MORPHOMETRY AND PALYNOLOGICAL STUDY OF THE GENUS COUSINIA SECT. COUSINIA (ASTERACEAE) IN IRAN

Z. Ahmad-Khanbeygi, M. Sheidai & F. Attar

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Ahmad-Khanbeygi, Z., Sheidai, M. & Attar, F. 2011 12 31: Morphometry and palynological study of the genus *Cousinia* sect. *Cousinia* (*Asteraceae*) in Iran. *–Iran. J. Bot. 17 (2): 158-166.* Tehran.

Morphological and palynological studies were performed on 16 populations of 8 species of the genus *Cousinia* sect. *Cousinia* in Iran, using morphological and pollen characteristics. The ANOVA test showed significant difference for almost all quantitative characters indicating the use of such characters in species delimitation. Trees obtained from morphological characters separated populations of *C. microcephala* from the other species, while the populations of *C. tabrisiana* and *C. chlorocephala* show affinity to each other. The species of *C. urumiensis*, *C. hamosa* and *C. tenuifolia* show affinity to each other and form a separate subcluster and the populations of *C. wilhelminae* and *C. seidlitzii* join these species with some distance. However, palynological tree did not agree with morphology tree.

Zahra Ahmad-Khanbeygi & Masoud Sheidai (correspondence <msheidai@yahoo.com, msheidai@sbu.ac.ir>*, Shahid Beheshti University, Faculty of Biological Sciences, GC, Tehran, Iran. -Farideh Attar, Biology Department, University of Tehran, Tehran, Iran.*

Key words. Cousinia, numerical taxonomy, pollen grains.

مورفومتری و مطالعه خصوصیات گرده در بخشه Cousinia از جنس Cousinia در ایران

زهرا احمد خان بیگی، دانشجوی کارشناسی ارشد دانشکده علوم زیستی دانشگاه شهید بهشتی.

مسعود شیدایی، استاد دانشکده علوم زیستی دانشگاه شهید بهشتی.

فریده عطار، دانشیار دانشکده زیست شناسی دانشگاه تهران.

مورفومتری و بررسی ویژگیهای گرده در ١٦ جمعیت از ٦ گونه *Cousinia* با استفاده از روشهای آماری یک و چند متغیره انجام گرفت. آزمون ANOVA اختلاف معنی دار گونه‌های مطالعه شده را در اکثر صفات کمی نشان داد که بیانگر کارایی این دسته از صفات در تفکیک گونه‌ها از یکدیگر می‌باشد. دندروگرام‌های بدست آمده از آنالیز صفات ریختی، جدایی جمعیت های *C. microcephala* را ازدیگر گونه‌ها نشان می‌دهند. همچنین نزدیکی گونه‌های *C. tabrisiana* و *C. chlorocephala* تایید می‌شود. گونه‌های *C. urumiensis* ، *C. hamosa* و *C. tenuifolia* در کنار هم و یک خوشه را می‌سازند، در حالیکه گونه‌های *C. wilhelminae* و *C. seidlitzii* با فاصله به این خوشه متصل می‌شوند. نزدیکی گونه‌ها در دندروگرام صفات گرده با نتایج مورفومتری همخوانی ندارد.

INTRODUCTION

The genus *Cousinia* (*Asteraceae*) is of worldwide distribution and comprises approximately 672 species, of which about 235 occur in central, western, eastern and southeastern Iran. According to Rechinger (1970), the section *Cousinia* contains 10 species growing mainly in the East and West Azerbaijan provinces out of which seven species are endemic to Iran.

Earlier reports on Iranian *Cousinia* have been confined mainly to cytotaxonomy and taxonomic studies (Afzal-Rafii 1980, Ghaffari & Djavadi 1998, Ghaffari et al. 2000, Djavadi 2005, 2007, Sheidai et al. 2006a, Attar & Ghahreman 2000, 2002, Attar et al. 2005, Mehregan & Kadereit 2008, López-Vinyallonga et al. 2009).

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 et 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

Table 1. *Cousinia* sect. *Cousinia* species studied and their voucher nos.

Species	Locality	Voucher no.
Cousinia microcephala 1	Sofian	8600203 (HSBU)
C. microcephala 2	Marand	8600204 (HSBU)
C. tabrisiana 1	Zonoz	8600202 (HSBU)
C. tabrisiana 2	Galeban	8600205 (HSBU)
C. tabrisiana 3	Ilkhchy	8600210 (HSBU)
C. urumiensis 1	Ahar road	8600206 (HSBU)
C. urumiensis 2	Haris	8600207 (HSBU)
C. tenuifolia 1	Sero	8600208 (HSBU)
C. tenuifolia 2	Raskhan	8600200 (HSBU)
C. tenuifolia 3	Bandar-Golmankhane	8600201 (HSBU)
C. tenuifolia 4	Oshnaviyeh	8600209 (HSBU)
C. wilhelminae 1	Zanjireh	9720 (TUH)
C. wilhelminae 2	Seyed-Hajin	21213 (TUH)
C. chlorocephala	Mako	21216 (TUH)
C. seidlitzii	Mahabad dam	70045 (TUH)
C. hamosa	Ghasemlo valley	70039 (TUH)

of the taxa which may lead to morphological divergence at the large scale (Wortley et al. 2007). Moreover, due to possibility of homoplasy in morphological characters, the use of additional pollen characters possibly avoid such limitation and improve the results obtained (Scotland et 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 *Barnadesioideae* and *Mutisioideae* and also found in *Calyceraceae* and some other families. Therefore this probably plesiomorphic in *Asteraceae* (Hansen 1991, Urtubey & Telleria 1998).

There have been very limited palynological studies in the genus *Cousinia*, particularly in the sect. *Cousinia* (Meo & Khan 2003, Zafar et al. 2007, Jafari & Ghanbarian 2007, Saber et al. 2009). These studies are either only light microscopy results or just contain pollen description of one to few species with no numerical analysis and no species relationships is studied. Therefore, the present study considers morphometry and palynological studies of the sect. *Cousinia* in Iran for the first time, trying to investigate the species affinities within the sect. *Cousinia* Based on pollen grains characters.

MATERIALS AND METHODS

Plant material

In total 16 populations of 8 *Cousinia* species 1- *Cousinia chlorocephala* C. A. Mey., 2- *C. hamosa* C. A. Mey., 3- *C. microcephala* C. A. Mey., 4- *C. seidlitzii* Bunge, 5- *C. tabrisiana* Bunge, 6- *C. tenuifolia* C. A. Mey., 7- *C. wilhelminae* Rech. f., and *C. urumiensis* Bornm., were studied. Three plants from each population were used for morphometery and palynological studies. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU) and University of Tehran. Details of localities and voucher numbers are provided in Table 1.

Morphometry

In total 29 morphological characters were used for morphometry (Table 2), including quantitative and qualitative characters taken from floras and personal observations in the field. Quantitative morphological characters were randomly measured in at least 3 plants and the means were used in analyses. Qualitative characters were coded as binary or multistate characters accordingly (Table 2).

UPGMA (Unweighted Paired Group using Arithmetic Average), Neighbor Joining (NJ) as well as ordination plots based on Principal Components Analysis (PCA) and Principal Coordinate Analysis (PCO) were used for grouping of the species studied. Cophenetic correlation and bootstrapping was performed to check the fit of dendrograms obtained (Podani 2000). For clustering, morphological data were standardized (Mean = 0, variance = 1) and used to determine Taxonomic and Euclidean distances (Podani 2000).

Palynology

Mature anthers were removed from 8 species. Scanning electron microscopy (SEM) studies were performed on pollen grains. The pollen samples used for SEM were mounted on aluminum stubs and coated with gold in an Emitech EMK 550 sputter. Observations and measurements were made with a Zeiss Axiophot light microscope and a Philips XL 20 SEM at 20kV. The

Table 2. Morphological characters and their coding in *Cousinia* sect. *Cousinia* spp.

character / code	code 1	2	3	4	5	6
basal leaves	crisped, sinuate, pinnate-lobate	pinnatisect	sinuate, pinnate-lobate	pinnatipartite		
stem leaves	sinuate-lobate	pinnatisect	sinuate,pinnate-lobate			
leaf color	concolourous	discolourous				
concord between basal leaves and stem leaves	similar	different				
base of stem leaves	decurrent	not decurrent	not exactly this not exactly that			
upper surface trichome	araneous, (+,-) glabrescent	araneous				
lower surface trichome	white- araneous-tomentose	araneous				
stem hair-covering	pilose	glabrous				
type of stem hair	white- araneous-glabrescent	white-floccose	glabrescent			
capitule	cylindric-campanulate	ovate-oblong	ovate-globose	ovate	almost globose	
involucral bracts	imbricate	erect-patulous	patulous-recurvate	recurvate	falcate-recurvate	arcuate-reflected
density of capitule on stem	dense	single on branch				
capitule frequency	numerous	not numerous				
spine at bracts margin	serrate	not serrate				
trichome in capitules	araneous	not araneous				
stem color	all over the stem is light	bottom of the stem is dark				
height	x <25 (cm)	25(cm) < x				
length of basal leaves	x <80 (mm)	80< x <100 (mm)	100< x <120 (mm)	120< x <150 (mm)	150< x (mm)	
width of basal leaves	x <35 (mm)	35< x < 50 (mm)	50< x (mm)			
length of median leaves	x<62 (mm)	x >62 (mm)				
width of median leaves	x <25 (mm)	x >25 (mm)				
no. of bracts	x< 70	70< x <120	x >120			
capitule diameter (including spines)	x <10 (mm)	10< x <14 (mm)	14< x <18 (mm)	x >18 (mm)		
no. of flowers per capitule	x< 10	10< x <20	20< x <30	x >30		
flower tube length	x <3 (mm)	3< x <4 (mm)	x >4 (mm)			
limb of corolla length	x <6.5 (mm)	x >6.5 (mm)				
lobe length	x <2 (mm)	x >2 (mm)				
corolla length	x <10 (mm)	10< x <13 (mm)	x >13 (mm)			
length of spines in basal leaves	x <2 (mm)	2< x <5 (mm)	x >5 (mm)			

Table 3. Pollen characteristics and their coding in *Cousinia* sect. *Cousinia* species.

	Character	Coding			
1	pollen shape	elliptical-rectangular (1)	not eliptical-rectangular (2)		
2	exine	verrucate-perforate (1)	non verrucate-perforate (2)		
3	lumen	have lumen (1)	no lumen (2)		
4	lumen density	less (1)	Medium (2)	High (3)	no lumen (4)
5	equatorial axis length	x < 35 µm (1)	35 µm < x > 38 µm (2)	x >38 µm (3)	
6	polar axis length	x < 26 µm (1)	26 µm < x > 27.05 µm (2)	x > 27.05 µm (3)	
7	E/P ratio	x < 1.4 (1)	x >1.4 (2)		
8	spine length	x < 0.3 µm (1)	0.3 < x > 0.4 (2)	x > 0.4 µm (3)	
9	spine base	x < 1 µm (1)	1 < x > 1.2 (2)	x > 1.2 µm (3)	
10	exine thickness	x < 5.5 µm (1)	5.5 < x > 6 (2)	x > 6 µm (3)	
11	pore width	x < 4 µm (1)	x > 4 µm (2)		
12	pore length	x < 4 µm (1)	x > 4 µm (2)		

measured characters were calculated from at least 20 pollen grains.

Image tool ver.3 software was used for pollen measurements and data obtained were coded accordingly (Table 3). For multivariate analyses 18 pollen characteristics including 13 quantitative and 5 qualitative characters were used. The mean of quantitative characters were directly used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean=0, variance=1) were used for multivariate statistical analyses (Podani 2000). The average taxonomic distance and squared Euclidean distance were used as dissimilarity coefficient in cluster analysis of data (Podani 2000). NJ tree and PCA plot were used to get species groupings (Podani 2000). NTSYS ver. 2.1 (1998) and DARwin ver. 5. (2000) was used for multivariate analyses.

RESULTS

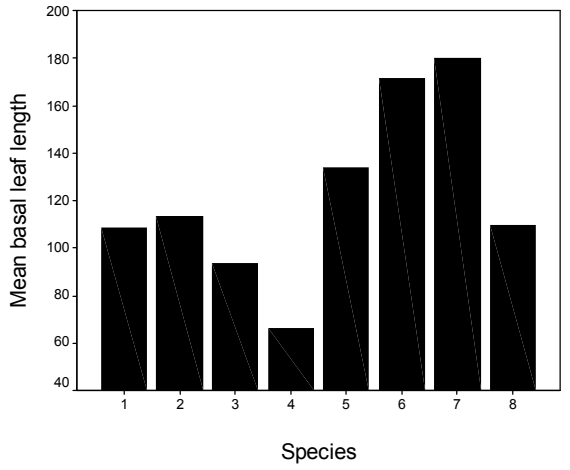
Morphometry

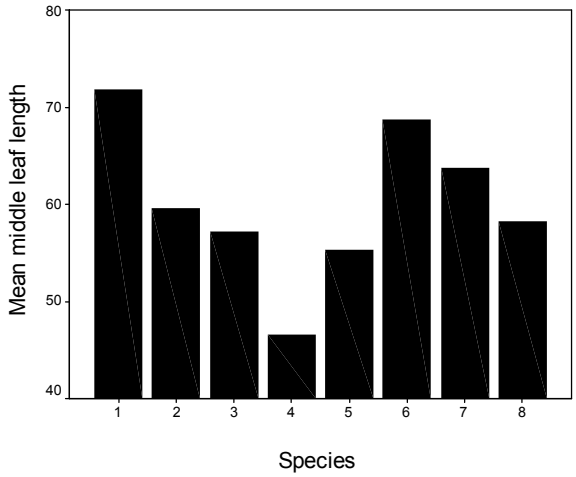
The ANOVA test performed for these quantitative characters among the species studied showed significant difference ($p<0.01$) for all quantitative characters except middle leaf width and lobe length in the flower leaves, indicating the use of such characters in species delimitation. Representative bar graphs showing separation of the species for quantitative characters are provided in Figs. 1-3. Pearson coefficient of correlation determined among quantitative morphological characters showed significant correlation (either positive or negative, $p<0.01$) among them indicating that the species determination may be easier just by choosing those characters separated by PCA analysis, as the other characters are highly correlated with them.

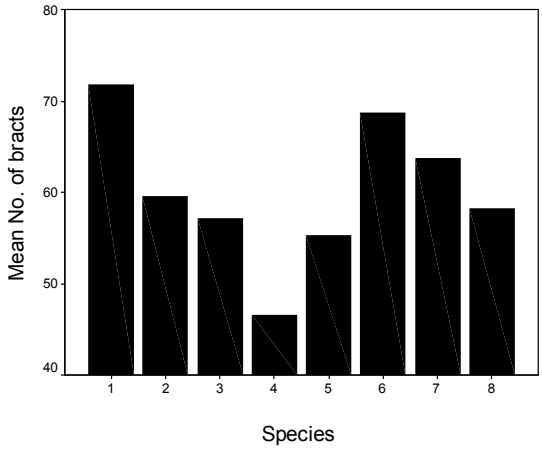
UPGMA and NJ trees of the morphological characters produced similar results (Figs. 4 & 5). The populations of *C. microcephala* are separated from the other species forming a single cluster, while the populations of *C. tabrisiana* and *C. chlorocephala* show affinity to each other and form a separate cluster. Close relationship of these three species is also supported by PCO and PCA plot (Fig. 5).

The species of *C. urumiensis*, *C. hamosa* and *C. tenuifolia* show affinity to each other and form a separate subcluster. The populations of *C. wilhelminae* and *C. seidlitzii* join these species with some distance. Close relationship of *C. urumiensis*, *C. hamosa* and *C. tenuifolia* is also supported by PCO and PCA plot, while *C. wilhelminae* and *C. seidlitzii* join them with some distance.

PCA analysis revealed that the first 3 factors comprise about 65% of total variance. In the first factor with about 33% of total variance, characters like stem and basal leaf characters, shape of middle bracts, capitulum position on the stem, density of capitulum on the stem, the no. of bracts, capitulum diameter and the no. of flowers, have the highest values of positive correlation ($r<0.70$) with this component. In the second component with about 19% of total variance, shape of the stem leaf and the mean of basal leaf width show the highest values of positive correlation ($r<0.70$) with the component. Therefore these are the most variable morphological characters among *Cousinia* species studied. As it is evident in PCA plot, variable morphological characters of the first PCA axis, separate the species of *C. microcephala*, *C. tabrisiana* and *C. chlorocephala* (which show affinity to each other) from the other species, while the variable morphological characters of the second PCA axis differentiate between these species. For example, *C. chlrocepha* is placed with some distance from *C. microcephala* and *C. tabrisiana* in the second PCA axis and *C. wilhelminae* which is placed with some distance from *C. tenuifolia*, *C. urumiensis* and *C. hamosa*.







Figs. 1-3. Bar plot showing the mean of basal leaf length (cm), middle leaf length (cm) and the mean no. of bracts in *Cousinia* species studied.

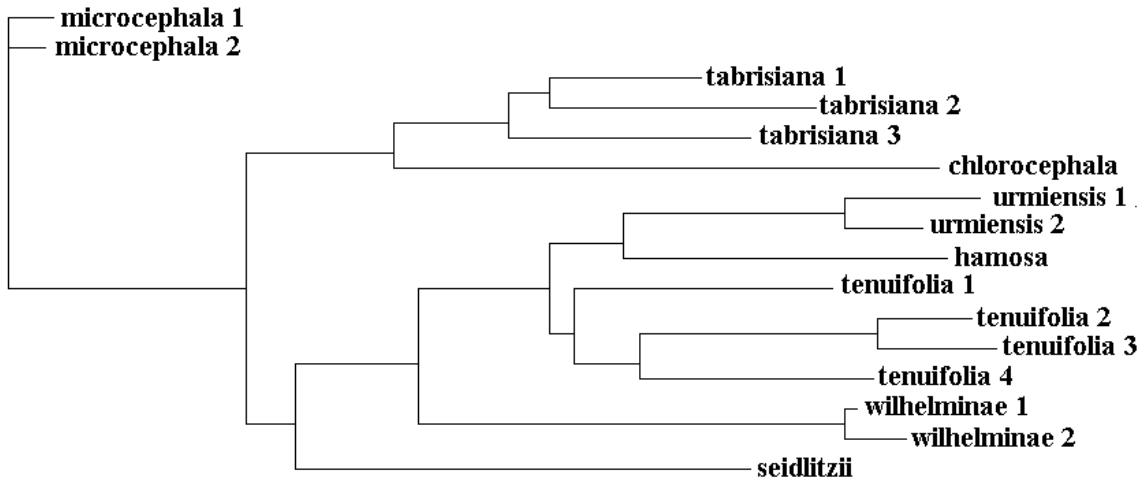


Fig. 4. NJ tree of morphological characters in *Cousinia* species.

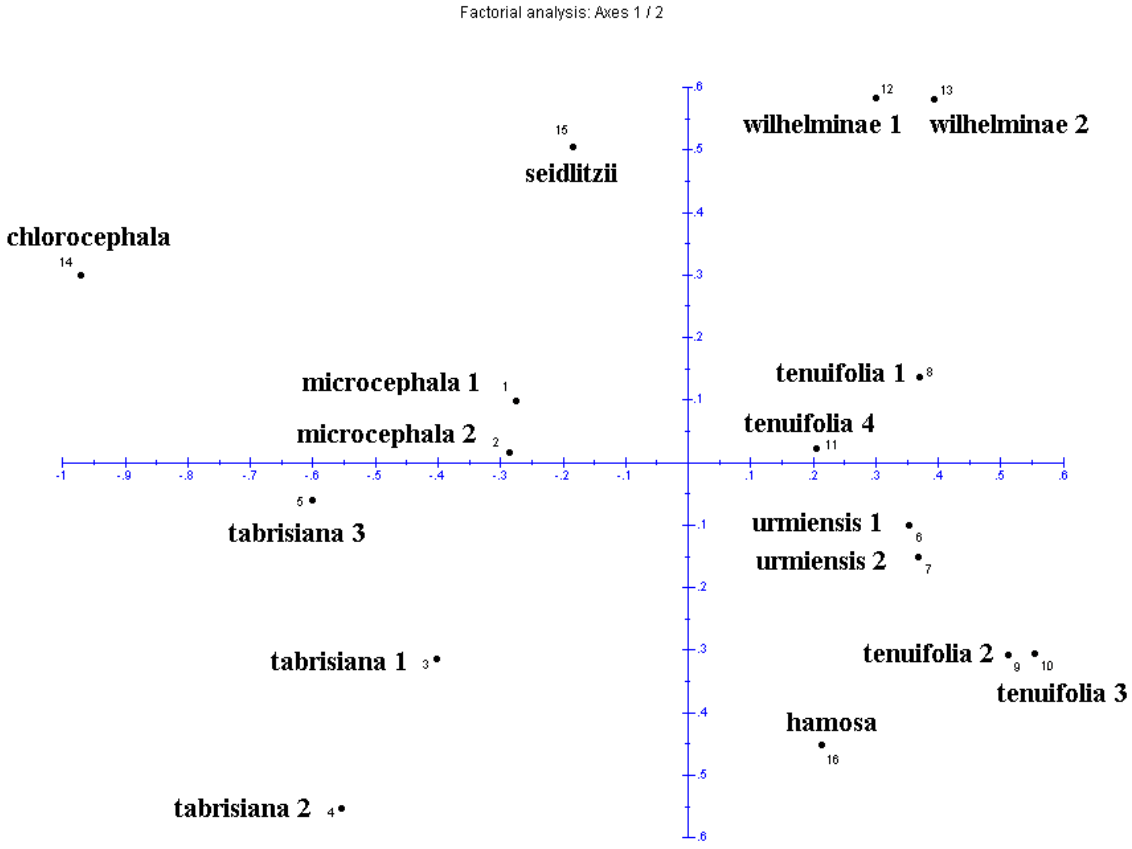


Fig. 5. PCA plot morphological characters in *Cousinia* species.

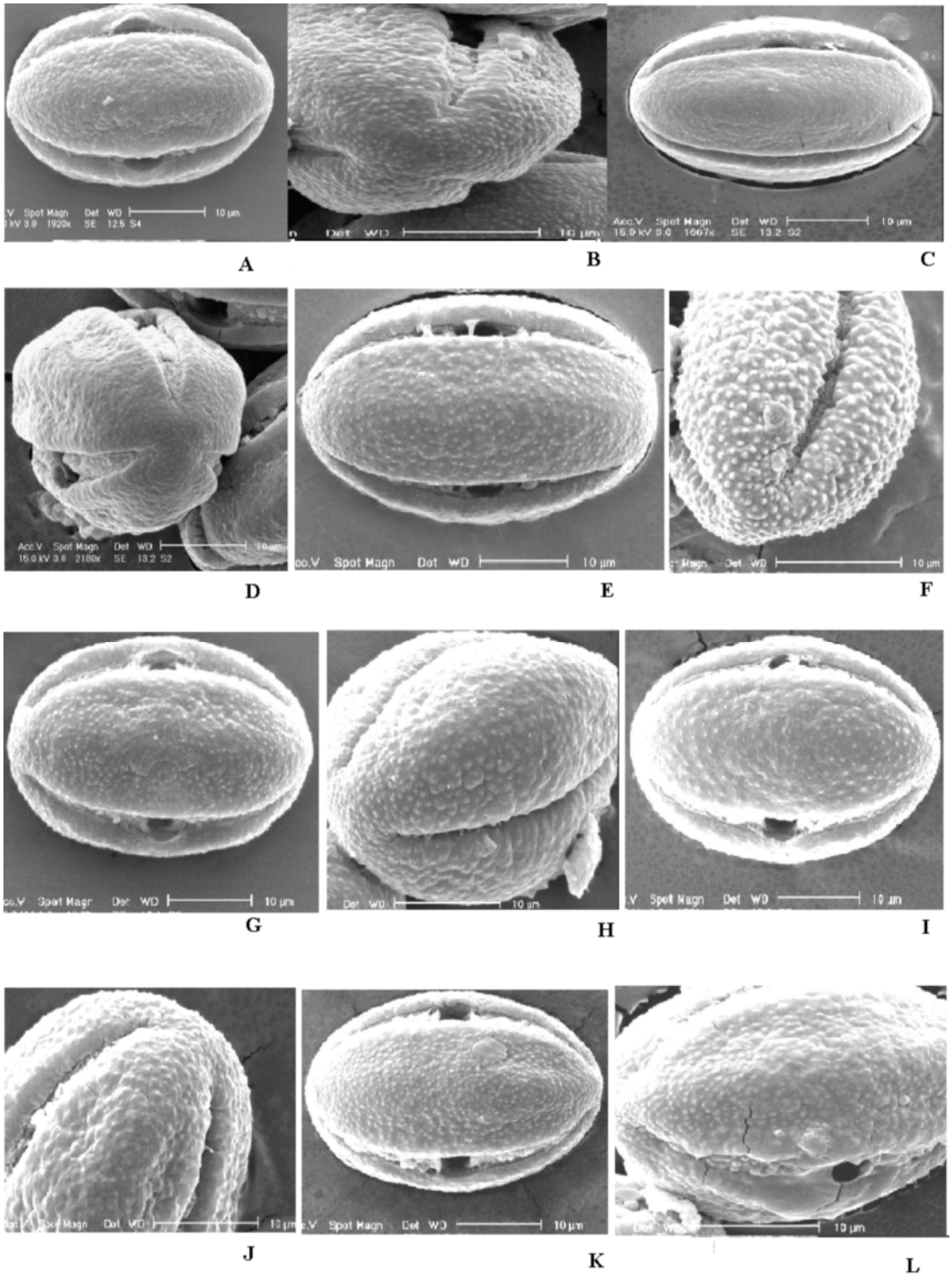


Fig. 6. Representative SEM photographs of *Cousinia* species studied. A & B= Equatorial and polar view of pollen grain in *C. chlorocephala* respectively. C & D= Equatorial and polar view of pollen grain in *C. hamosa* respectively. E & F= Equatorial and polar view of pollen grain in *C. urumiensis* respectively. G & H= Equatorial and polar view of pollen grain in *C. wilhelminae* respectively. I & J= Equatorial and polar view of pollen grain in *C. tabrisiana* respectively. K & L= Equatorial and polar view of pollen grain in *C. seidlitzii* and *tabrisiana* respectively.

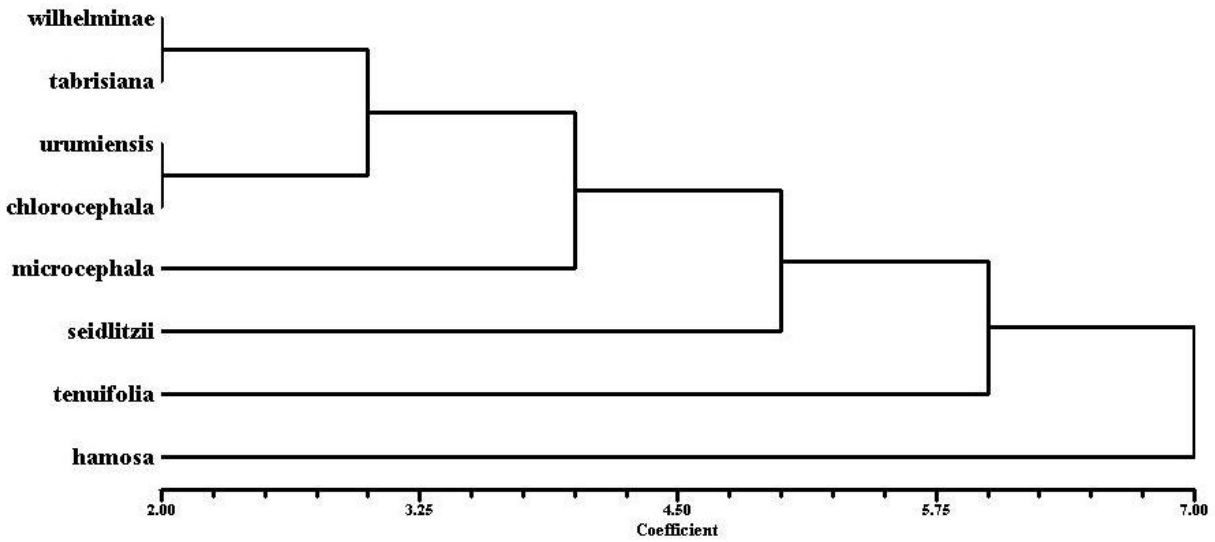


Fig. 7. NJ tree of palynological characters in *Cousinia* species.

Palynology

Pollen grains of *Couinia* species were spherical to cylindrical in form (Fig. 6), tricolpate, with the colpi tapering towards the poles. The exine is thicker than nexine, tapering towards the poles, with the thickness range of 5.00-6.56 µm. The exine surface is verrucated-perforated and the mean size of pores 3.52-3.82 µm.

The mean value of equatorial axis varied from 33.92 µm in *C. chlorocephala* to 39.04 µm in *C. wilhelminae*, while the mean size of polar axis varied from 23.51 µm in *C. chlorocephala* to 28.47 µm in *C. wilhelminae*.

UPGMA and NJ trees of the palynological characters produced similar results (Fig. 7), which is not in agreement with our morphometry. For example, *C. microcephala* is close to *C. seidlitzii*, while *C. tenuifolia* and *C. hamosa* join them with some distance. The latter two species also show affinity in morphological characters.

C. tabrisiana and *C. wilhelminae* are placed close to each other and join the above said cluster. Two species of *C. urumiensis* and *C. chlorocephal* show affinity and form a separate cluster far from the other species studied.

DISCUSSION

The ANOVA test and bar graphs show that quantitative morphological characters can be used along with other characters (particularly those identified by PCA) in taxonomy of the genus *Cousinia*. This is further supported by the grouping of the populations in each species separate from the other species studied. The species affinities revealed by NJ are in agreement with Flora Iranica taxonomic treatment of the genus.

Palynological data are considered as important characters in taxonomy and phylogeny of *Asteraceae* in general (for example, Hansen 1991, Urtubey & Telleria 1998, Scotland et al. 2003, Wortley et al. 2007, Lundberg 2009). *Asteraceae* is a eurypalynous family (Meo & Khan 2003) and most of its genera possess zonocolporate pollen grains. The pollen grains are helianthoid, spherical or slightly flattened, mainly tricolporate, echinate with variation in size and colpus number (Meo & Khan 2003).

Palynological studies in the genus *Cousinia* are confined to few studies. For example, Saber et al. (2009) studied 25 species of the sect. *Stenocephalae* and separated them in two groups based on pollen characteristics like the no. of verrucates, Sheidai et al. (2006b) carried out biosystematic study of the sect. *Serratuloideae* in Iran and separated the species based on cytology and palynology in two groups of x=24 and 26. Meo & Khan (2003) studied pollen characteristics of *Cousinia minuta* Boiss., reporting the presence of pollen grains which are semi-angular in polar view and prolate to spheroidal in equatorial view. The number of spine rows between colpi were 4 to 7, with no spines and exine thickness was 9.8 µm. However, Zafar et al. (2007) reported the exine thickness of 4-6.5 µm for the same species. Similarly Jafari & Ghanbarian (2007) studied pollen morphology of 30 species of 24 genera of 6 tribes of *Asteraceae* containing only one species of *Cousinia* i.e. *C. eriobasis* Bunge., by using light microscopy. They reported pollen grains with subporolate shape which are verrucate, polar axis of 54-58.4 µm and equatorial axis of 41.5-44.5 µm and P/E ratio of 1.3-1.31 µm in this species.

The present study shows that species relationship obtained from palynology does not agree with morphological analysis and we need other type of study including molecular investigation, to clarify the species affinity within the genus *Cousinia* sect. *Cousinia*. However, at present, the species determination might be done by using both morphological and palynological characters including stem and basal leaf characters, shape of middle bracts, capitulum position on the stem, density of capitulum on the stem, the no. of bracts, capitulum diameter and the no. of flowers, shape of the stem leaf and the mean of basal leaf width as the most variable morphological characters among *Cousinia* species studied as well as pollen exine thickness, size of equatorial and polar axes.

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