Carduinae: an analysis based on ITS sequence data.-Willdenowia, 29: 27-39.

Hansen, HV. 1991: SEM-studies and general comments on pollen in tribe Mutisieae (Compositae) sensu Cabrera. -Nord. J. Bot, 10: 607-623.

Nouroozi, M., Sheidai, M., Attar, A. & Noormohammadi, Z. 2012: pollen morphological studies on the genus Cirsium Mill.(Asteraceae) in Iran. -J. Jpn. Bot. 87: 272-283.

Pereira-Coutinho, A. 1996: Palinologia do género *Carduus* L. (Asteraceae) em Portugal. -Anales Jardin Botanico de Madrid, 54: 347-354.

Podani, j. 2000: Introduction to the Exploration of Multivariate Biological Data, English translation, pp 407. -Backhuys publishers, Leiden.

Rechinger, K. H. 1979: Flora Iranica. *Carduus*, -Akademische Druck-U. Verlagsanstalt, Graz, Pp.221-231.

Scotland, RW., Olmstead, RG. & Bennett, J R. 2003: Phylogeny reconstruction: the role of morphology. -Sys. Bio. 52: 539-548.

Susanna, A. 2006: The Cardueae (Compositae) revisited: Insights from ITS, trnL-trnF, and matK nuclear and chloroplast DNA analysis. -Annals of Missouri Botanical Garden 93: 150.

Tormo, R. & Ubera, J. 1995: Tipos polínicos de la tribu Cardueae en la Península Iberica. -Monogr. Jard. Bot. Córdoba 2: 5-528.

Urtubey, E. & Telleria, MC. 1998: Pollen morphology of the subfamily Barnadesioideae (Asteraceae) and its phylogenetic and taxonomic significance. -Review of Palaeobotany and Palynology. 104: 19-37.

Wortley, AH., Funk, VA., Robinson, H., Skvarla, JJ. & Blackmore, S. 2007: A search for pollen morphological synapomorphies to classify rogue genera in Compositae (Asteraceae). -Review of Palaeobotany and Palynology, 146: 169-181.