

# MICRO-MACROMORPHOLOGICAL STUDY OF THE GENUS CICER L. (FABACEAE) IN IRAN

F. Sharifnia, T. Farhani & F. Salimpour

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In this study, eleven species of genus *Cicer* L. were studied and morphological and micro-morphological characters were evaluated. These species are classified in 4 sections: *Monocicer*, *Chamaecicer*, *Polycicer* and *Acanthocicer*. The understudied species assessed by biometric study, and about 40 quantitative and 38 qualitative characters were assessed. Phenetic analysis was carried out using SPSS software, and phenograms of these species were prepared. Furthermore, PCA analysis was carried out and the most variable characters were determined. Finally, the seeds and pollen of these species were also investigated using S. E. M electronic microscope, and the tables of characters were formed individually for them. Afterward, phenetic analysis and phenogram preparation were done based on morphological characters of seeds and pollen of the species, and the following results were obtained:

1. Distinction of *C. kermanense* Bornm., as an independent species, from *C. spiroceras* Juab. & Spach, in contrary to what mentioned in Flora of Iran. 2. Transferring *C. subaphyllum* Boiss. from *Polycicer* section to the *Acanthocicer* section. 3. In spite of leaf polymorphism seen in *C. tragacanthoides* Jaub. & Spach, it seems that the pollen characters are sufficient to divide it to varieties. 4. Division of species of this genus using quantitative and qualitative specifications of seeds. 5. Preparation of the table of characters for pollen and seeds of all species.

Fariba Sharifnia, Tayebbeh Farhani & Fahimeh Salimpour, Department of Biology, Faculty of Science, Islamic Azad University, North Tehran Branch, P. O. BOX 19585-936 Tehran, Iran.

**Key words.** Micro-macromorphology, phenetic analysis, Iran, *Cicer*.

## مطالعه میکرو-ماکرومورفولوژی گونه‌های جنس نخود (*Cicer* L.) در ایران

فربیا شریف‌نیا، طیبه فرهانی و فهیمه سلیم‌پور

در این مطالعه ۱۱ گونه از جنس *Cicer* L. مورد مطالعه و بررسی مورفولوژیکی و میکرومورفولوژی قرار گرفت. کلیه این گونه‌ها در چهار بخشه *Monocicer*، *Chamaecicer*، *Polycicer* و *Acanthocicer* قرار دارند. گونه‌ها مورد مطالعه بیومتری قرار گرفت و تعداد چهل صفت کمی و سی و هشت صفت کیفی مورد سنجش قرار گرفت. آنالیز فنتیکی با استفاده از نرم‌افزار SPSS انجام شد و فنوگرام گونه‌ها تهیه گردید. همچنین آنالیز PCA انجام گرفت و متغیرترین صفات مشخص شدند. در نهایت دانه و گرده‌های این گونه‌ها هم مورد بررسی با میکروسکوپ الکترونی S. E. M. قرار گرفت و جدول صفات جداگانه‌ای برای آنها تشکیل شد، سپس آنالیز فنتیکی و تهیه فنوگرام براساس صفات مورفولوژی بذر و گرده گونه‌ها انجام شد و نتایج زیر به دست آمد:

۱- جدا شدن آرایه *C. kermanense* Bornm. به عنوان یک گونه مستقل و جدا از گونه *C. spiroceras* Juab. & Spach برخلاف آنچه که در فلور ایران آمده است.

۲- جدایی گونه *C. subaphyllum* از بخشه *Polycicer* و انتقال آن به بخشه *Acanthocicer*.

۳- با وجود پلی مورفیسم برگگی در گونه *C. tragacanthoides* Jaub. & Spach به نظر می رسد صفات گرده قادر به تفکیک واریته‌های گونه *C. tragacanthoides* می‌باشد.

۴- تفکیک گونه‌های این جنس با استفاده از خصوصیات کمی و کیفی دانه‌ها.

۵- تهیه جدول خصوصیات گرده و دانه کلیه گونه‌ها.

## Introduction

Genus *Cicer* L. belongs to the family *Fabaceae* and the tribe *Cicereae* Alef., and it consists of 43 species, ten annuals and 33 perennials. About 39 species grow in Middle Asia and West Asia, and about 4 species in certain regions of north and northwestern areas of Africa and Europe (van der Maesen 1987).

The basic chromosomal number for them is  $x = 8$ . This genus consists of some herbaceous and shrubby species which are classified into 4 sections on the basis of morphological specifications and life cycle characteristics (van der Maesen, 1987 and 1972).

1. The section *Monocicer* consists of the agronomic species *C. arietinum* and 7 annual species including *C. reticulatum*, *C. bijugum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, *C. cuneatum*, *C. yamashitae*.

2. The section *Chamaecicer* consists of one annual species and one perennial species which grow in mountain areas in West Asia and Kert Island.

3. The section *Polycicer* consists of 25 perennial species.

4. The section *Acanthocicer* consists of 7 perennial species growing in mountain regions of Iran, Afghanistan and Middle Asia (van der Maesen, 1987; Popov, 1976).

In this paper, micro-macromorphological data was subjected to cluster analysis in order to indicate the

species inter-relationship, to evaluate the previous taxonomic treatment of the genus *Cicer* in Iran, and provide the evidence for efficacy of micro-macromorphological data in taxonomic treatment of genus *Cicer* at sub-generic level.

## Materials and Methods

In order to study morphological characters of plant specimens in each species (at least 3 specimens), we chose about 40 quantitative and 38 qualitative characters.

The table of morphological characters was prepared based on these qualitative and quantitative characters (Table 1).

For statistical analysis, we initially encoded the qualitative characters according to the multi-state method, and the related means were considered for quantitative characters, and then these were standardized. Phenetic analysis was carried out using SPSS, ver. 9 software and Ward method (Norusis1999). Based on this method, we delivered a cluster analysis for morphological characters of species and then hierarchical phenograms of species were prepared. PCA analysis was performed as well and the most variable characters were specified.

Table 1. List of characters and related numerical codes used in morphological studies.

No.	Characters	Numerical code
1	Plant height	Cm
2	Stem diameter	Mm
3	Rachis length	Mm
4	Number of leaflets	In no.
5	Leaflet length	Mm
6	Leaflet width	Mm
7	Leaflet length / width ratio	In no.
8	Length of leaflet teeth	Mm
9	Number of leaflet teeth	In no.
10	Number of stipule teeth	In no.
11	Stipule length	Mm
12	Stipule width	Mm
13	Stipule length / width ratio	In no.
14	Calyx length	Mm
15	Length of calyx tube	Mm
16	Length of calyx teeth	Mm
17	Number of calyx teeth	In no.
18	Peduncle length	Mm
19	Pedicele length	Mm
20	Corolla length	Mm
21	Standard length	Mm
22	Standard width	Mm
23	Standard length / width ratio	In no.
24	Wing length	Mm
25	Wing width	Mm

No.	Characters	Numerical code
26	Wing length / width ratio	In no.
27	Keel length	Mm
28	Keel width	Mm
29	Keel length / width ratio	In no.
30	Pod length	Mm
31	Pod width	Mm
32	Pod length / width	In no.
33	Arista length	Mm
34	Seed length	Mm
35	Seed width	Mm
36	Seed length/width ratio	In no.
37	Pedicel / peduncle length ratio	In no.
38	Pedicel & peduncle/rachis length ratio	In no.
39	Calyx teeth / tube length ratio	In no.
40	Corolla / calyx length ratio	In no.
41	Growth period	1- annual 2- perennial
42	Growth habit	1- erect-semi erect 2- prostrate to erect 3- prostrate 4- shrubby 5- shrubby – cushion
43	Stem shape	1- straight to slightly flexuous 2- straight – flexuous 3- flexuous
44	Epigeal stem	1- absence 2- presence
45	Stem hairs	1- glandular and simple 2- mostly glandular, rarely simple 3- mostly simple, rarely glandular 4- simple 5- glandular
46	State of stem hairs	1- less 2-mean 3-more
47	Size of stem hairs	1-long 2-long and short 3-short
48	Leaflets arrangement	1-imparipinnate 2- paripinnate and imparipinnate 3- paripinnate
49	End of rachis	1- unileaflet 2- in upper leaves, curled or simple and ramified tendril. in lower leaves, leaflet 3-curled or simple and ramified tendril 4- curled or simple tendril 5-spinelet or curl 6-spinelet
50	Leaflet shape	1-elliptic-obovate 2-obovate-oblong 3-cuneate-flabellate 4-obovate 5-cuneate-ebovate 6-rounded- flabellate 7-spiny 8-upper leaflet, spiny lower leaflet, fan shaped 9-ovate-flabellate
51	Leaflet base	1-cuneate 2-rounded-cuneate 3-broadly cuneate
52	Leaflet apex	1-rounded or acuminate 2- rounded or truncate 3-rounded 4-truncate 5-acute 6-upper leaflets acute and lower leaflets, rounded
53	Leaflet margin	1-serrate 2-entire 3- dentate at the apex
54	Leaflet hairs	1- glandular and simple 2- mostly glandular, rarely simple 3- mostly simple, rarely glandular 4- simple 5- glandular
55	Shape of upper stipules	1-hastate 2- entire 3- lanceolate
56	Shape of lower stipules	1- ovate or semiovate 2- ovate 3- oblique – triangular incised 4-semiovate 5- triangular 6- lanceolate - triangular
57	Calyx gibbosity	1- faintly gibbous 2- middle gibbous 3- strongly gibbous
58	Shape of calyx teeth	1- lanceolate 2- lanceolate–triangular 3- broadly lanceolate 4- lanceolate – acuminate
59	Position of calyx teeth	1- almost equal 2- unequal
60	Calyx hairs	1- glandular and simple 2- mostly glandular, rarely simple 3- mostly simple, rarely glandular 4- simple 5- glandular
61	State of calyx hairs	1- less 2-mean 3-more
62	Number of flowers per peduncle	1- 1 2- 1-2 3- 1-4
63	Pedicel hairs	1- glandular and simple 2- mostly glandular, rarely simple 3- mostly simple, rarely glandular 4- simple 5- glandular
64	State of pedicel hairs	1- less 2-mean 3-more
65	Corolla color	1- blue, pink, white 2- purplish pink 3- cream + violet 4- purplish 5- purplish blue 6- lilac – white 7- lavender 8- pink – white 9- white
66	Standard shape	1- obovate 2- ovate 3- elliptic – ovate

No.	Characters	Numerical code
67	Standard base	1- broad 2- spatula-shaped 3- non
68	Standard apex	1- less emarginated 2- mean emarginated
69	Wings shape	1- obovate 2- obovate – oblong 3- triangular 4- oblong
70	Wings base	1- auriculate 2- short auriculate 3- long auriculate
71	Keel shape	1- rhomboid 2- oblong
72	Position of keel adnate	1- 2/3 of ventral margin 2- 3/4 of ventral margin
73	Pod shape	1- elliptic - rhomboid 2- elliptic oblong 3- ovoid 4- elliptic obovate 5- elliptic
74	Pod hairs	1- glandular and simple 2- mostly glandular, rarely simple 3- mostly simple, rarely glandular 4- simple 5- glandular
75	State of pod hairs	1- less 2-mean 3-more
76	Seed shape	1- circular 2- subcircular 3- obovate 4- cordate
77	Seed color	1- cream 2- brownish with black spot 3- grey brown with black point 4- light brown 5- black brown 6- brown 7- reddish brown
78	Seed coat texture	1- wrinkled reticulate 2- spiny hairs + conical projection 3- shallow protuberance 4-tuberculated 5- wrinkled tuberculated + acuminate projection 6- tuberculated + irregularly curved 7- rugose – reticulate + wrinkled tubercle 8- wrinkled tuberculated 9- flat tuberculated 10- tuberculated reticulate 11- rugose

For morphological study of pollen and seed, we used herbarium specimens and fresh materials in the field (Table 2 & 3). Pollen grains and seeds were stabilized on aluminum stocks and coated with a thin layer of gold using coating equipment. Then, the specimens were observed under S. E. M. electronic microscope, model LEO 440 at the Islamic Azad University, Research and Sciences Branch. For each species, about 3 specimens of pollen and seeds were studied, and finally the related images and tables of morphological characters of pollen and seeds were prepared.

Moore 1991, was utilized for the terminology of the pollen as reference and Zohary & Heller, 1984 and Javadi & Yamaguchi, 2004 were utilized for terminology of the seeds as references.

### Result and Discussion

According to the phenogram, (fig. 1) in the linkage distance 11, two original clusters are distinguishable. In the first cluster, the *C. incisum* and *C. chorassanicum* pertaining to the section *Chamaecicer* are located close to each other (subcluster 1). Furthermore, two species of *C. arietinum* and *C. bijugum* which belong to the section *Monocicer* are also located adjacent to each other (subcluster 2).

In turn, the 2nd cluster is divided into two subclusters 1 and 2. The subcluster 1 consists of *C. anatolicum*, *C. oxyodon*, *C. kermanense*, and *C. spiroceras* which are located adjacent to each other. These four species are located into *Polycicer* section,

and in the subcluster 2, *C. tragacanthoides* (var. *tragacanthoides* and var. *turcomanicum*) and *C. stapfianum* which belong to the section *Acanthocicer*, are located. However *C. subaphyllum* from *Polycicer* section appears in this group. In fact, this species due to having some specifications such as spinous leaflets, presence of spine or curl at the end of rachis and its suffruticose appearance becomes distinguishable from the other species in the section *Polycicer*, and instead it should be placed in *Acanthocicer* section.

The differences between these two original clusters mainly underlies even or odd arrangement of leaves, status of the end of rachis and the calyx gibbosity positioning.

The PCA analysis revealed that the most variable characters are calyx gibbosity positioning, specifications of ending of rachis, type of peduncle hairs, growth habit, length of vexillum, length of carina, type of pod and calyx hairs, shape of lower stipules, type of division of leaf, length of wing and shape of leaflet, respectively. The ordination of the species based on PCA is also compatible highly with the related phenogram (Fig. 1).

In order to study relationship among the species based on pollen morphology, (Figs. 4-9) the cluster analysis under Ward method and based on understudied characters was performed and the result is delineated in Fig. 2. According to this phenogram, in the linkage distance 12, two main clusters are observed. The first cluster includes two subclusters 1 and 2. In the first subcluster, *C. bijugum* from the section *Monocicer* is

Table 2. *Cicer* species, their localities and voucher numbers in pollen study.

Species	Locality
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Section 1: <i>Monocicer</i>	
<i>C. arietinum</i> L.	Tehran: Karaj, farm of Genebank 1321m, Mesbah & Roohnavaz 1400.
<i>C. bijugum</i> Rech. f.	Kermanshah: Gahvareh, 5 km. E. of Changar, 1880m, Jalilian et al. 1402.
Section 2: <i>Chamaecicer</i>	
<i>C. chorassanicum</i> (Bge.) M. Pop.	Khorasan: Esfaraïen to Sabzevar, 5-20 km Sabzevar, Termeh 39956-E.
<i>C. incisum</i> (Willd.) K. Maly.	Lorestan: Oshtoran kouh, Ghaleh Rostam to Gahar, 2400-2700m, Iranshahr 14626.
Section 3: <i>Polycicer</i>	
<i>C. anatolicum</i> Alef.	Hamadan: Gardaneh Asadabad, Galehbour, 2250, Kalvandi et al. 1403.
<i>C. kermanense</i> Bornm.	Kerman: Baft, Siah kouh, Hamzehnejad H-1529.
<i>C. spiroceras</i> Jaub. & Spach	Bakhtiari: Ardal, Sarhang Mahmoud, 1800m, Iranshahr & Moussavi 39966-E.
<i>C. oxyodon</i> Boiss. & Hoh.	Tehran: Shahrestanac, 2300m, Pakravan 1408.
<i>C. subaphyllum</i> Boiss.	Fars: Shiraz, near Marvdasht, Kouh – e- Ayyoub, 2100m, Neamati & Jalilian 1412.
Section 4: <i>Acanthocicer</i>	
<i>C. stapfianum</i> Rech. f.	Fars: Shiraz, Eghlid, Kouh-e-Bel, Sardab, 2900m, Jalilian et al. 1413.
<i>C. tragacanthoides</i> Jaub. & Spach	
Var. <i>tragacanthoides</i>	Semnan: Shahroud, Gharieh-e-Tash to Kouh-e-Shahvar, 2600-3300m, Moussavi & Karavar 33605-E.
var. <i>turcomanicum</i> M. Pop.	Khorasan: Kopet Dagh, 45km N.N.E of Shirvan, Kouh-e-Alam, 2400m, Edmonson 26921-E.

Table 3. *Cicer* species, their localities and voucher numbers in seed study.

Spices	Locality
Section 1: <i>Monocicer</i>	
<i>C. arietinum</i> L.	Azerbaijan: Tabriz, Benis, Jalilian 1401.
<i>C. bijugum</i> Rech. f.	Kermanshah: Gahvareh, 5 km. E. Changar, 1880m, Jalilian et al 1402.
Section 2: <i>Chamaecicer</i>	
<i>C. chorassanicum</i> (Bge.) M. Pop.	Khorasan: Esfaraïen to Sabzevar, 5-20 km Sabzevar, Termeh 39956-E.
<i>C. incisum</i> (Willd.) K. Maly.	Lorestan: Oshtoran kouh, Ghaleh Rostam to Gahar, 2400-2700m, Iranshahr 14626.
Section 3: <i>Polycicer</i>	
<i>C. anatolicum</i> Alef.	Hamadan: Gardaneh Asadabad, Galehbour, 2250, Kalvandi et al. 1403.
<i>C. kermanense</i> Bornm.	Kerman: Mahan, Darreh-e-Kahnouj, Farhani 1411.
<i>C. spiroceras</i> Jaub. & Spach	Bakhtiari: Ardal, Sarhang Mahmoud, 1800m, Iranshahr & Moussavi 39966-E.
<i>C. oxyodon</i> Boiss. & Hoh.	Ghazvin: Alamout 2300-2400m, Farhani 1404-A.
<i>C. subaphyllum</i> Boiss.	Fars: Shiraz, near Marvdasht, Kouh – e Ayyoub, 2100m, Neamati & Jalilian 1412.
Section 4: <i>Acanthocicer</i>	
<i>C. stapfianum</i> Rech. f.	Fars: Shiraz, Eghlid, Kouh-e-Bel, Sardab, 1900m, Jalilian et al. 1413.
<i>C. tragacanthoides</i> Jaub. & Spach	
var. <i>tragacanthoides</i>	Semnan: Damghan to Shahroud, Gharieh-e-Tazareh, Kouh-e-Sefid-Shekar, 2700-3000, Moussavi & Karavar 33590E.
var. <i>turcomanicum</i> M. Pop.	Khorasan: Kopet Dagh, 45km N. N. E of Shirvan, Kouh-e-Alam, 2400m, Edmondson 26921-E.

located near the species *C. incisum* and *C. chorassanicum* from the section *Chamaecicer* which they are nearly incompatible with the morphological phenogram. In the second subcluster, the species *C. subaphyllum* from the section *Polycicer* is located close to *C. tragacanthoides* var. *turcomanicum*, belonging to

the section *Acanthocicer*. And the two species *C. stapfianum* and *C. tragacanthoides*, var. *tragacanthoides* from the section *Acanthocicer* are located close to each other, as well. These species which belong to the two mentioned sections are located near together according to the classic classification.

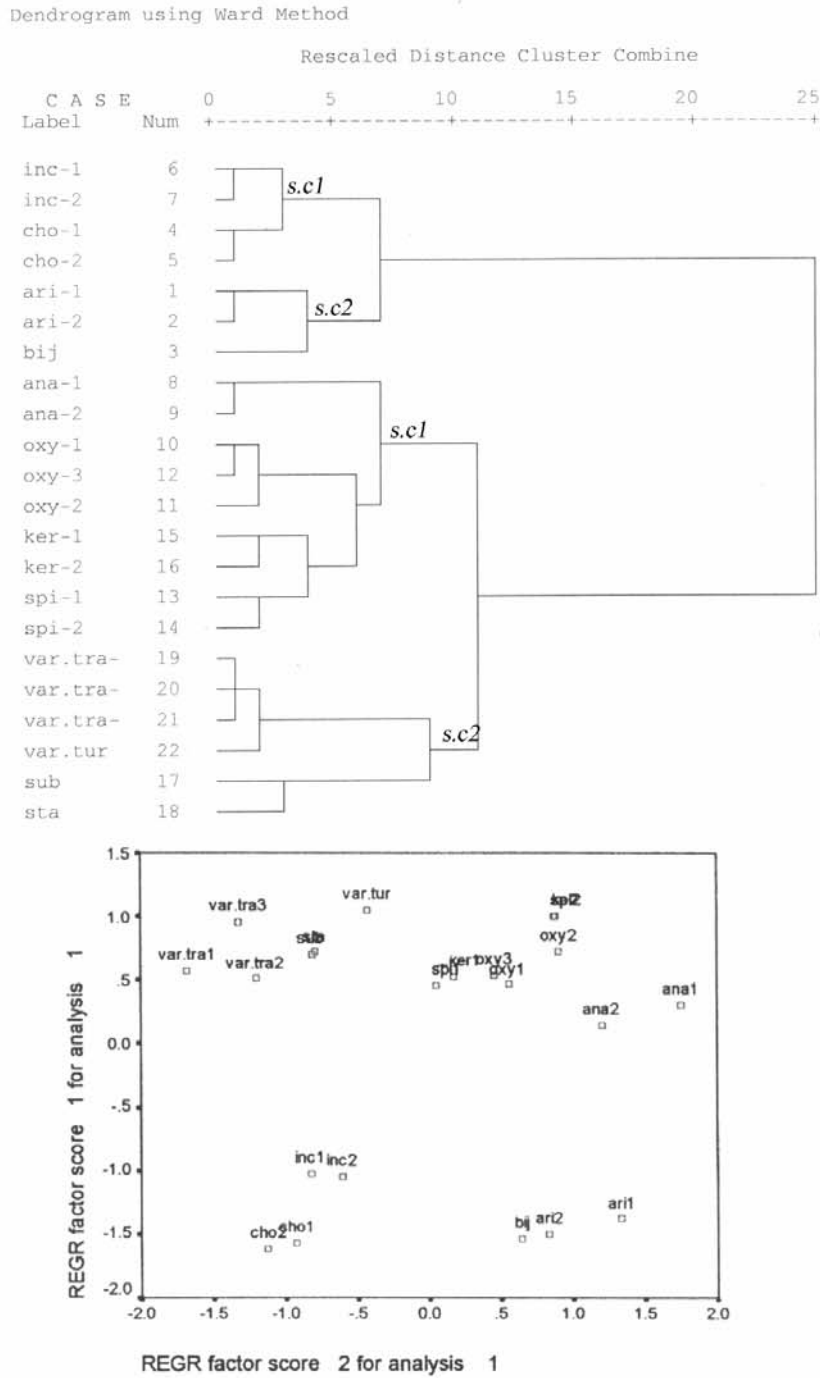


Fig. 1. Phenogram and ordination based on morphological data of *Cicer* species. –Abbreviations, inc= *C. incisum*; cho= *C. chorassanicum*; ari= *C. arietinum*; bij = *C. bijugum*, ker= *C. kermanense*; spi= *C. spiroceras*; ana= *C. anatolicum*; oxy= *C. oxyodon*; var. tra= *C. tragacanthoides*, var. *tragacanthoides*, var. tur=*C. tragacanthoides* var. *turcomanicum*; s.c= subcluster

Dendrogram using Ward Method

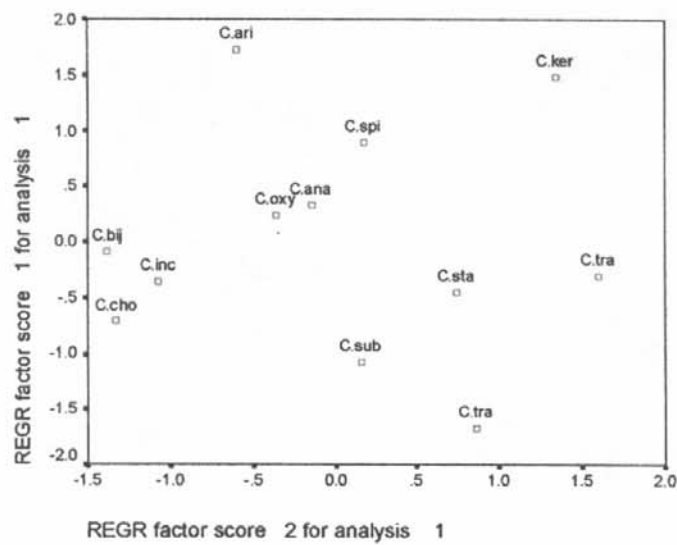
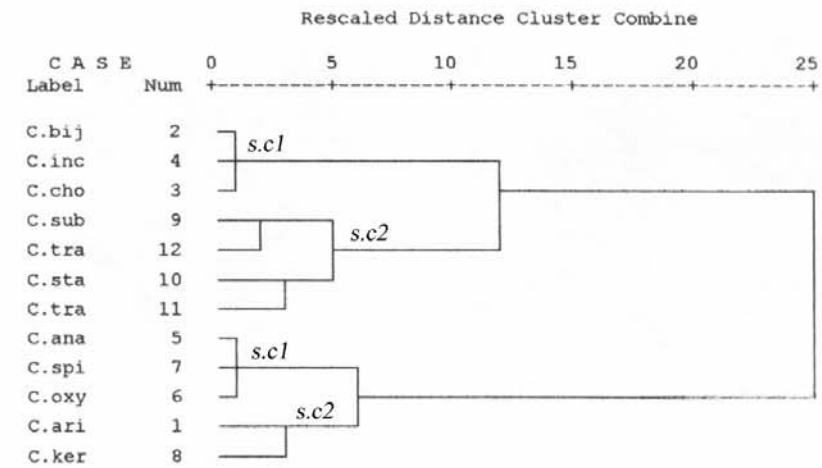


Fig. 2. Phenogram and ordination based on palynological data of *Cicer* species. Abbreviation as in fig. 1

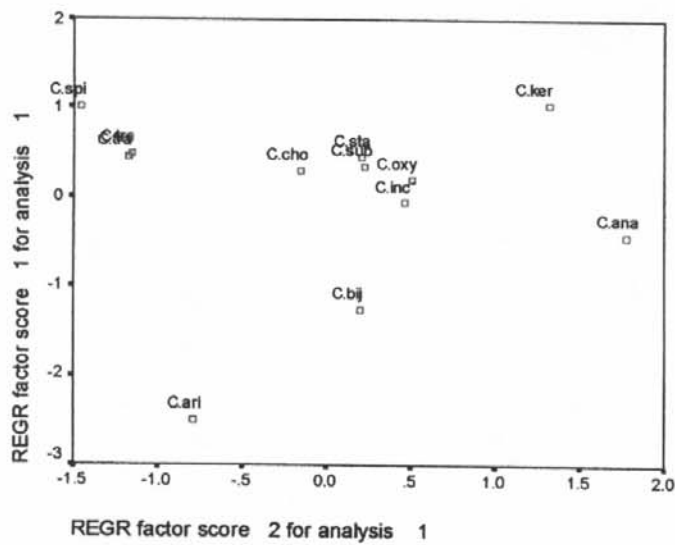
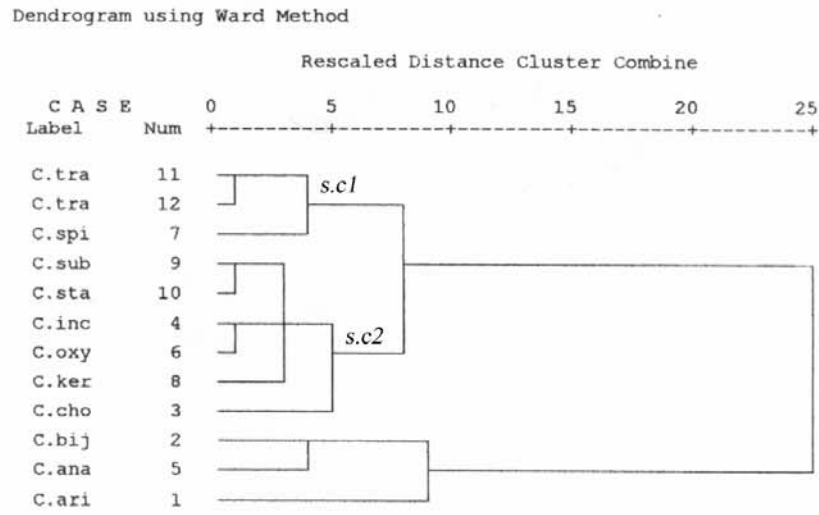


Fig. 3. Phenogram and ordination based on seed characters of *Cicer* species. Abbreviation as in fig. 1



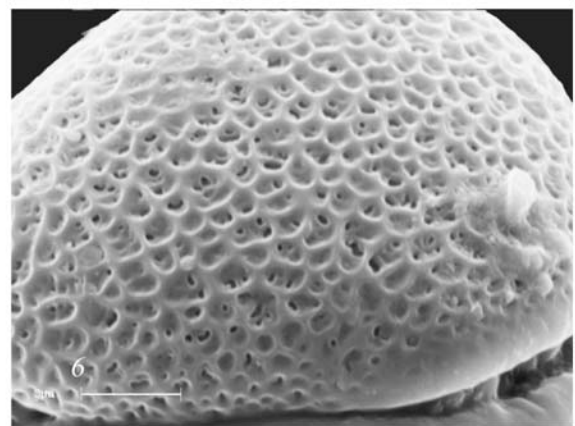
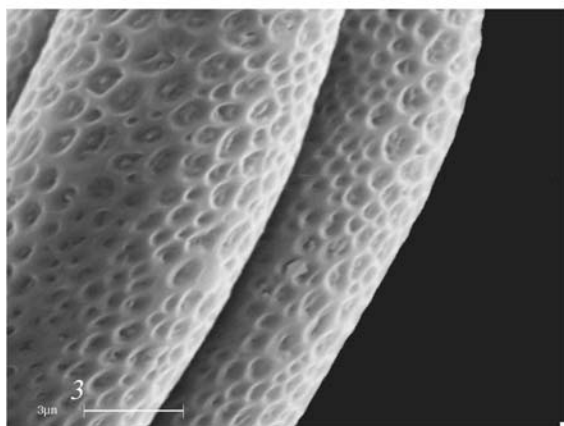
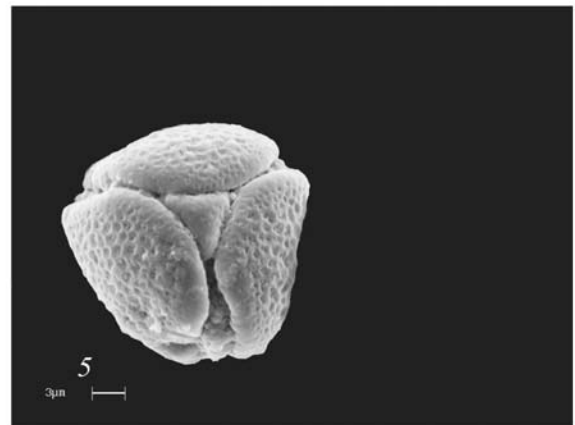
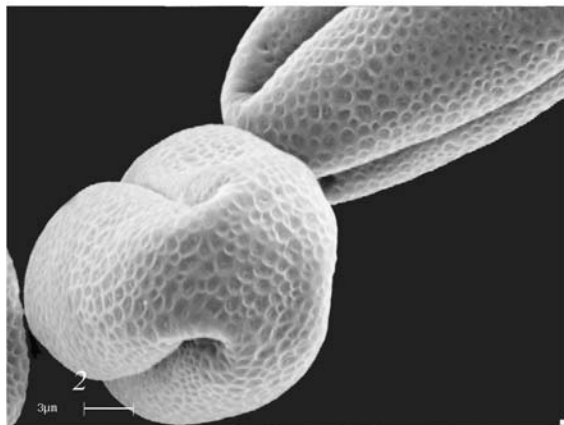
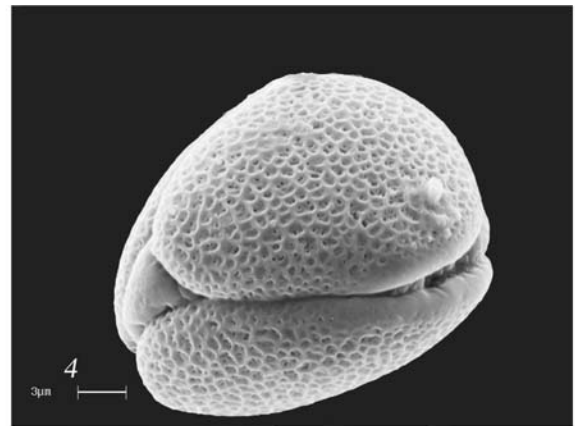
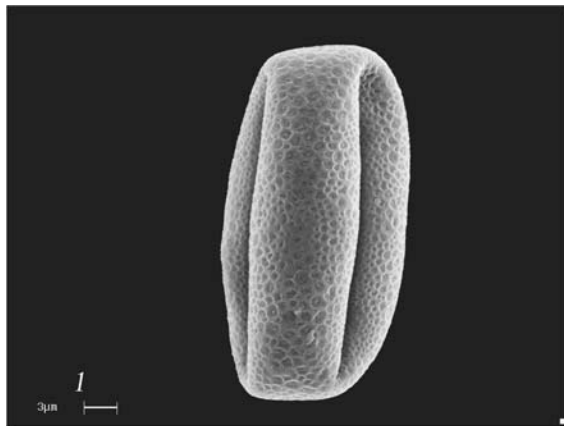


Fig. 4. A. Pollen grains of *Cicer* species. 1-3. *Cicer arietinum*, 1) Equatorial view (x 1300), 2) Polar view (x 2000), 3) Ornamentation (x 4000); 4-6. *Cicer bijugum*, 4) Equatorial view (x 2000), 5) Polar view (1300), 6) Ornamentation (x 1400).

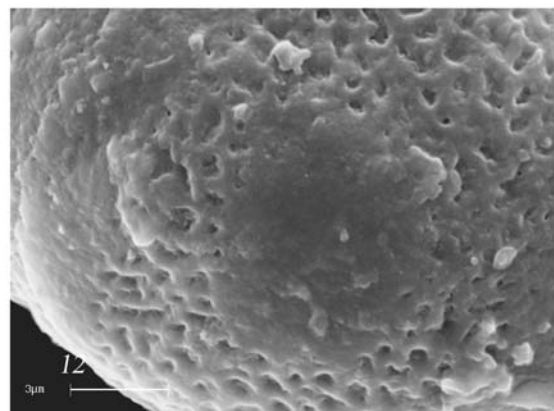
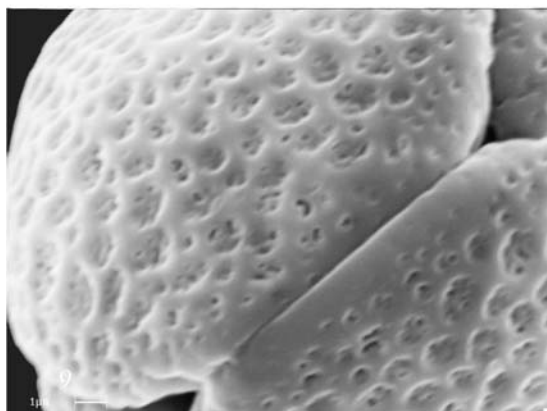
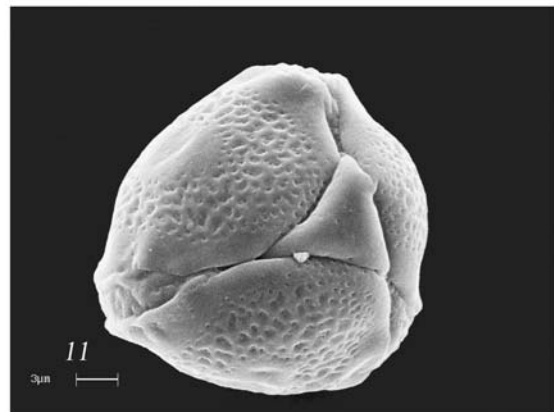
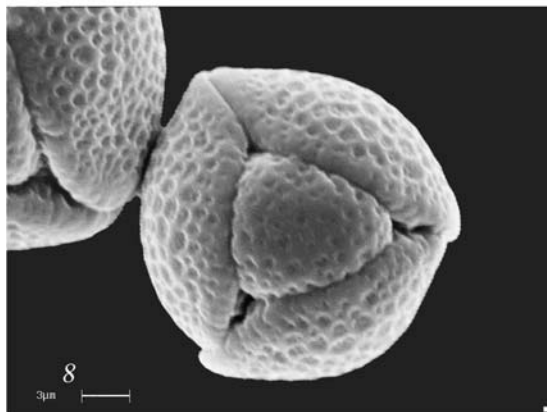


Fig. 5. Pollen grains of *Cicer* species. 7-9. *Cicer chorassanicum*, 7) Equatorial view (x 2000), 8) Polar view (x 2000), 9) Ornamentation (x 4000); 10-12. *Cicer anatolicum*, 10) Equatorial view (x 1300), 11) Polar view (x 1800), 12) Ornamentation (x 4300).

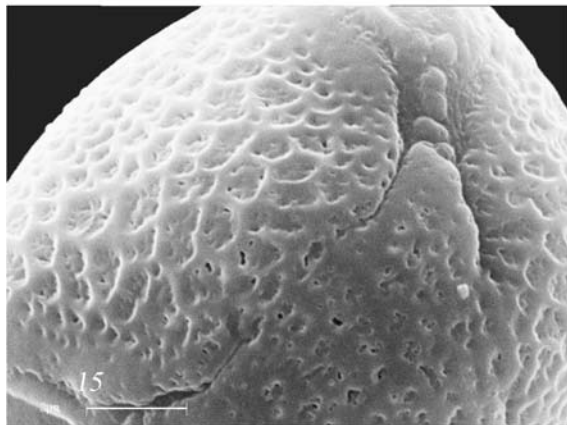


Fig. 6. Pollen grains of *Cicer oxyodon*. 13) Equatorial view (x 1500), 14) Polar view (x 2000), 15) Ornamentation (x 4300).

Therefore, pollen morphology phenogram also confirm this similarity. The second cluster is also consisted of two subclusters (subclusters 1 and 2). The first subcluster, consists of *C. anatolicum*, *C. spiroceras*, and *C. oxyodon* from the section *Polycicer*, and also the 2nd subcluster including *C. arietinum* from the section *Monocicer* are located near the species *C. kermanense* from the section *Polycicer*. According to the table of morphological characters of pollen (table 4), these two latter species, in spite that they belong to separate sections but have similar pollen specifications including similarity in equatorial view, polar view, colpi position and length of them. Therefore, these two species are isolated from the other related species of sections in terms of pollen specifications, and this status does not confirm the classic classification. With regard to the results obtained, we observed that two taxa of *C. kermanense* and *C. spiroceras* are different in all pollen specifications, as it was true for differences in morphological specifications. Therefore, it validates the distinction of them as two separate species.

Meanwhile, in this phenogram similar to morphological phenogram *C. subaphyllum* appears isolated from other species of the section *Polycicer* within the 2nd cluster, indicating that this species is different from the other species of *Polycicer* section in terms of pollen specifications. This is true as the ornamentation of exin surface in *C. subaphyllum* is reticulate but, in the other 3 species of the section is reticulate-areolate. Also, the shape of pollen in this species is oblate-spheroidal and in the other 3 species is prolate. PCA analysis was also carried out and the most variable characters are: P/E ratio, length of polar axis and shape of pollen grains.

The ordination of species based on PCA is also comparable highly with the pollen morphological phenogram (Fig. 2).

The seed morphology of *Cicer* species were studied (Fig. G-L)

Cluster analysis using Ward method and based on understudied seed characters was also carried out. The result of this analysis is shown on phenogram 3. According to this phenogram in the linkage distance 9, two main clusters observed. The first, includes two subclusters which in the first subcluster, two varieties of *C. tragacanthoides* are observed. As expected, they are completely similar and are located near each other. Near these two varieties *C. spiroceras* belonging to *Polycicer* section is located. Comparing to table 5, the specifications keeping these 3 species belonging to two different sections close to each other, are mainly seed circular shape and after that seed coat texture and seed color which are similar in high extent in these 3 species.

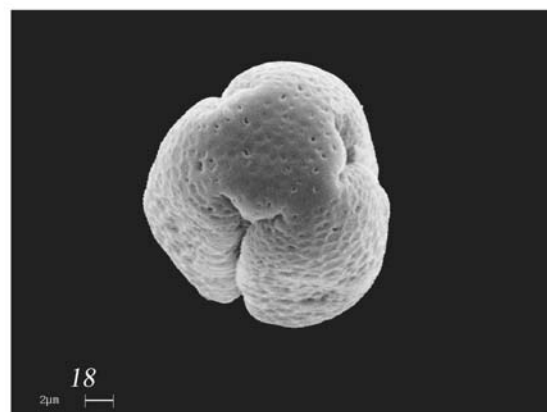
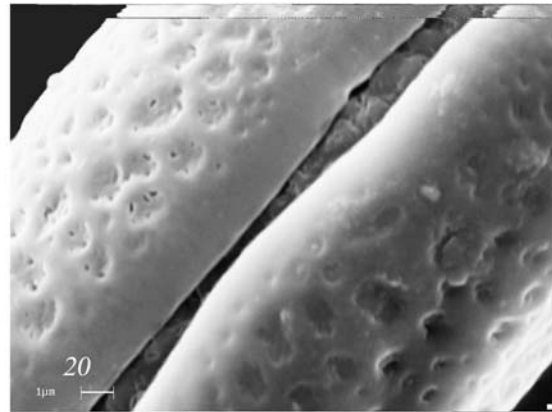
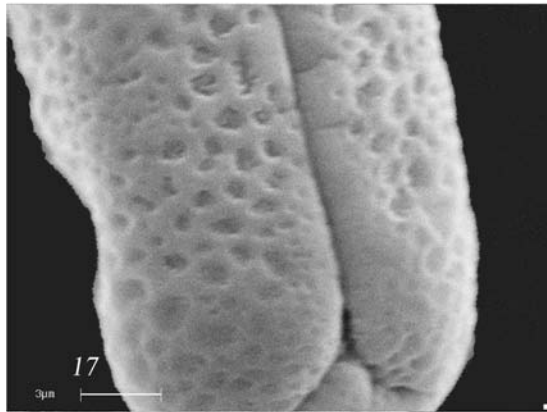
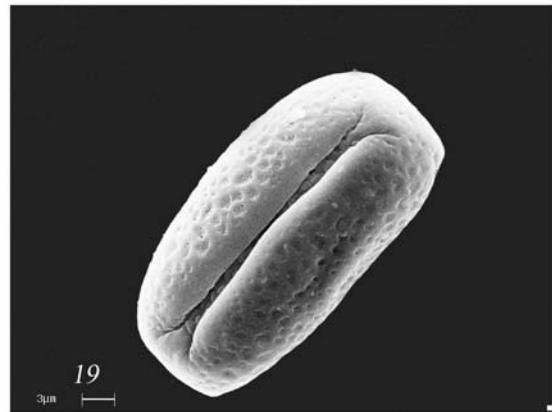


Fig. 7. Pollen grains of *Cicer* species. 16-18. *Cicer kermanense*, 16) Equatorial view (x 1300), 17) Ornamentation (x 3500), 18) Polar view (x 2000); 19-20. *Cicer spiroceras*, 19) Equatorial view (x 1300), 20) Ornamentation (x 4000).

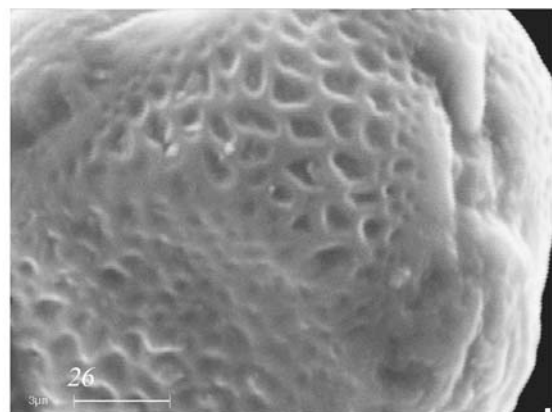
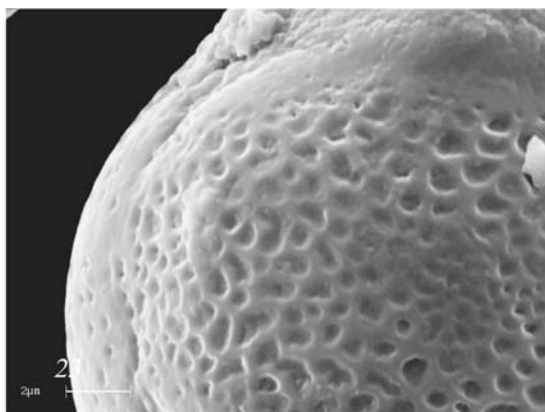
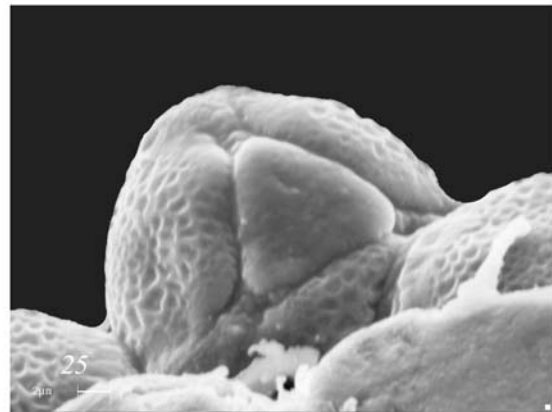


Fig. 8. Pollen grains of *Cicer* species. 21-23. *Cicer Subaphyllum*, 21) Equatorial view (x 2000) 22) Polar view (x 1800), 23) Ornamentation (x 4000). 24-26. *Cicer stapfianum*, 24) Equatorial view (x 1300), 25) Polar view (x 2200), 26) Ornamentation (x 4100)

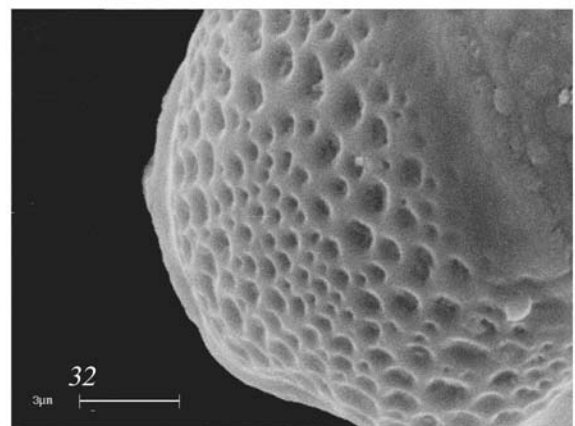
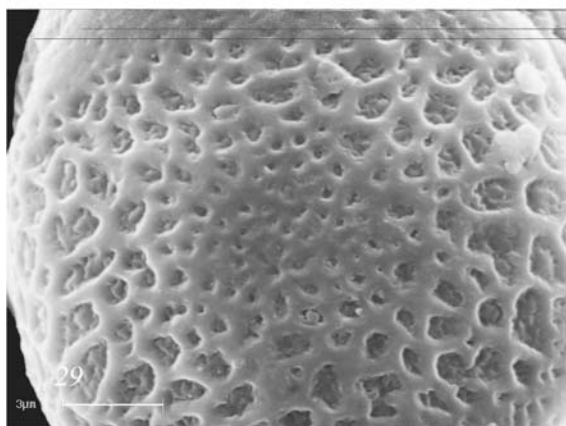
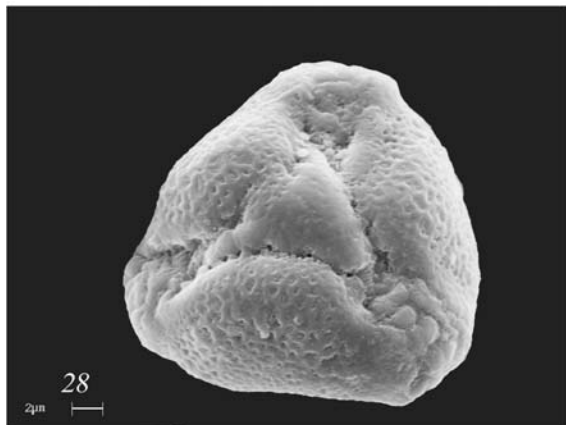
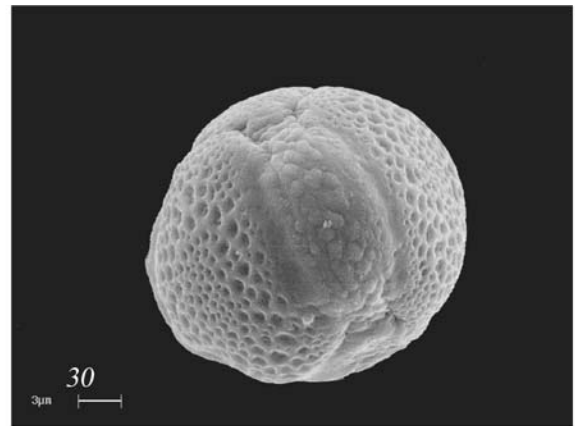


Fig. 9. Pollen grains of *Cicer* species. 27-29. *Cicer tragacanthoides*, var.