0STUDIES OF POLLEN GRAINS IN THE SECT. STENOCEPHALAE (COUSINIA CASS.-ASTERACEAE) IN IRAN

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Pollen morphology of 25 species of section *Stenocephalae* Bunge from genus *Cousinia* Cass. is described and compared using Light and Scanning Electron Microscope. The pollen grains are prolate, isopolar, tricolporate and exine is densely or loosely verrucate. Based on morphological characters, species of this section can be divided to two groups: 1. species with narrow cylindrical heads and leathery leaves, 2. species with ovate heads and herbaceous leaves. The pollen grains of the first group with more than 20 verrucae and the second group with 3-30 verrucae in each 25 µm² of pollen surface are differentiated from each other. Indeed, these two detached grouping for species of sect. *Stenocephalae* are somewhat supporting each other.

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Key words. Cousinia, sect. Stenocephalae, pollen morphology, Iran.

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مورفولوژی دانه گرده ۲۵ گونه از بخش استنوسفاله از جنس کوزینیا با استفاده از میکروسکوپ نوری و میکروسکوپ الکترونی اسکنینگ شرح داده شده و با یکدیگر مقایسه میشوند. شکل دانههای گرده در گونههای این بخش بیضوی کوتاه یا بلند، جور قطب و سه شیار منفذی است. اگزین به طور متراکم یا تنک زگیلدار میباشد. بر اساس صفات ریختشناسی، گونههای این بخش به دو گروه تقسیم میشوند: ۱-گونههای دارای کپه استوانهای باریک و برگهای چرمی، ۲- گونههای دارای کپه تخممرغی و برگهای علفی. علاوه بر صفات مطالعه شده، دانههای گرده گروه اول با بیش از ۲۰ زگیل و گروه دوم با ۳۰-۳ زگیل در هر ۲۵ میکرومتر مربع از سطح اگزین از یکدیگر متمایز میگردند. بنا بر این، گروهبندی بر اساس صفات گردهای تا حدودی تاییدکننده تقسیمبندی مرفولوژیک نیز می باشد.

Introduction

The genus *Cousinia* Cass. from Arctium group tribe *Cardueae* subtribe *Carduinae* (Häffner 2002, Susanna et al. 2003) with nearly 250 species (Attar & Ghahreman 2006) is the second largest genus in Iran. The species of this genus are the main element of Irano-Turanian region distributed mostly in mountains and semiarid places. Among 43 sections of this genus in Iran (Rechinger 1972, 1979), sect. *Cynaroides* (Attar & Ghahreman 2007) Bunge, with nearly 90 species (Attar & Ghahreman 2006, 2007) and sect.

Stenocephalae Bunge, with about 32 species (Djavadi & Attar 2006) are the largest sections respectively.

All the species belonged to sect. *Stenocephalae* are characterized by ovate or cylindrical heads, with up to 20 pink or yellow flowers and decurrent herbaceous or leathery leaves. Some species are easily distinguishable from the others (for example, *C. recurvata* is readily distinguished by having long recurved bracts) whereas, identification of some species are more difficult. Therefore, using pollen morphology can be useful in separation and determination of the crucial species.

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Some taxonomists such as Bunge (1865), Boissier (1875), Winkler (1897), and Rechinger (1972, 1979) are believed that *Cousinia* is homogeneous and considered all species of the genus in one group, whereas, some others such as Kuntze (1891) considering *Cousinia* as a heterogeneous group similar to the genus *Arctium*.

The pollen morphology of tribe *Cardueae*, including genus *Cousinia*, has been extensively studied by Schtepa (1966). According to Schtepa (1966), all genera belong to the subtribe *Carduinae*, except *Cousinia*, have almost one pollen form. But, there are two distinct types of pollen in the genus *Cousinia* as follows:

Type I, Arctioides. This type which is also the main pollen type of subtribe *Carduinae*, is very similar to that of *Arctium*. The most important characters of this type are: spheroid or triangular-spheroid pollen with short polar axis, spinulose exine which is ornamented with large or small tubercles. Besides *Arctium* and some subgenera of *Cousinia, Jurinea* and *Circium* have this pollen type (*Arctiastrum* pollen type).

Type II, Orientalis: Some genera of subtribe *Carduinae* such as *Polytaxis* and *Ptilostemon* as well as *Cousinia* subgenus *Cousinia* have this type of pollen which is characterized by elliptic-oblong pollen grain with long polar axis and smooth-spinulose exine (*Cousinia* pollen type)

Kuprianova and Tscherneva (1982) with accepting that *Cousinia* is heterogeneous, admitted two pollen types for the genus but introduced them with names as follows:

Type Cousinia. The pollen grains of this type are tricolporate, length of polar axis (P) and length of equatorial axis (E) are (32.4) 43.2 -64.6 (72) μ m and (28.6) 32.4-36 (45) μ m respectively, P/E=1.5-1.9, colpus up to 13.6 μ m, exine thick, ornamented with small or large spinules, between spinules smooth or with micro-pores, thickness of exine in equatorial and polar regions are 9-11.9 μ m and 3.4 μ m respectively.

Type Arctiastrum. This type of pollen grains are tricolporate, widely elliptic or subspheroid, length of polar axis (P) and length of equatorial axis (E) are (43.2) 50.4-54 (65.8) μ m and 36-54 μ m respectively, P/E=1-1.2, thickness of exine up to (7) 8-10 (11.9) μ m in equatorial region and 5.1-5.6 μ m in polar region, exine ornamented with large acute tubercles, granulate between tubercles.

Apart from the above-mentioned studies on this genus, we usually cross with some sporadic researches in the literature viz. Meo 2005, Zafar et al. 2007, Jafari & Ghanbarian 2007. These studies have been mainly carried out in different tribes and genera of *Asteraceae* including few species of *Cousinia*.

Materials and Methods

The pollen grains were obtained from fresh and herbarium materials of herbaria TUH (Central Herbarium of Tehran University), IRAN (Herbarium Ministerii Agriculturae) and FUMH (Mashhad University Herbarium). The locality and voucher specimen references are presented in Table 1.

For Scanning Electron Microscopy, unacetolized pollen grains of fully heads were transferred to stubs and coated with gold with Sputter Coater. For preparing light Microscope, the pollengrains were acetolized (Erdtman 1952), transferred to slides mounted with glycerin jelly. The measurements (average amounts and their variation) were carried out using Light Microscopy and Excel software.

Results

Characters of pollen grains are shown in Tables 2 and 3 and Figs. 1-58. The pollen grains are elliptic or elliptic-oblong in shape, monad, tricolporate, attenuate from middle toward the poles, colpi acute at both ends. They are radially symmetric, isopolar and with branched columella. The exine sculpturing is densely or loosely verrucate-porate, verrucae wide at the base and obtuse or acute at the tip. Polar axis is longer than equatorial axis. Thus, the main symmetry is polar symmetry. The ratio of polar length to equatorial width (P/E) is more than 1 (1.23-1.55). According to the previous researches (Moore et al. 1991), pollen grains with P/E of 1.14-1.33 and 1.2-1.33 are respectively subprolate and prolate. The palynological observations revealed that the pollen grains of most species of sect. Stenocephalae are prolate and some have subprolate pollen grains.

In Scanning Electron Micrograph (SEM) analysis, pores in some species, C. lucida, C. commutata, C. aggregata are distinct. But are indistinct in species as C. tenuiramula, C. gauba, C. wendelboi. The thickness of exine in equatorial region is up to $12 \mu m$, thinner in polar region (up to 4.5 µm). Nexine is thickened at the margins of equatorial region. The size of pollen grains is different in various species, therefore not suitable to use as a character to delimitate the species. The smallest pollen size is seen in C. commutata and C. bijarensis and the largest one in C. prasina. The average of P/E is variable between 1.23 (in C. renominata) and up to 1.55 (in C. nujianensis). The thickest sexine, in equatorial region, belongs to C. nujianensis and the thinnest is in C. manouchehrii. The thickest and thinnest nexine in polar region are observed in C. hypopolia and C. bijarensis respectively.

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Species	collector	herb. No.
<i>C. aggregata</i> DC.	Attar, Zarre & Saber	34413-TUH
C. alexeenkoana Bornm.	Ghahreman & Attar	20555-TUH
C. assyriaca Jaub. & Spach	Zarre & Saber	34385-TUH
C. bijarensis Rech. f.	Zarre & Saber	34381-TUH
C. calolepis Boiss.	Saber & Zarre	34380-TUH
C. commutata Bunge	Djavadi	18888-IRAN
C. cylindracea Boiss.	Zarre & Saber	34390-TUH
C. cylindrocephala Jaub. & Spach	Djavadi	50099-IRAN
C. decipiens Boiss. & Buhse	Ghahreman & Attar	25437 – TUH
C. esfandiarii Rech. f. & Aell.	Saber & Moazzeni	34367-TUH
<i>C. gaubae</i> Bornm.	Saber & Zarrei	34376-TUH
C. glaucopsis Bornm. & Rech. f.	Attar, Zarre & Saber	34398-TUH
C. hypopolia Bornm. & Sint.	Ghahreman & Attar	21904-TUH
<i>C. lucida</i> DC.	Ghahreman & Attar	21850-TUH
C. manouchehrii Rech. f. & Esfand.	Saber & Zarrei	34415-TUH
C. nekarmanica Rech. f.	Saber & Moazzeni	34365-TUH
C. nujianensis Attar et al.	Ghahreman & Attar	21830-TUH
C. prasina Jaub. & Spach	Djavadi & Ghanbari	29277-IRAN
<i>C. recurvata</i> DC.	Ghahreman & Attar	34373-TUH
C. renominata Rech. f.	Saber & Moazzeni	34369-TUH
C. stahliana Bornm. & Gauba	Joharchi	20761-FUMH
C. stenocephala Boiss.	Iranshahr & Dezfulian	9279-IRAN
C. tenuiramula Rech. f.	Ghahreman & Attar	21886-TUH
C. thamnodes Boiss. & Hausskn.	Djavadi & Ghanbari	29277-IRAN
<i>C. wendelboi</i> Rech. f.	Saber & Moazzeni	34375-TUH

Table 1. Voucher specimens of Cousinia species used in this study.

The average thickness of nexine in equatorial region is varied between 2.4 in *C. alexeenkoana* and 3.6 in *C. hypopolia*. The thickest nexine in polar region belongs to *C. calolepis* and the thinnest is in *C. aggregata*. The exine sculpturing of *C. aggregata* has very dense verrucae but in *C. esfandiarii* they are very loose.

Discussion

Based on two types of pollen grains in *Cousinia* (Schtepa 1966, Kuprianova & Tscherneva 1982), our studies showed that the pollen of sect. *Stenocephalae* have *Cousinia* pollen type.

Based on the number of verrucae on the surface of exine, following groups are distinguished:

Group 1. Species with cylindrical, aggregated heads, number of verrucae more than 20 in 25 μ m² of exine surface, including: *C. aggregata, C. lucida, C. cylindracea, C. manouchehrii, C. nekarmanica* and *C. alexeenkoana*.

Group 2. Species with solitary or 2-3 ovate heads, number of vertucae 3-30 in 25 μ m². This group is divided to three subgroups as follows:

Subgroup I. Number of vertucae more than 15 in 25 μ m². The most important morphological characters of

this subgroup are: leaves arachnoid on both sides, bracts more than 70 in number, erect, spreading-recurved, flowers c. 20, corolla yellow, including: *C. gilanica*, *C. hypopolia* and *C. stahliana*.

Subgroup II. Number of verrucae 3-12 in 25 µm². The most common characters of this subgroup are leaves long decurrent, herbaceous or leathery-herbaceous, arachnoid on both sides, flowers up to 27, corolla purple or yellow-purple in an individual head (discolor head), bracts imbricate at base, spreading or recurved at the tip, including: *C. esfandiarii, C. commutata, C. calolepis, C. renominata, C. decipiens* and *C. recurvata.*

Subgroup III. Number of verrucae more than 20 in 25 µm². The largest and smallest size of pollen grains is observed in this subgroup. The most important characters of this subgroup are leaves shortly decurrent, cordate at base, discolor (except *C. bijarensis*), bracts appressed, heads solitary or 2, ovate, flowers 7-15, including: *C. assyriaca, C. bijarensis, C. tenuiramula, C. prasina, C. gaubae* and *C. thamnodes*.

It is noticeable that this grouping is also supported by anatomical and morphological results.

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Table 7	Part of 1	nollen	characters	1n	Cousinia	species	arranged	according	o to	their	relation	shin
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Species	Shape	Distance between	Number of verruca
-	1	verruca	per 25µm ² .
C. lucida	prolate (narrow elliptic)	0.8 - 1	22 - 25
C. aggregata	prolate (narrow elliptic)	0.8 - 0.9	30 - 37
C. stenocephala	prolate (wide elliptic)	1 – 1.3	23 - 26
C. cylindracea	prolate (wide elliptic)	0.8 - 1.15	24 - 26
C. manouchehrii	subprolare (wide elliptic)	1 – 1.22	23 - 25
C. wendelboi	prolate (narrow elliptic)	0.9 - 1	31 - 30
C. nekarmanica	prolate (narrow elliptic)	1.05 - 1.2	17 -22
C. alexeenkoana	subprolate (narrow elliptic)	0.9 - 1.05	24 - 25
C. glaucopsis	subprolate (narrow elliptic)	0.9 - 1.2	12 - 17
C. assyriaca	subprolate (narrow elliptic)	0.8 - 1	20 - 23
C. bijarensis	subprolate (narrow elliptic)	0.8 - 1	24 - 25
C. tenuiramula	subprolate (narrow elliptic)	0.9 - 1.05	28 - 32
C. prasina	prolate (wide elliptic)	0.9 - 1.5	17-21
C. gaubae	sub prolate (wide elliptic)	1.7 - 2	30
C. thamnodes	prolate (narrow elliptic)	0.9 - 1.22	19 -22
C. cylindrocephala	prolate (wide elliptic)	1 – 1.2	20 - 25
C. esfandiarii	sub prolate (narrow elliptic)	2.5 - 2.9	3 - 6
C. commutata	sub prolate narrow elliptic)	1.4 - 1.6	9 - 12
C. calolepis	prolate (narrow elliptic)	1.1 – 1.2	13 - 15
C. decipiens	prolate (wide elliptic)	1.2 - 1.4	12 - 13
C. renominata	sub prolare (elliptic)	1.3 – 1.6	10 - 12
C. stahliana	prolate (elliptic)	1.2 - 1.5	10 - 15
C. hypopolia	prolate (narrow elliptic)	0.9 - 1	14 - 16
C. recurvata	prolate (elliptic)	0.9 - 1	15 - 17

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Table 3. The main characters used in palynological studies of *Cousinia* sect. *Stenocephalae* Bunge. -Abbreviations: P=Polar length, E=Equatorial width, P/E=Polar length / Equatorial width, Se..E=Sexine thickness in equatorial region, Ne. E=Nexine thickness in equatorial region, Se. P=Sexine thickness in polar region, Ne. P=Nexine thickness in polar region.

Species	Р	Е	P/E	Se.E	Ne.E	Se.P	Ne.p
C. esfandiarii	43 (48.7 ± 2.9) 52	34 (37±1.9) 40	1.2 (1.3±0.08) 1.52	$4(5.1 \pm 0.5)6$	2 (3.2±0/7) 4.5	2 (2.4±0/4) 3	1 (1.2±0/3) 3
C. renominata	50 (52.5±1.83) 55	40 (42.5±1.3)45	1.18 (1.23±0.04) 1.4	4 (5.2±0.5) 6.5	3 (3.7±0.4) 4.2	2 (2.9±0.4) 3.2	1.5 (1.87±0.2)2
C. decipiens	54 (56.8 ± 1.9) 61	39 (41 ± 1.2) 43	1.27 (1.38± 0.05)1.48	$5(5.4 \pm 0.4)6$	3 (3.9±0.63) 9	1.8 (2.3±0.3) 3	1.2 (1.8±0.3)2.1
C. hypopolia	55 (59 ± 2.6) 64	39 (43.6 ± 2.5) 50	1.24 (1.35±0.07)1.53	5 (6.7±0.7) 8	4 (4.9±0.7) 6.5	2.8 (3.6±0.6) 5	1.2(1.86±0.4)2.
C. wendelboi	53 (56.8±1.3) 59	36(37.7±1.4) 40	1.42 (1.5±0.05) 1.59	$5(6 \pm 0.4)7$	4 (4.3±0.4)5.5	2 (2.81±0.3) 3	1.1 (1.6±0.3) 2
C. stenocephala	49 (52.4±2.3) 56	35 (40±1.53) 42	1.12 (1.34±0.07) 1.4	5.5 (6.1±0.4) 7	4 (4.8 ±0.6) 5.5	2 (2.9±0.3) 3.8	1 (1.3±0/4) 2
C. recurvata	44 (47.9±2.7) 53	32 (36.8±3.2) 44	1.17 (1.3±0.1) 1.42	4.5 (6.3±0.8) 8	4 (4.8±0.8) 7	1.8 (2.4±0.2)2.8	1 (1.4±0.3) 2
C. assyriaca	42 (45.7±2.9) 51	32 (35.6±1.8) 39	1.15(1.28±0.1) 1.5	4 (5.37±0.6)7	2.5 (3.4±0.5)4	1.5 (2.3±0.4) 3	1 (1.2±0.3) 2
C. prasina	60 (64 ± 2.9) 71	41 (44.2 ± 2.4) 49	1.32 (1.45±0.07) 1.6	$6(6.8\pm0.8)$ 9	3 (4.2±0.7) 6	3 (3.5±0.6) 5	1.3 (1.9 ±0.2) 2
C. gaubae	44 (49±2.7) 54	35 (39±2.9) 46	1.15 (1.3±0.1) 1.38	3.5 (4.9±0.91)7	3 (3.43±0.67) 5	1.6 (2.5±0.4) 3	1 (1.21±0.3)2
C. commutata	35 (39±2.2) 43	26 (29.9 ±2.2) 35	1.16(1.31±0.1)1.4	4.2 (4.9±0.4)5.5	2 (3±0.5)4	1.6 (2±0.2)2.7	$0.7(09\pm0.2)1.1$
C. calolepis	56 (60.8±2.6) 66	42 (44.9±2.59) 50	1.26 (1.3±0.07) 1.47	5 (6.5±0.6) 7.5	3 (3.9±0.7) 5	2.5 (3.1±0.3) 4	1.5 (1.1±0.2)2
C. alexeenkoana	46 (52.7±3.4) 60	35 (39±2.2) 43	1.3 (1.4±0.1) 1.5	4 (5.5±0.8) 7	1.8 (2.4±0.6)3.5	2 (2.2±0.35) 3	1 (1.1±0.21)1.8
C. glaucopsis	51 (53±3.2) 62	37 (40±2) 44	1.33 (1.43±0.07) 1.56	5 (6 ±0.6)7	3.5 (4±0.7) 6	2 (2.5±0.3)3.2	1 (1.3±0.3) 2
C. cylindracea	45 (48.3±1.9) 51	30 (33.2±1.7) 37	1.27 (1.4±0.1) 1.58	$4(4.5\pm0.4)5$	$2.2(3.2\pm0.5)4$	1.1 (2.2±0.9)2.7	$1(1\pm0.1)1.2$
C. manouchehrii	39 (41.4±1.8) 45	30 (32.8 ± 2) 37	1.1 (1.26 ±0.6) 1.42	4 (4.1±0.58) 6	2.5 (4.6±0.9)6	1.2 (1.9±0.3)2.2	1 (1.3±0.3) 1.8
C. lucida	41 (45.2 ±21.4)48	30 (33.7 ± 2.2)39	1.18 (1.34±0.1) 1.54	$3.5(4.6 \pm 0.6)6$	$2.2(3.3 \pm 0.6)5$	1.4 (1.9±0.2)2.5	1 (1.6±0.3) 2
C. nekarmanica	43 (50.4 ±4.3) 56	30 (34.4 ± 1.2) 39	1.26 (1.45±0.1) 1.69	$4(5.2\pm0.5)$ 6	2.2 (3.3±0.5) 4	2 (2.4±0.4) 3	0.5 (1±0.1) 1.5
C. thamnodes	45 (49.5 ± 2.3)53	32 (33.9 ± 0.9)35	1.33 (1.45±0.06)1.56	$4(5.7\pm0.5)6.5$	2.1 (4±0.8)5	1.5 (2±0.2) 2.5	$1.1(1.5\pm0.3)2$
C. nujianensis	58 (64 ± 2.3) 57	38 (41±1.7) 44	1.45 (1.55±0.06) 1.65	6 (6.8±0.58) 8	4 (4.9±0.5) 6	2 (2.5±0.3) 3	1.2 (1.7 ±0.4) 2
C. aggregata	$40(42.\pm 2)46$	30 (32.4 ± 2)36	1.24(1.3±0.06)1.5	3.8 (4.7±0.5) 6	2.2 (3.3±0.8 4.5	1.8 (2.1±0.3)2.8	0.5 (0.8±0.2)1.1
C. tenuiramula	41 (43.4 ± 1.4)46	30 (32.2 ± 1.3)35	1.24(1.35±0.06)1.5	4.5(5.1±0.4) 6	3 (3.4±0.5)4	2 (2.38±0.3)3	1(1.5±0.3)2.2
C. bijarensis	38 (40.87±1.1) 43	27 (28.97±1.2) 31	1.33 (1.4±0.07) 1.55	4.5(5.07±0.36)6	2.6 (3.1±0.4) 4	1.2(1.76±0.2)2	0.8 (1±0.1)1.2
C. stahliana	47 (53.3±2.8) 57	34 (36.9±2.43) 42	1.25 (1.44±0.01) 1.65	5 (5.6 ±0.6)7	1.8 (2.5±0.3) 3	1.7 (2±0.2)2.5	1 (1.2±0.1) 1.6

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Figs. 1-6: Light microscope photographs, x 100: 1. Cousinia aggregata, equatorial view; 2. C. manouchehrii, equatorial view; 3. C. lucida, equatorial view; 4. C. cylindracea, exine sculpture; 5. C. cylindracea, equatorial view; 6. C. nekarmanica, equatorial view.



Figs. 7-12: Light microscope photographs, x 100: 7. *Cousinia alexeenkoana*, equatorial view; 8. *C. bijarensis*, equatorial view; *C. decipiens*, polar view; 10. *C. decipiens*, equatorial view; 11. *C. assyriaca*, equatorial view; 12 *C. glaucopsis*, exine sculpture.



Figs. 13-18: Light microscope photographs, x 100: 13. *Cousinia thamnodes*, equatorial view; 14. *C. prasina*, equatorial view; 15. *C. stenocephala*, equatorial view; 16. *C. calolepis*, equatorial view; 17. *C. commutata*, equatorial view; 18. *C. commutata*, polar view.



Figs. 19-24: Light microscope photographs, x 100: 19. *Cousinia esfandiarii*, equatorial view; 20. *C. esfandiarii*, polar view; 21. *C. hypopolia*, equatorial view; 22. *C. wendelboi*, equatorial view; 23. *C. tenuiramula*, equatorial view; 24. *C. tenuiramuls*, polar view.



Figs. 25-30: SEM photographs: 25. *Cousinia aggregata*, equitoraial view; 26. *C. aggregata*, exine sculpture; 27. *C. lucida*, equitoraial view; 28. *C. lucida*, exine sculpture; 29. *C. lucida*, polar view; 30. *C. cylindracea*, equitoraial view.



Figs. 31-36: SEM photographs: 31. *Cousinia alexeenkoana*, polar view; 32. *C. nekarmanica*, exine sculpture; 33. *C. nekarmanica*, equatorial view; 34. *C. assyriaca*, equatorial view; 35. *C. assyriaca*, exin sculpture; 36. *C. assyriaca*, polar view.



Figs. 37-42: SEM photographs: 37. Cousinia calolepis, equatorial view; 38. C. calolepis, exine sculpture; 39. C. calolepis., polar view; 40. C. commutata, equatorial view; 41. C. commutata, polar view; 42. C. commutata, exine sculpture.



Figs. 43-48: SEM photographs: 43. *Cousinia decipiens*, polar view; 44. *C. decipiens*, equatorial view; 45. *C. decipiens*, exine sculpture; 46. *C. esfandiarii*, equatorial view; 47. *C. esfandiarii*, exine sculpture; 48. *C. renominata*, equatorial view.



Figs. 49-54: SEM photographs: 49. *Cousinia renominata*, exin pores; 50. *C. renominata*, exine sculpture; 51. *C. tenuiramula*, equatorial view; 52. *C. tenuiramula*, exine sculpture; 53. *C. wendelboi*, equatorial view; 54. *C. wendelboi*, exine sculpture.



Figs. 55-58: SEM photographs: 55. Cousinia thamnodes, polar view; 56. C. glaucopsis, equatorial view; 57. C. glaucopsis, exine sculpture; 58. C. prasina, polar view.