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Investigating the relationship between three sections of Cousinia using macroscopic and microscopic characters

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ABSTRACT

Cousinia is the one of largest genera in Asteraceae family, after *Astragalus*, *Cousinia* is the largest genus, with over than 270 species and 43 sections in Iran. Morphological studies and micro-morphological studies of the leaf characters were performed in 31 species of the genus *Cousinia* (sections *Albidae*, *Cousinia* and *Stenocephalae*) growing in Iran. In total 17 morphological characters and 11 micro-morphological characters of the leaf epidermises were scored. UPGMA clustering and PCA ordination based on morphological characters almost separated the three sections. However, the same analyses based on micro-morphological characters did not completely agree with morphological results. Combined analyses of micro and morphological characters suggest that *C. chlorocephala* has closer affinity with the section *Stenocephalae*. It seems use of both micro-morphological and morphological characters for analysis, show species relationship clear than only morphological result data analysis. According to the results of the relationship of the species using the total characteristics of the grouping of this taxon, it needs to be revised again and molecular studies in this field are needed for more certainty.

KEYWORDS

Cousinia, Asteraceae, SEM, Leaf.

1. Introduction

Cousinia Cass. Is one of the largest genera (after *Senecio* and *Vernonia*) in the Asteraceae. (Frodin 2004, Rechinger 1986, Susanna & Garcia-Jacas 2007). It contains 600 to 700 species in Southwest and Central Asia. The generic distribution area of *Cousinia* is nearly identical to the Irano–Turanian region (Knapp 1987). Further, the modern geographical range of *Cousinia* correlates almost perfectly with the Irano-Turanian floristic region (Figure 1) (Djamali *et al*., 2012). The overall number of *Cousinia* sections and species is difficult to estimate. However, a careful comparison of the systems of Rechinger (1986) and Tscherneva (1962) leads to an approximate number of 630 species in 70 sections.

In Iran, after *Astragalus*, *Cousinia* is the largest genus, with over 270 species and 43 sections. *Cousinia* species are distributed in mountainous parts of Iran, especially in Zagros and Alborz ranges. Iran is well known for its high diversity in flowering plant flora, with the total number of 8000 species, of which 1800 are endemic (Ghahreman and Attar 1999). This is also reflected by the species richness in the genus *Cousinia*.

There are several morphological, cytogenetical, and molecular studies on some *Cousinia* species (Attar 2011, Djavadi 2012, López-Vinyallonga *et al*., 2010, 2011, Sheidai *et al*., 2006, Susanna *et al*., 2003). However, we found no report on a micro-morphological investigation of leaf characters in the *Cousinia* species of the country.

The leaf epidermis is often a useful character for the classification and identification of taxa such as *Salix* (Chen *et al*., 2008; Ghahremaninejad *et al*., 2012), *Malus* (Ganeva and Uzunova, 2010), *Sibbaldia* (Tahir and Rajput, 2009) and *Rosa* (Wissemann, 2000), and on some genus of Asteraceae (Adedeji and Jewoola 2008).

To the best of our knowledge, no studies have been conducted on leaf epidermal characters of Cousinia. Hence, the current study aims to provide new insights by examining leaf epidermal traits in 31 species within the Cousinia genus. The primary objective is to analyze species relationships using the generated epidermal data. Additionally, a comparison will be drawn between the derived species groups and those resulting from morphometric analyses.

2. Material and methods

Morphological studies were performed on 31 species of *Cousinia* from 3 sections namely: Section *Albidae* (3 species), Section *Cousinia* (8 Species), and Section *Stenocephalae* (20 Species). The voucher specimens have been deposited in Tehran University Herbariums (TUH, Table 1). In total 17 morphological characters including 10 quantitative and 8 qualitative characters were studied (Table 2). These were selected based on the species description available in Flora Iranica (Rechinger 1986), as well as our observations.

In micro-morphological studies, we used stem leaves and considered the abaxial surface of leaves after removing trichomes. Furthermore, dried leaves were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5–15 nm of gold. Subsequently, the coated leaves were examined and photographed with Cam Scan KYKY-EM3200 Electron Microscope.

In total 11 leaves micro-morphological characters were studied, including 8 quantitative and 3 qualitative characters (Table 3). Data were standardized (mean = 0. Variance=1) and used for clustering. Grouping of the species was done by using UPGMA (unweighted paired group using average) clustering method as well as PCA ordination (Podani 2000). Principal components analysis (PCA) was used to identify the most variable morphological and leaf micro-morphological characters. For statistical analysis, we used PAST (2001) software.

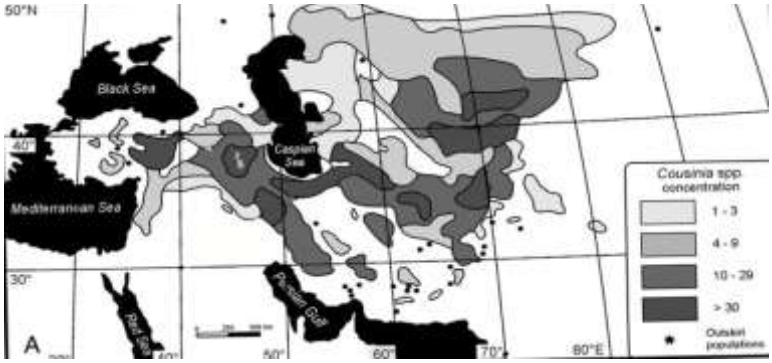


Figure 1. Map showing the distribution of *Cousinia* species richness (After Knapp 1987).

Table1. *Cousinia* species studied and their locations and voucher numbers

R	Taxa	Section	Locality	Abbreviation
1	*Cousinia bachtiarica*	*Albidae*	Lorestan,sepiddasht	bch
2	*C. eriorhiza*	*Albidae*	Hamedan, Assadabad	erz
3	*C. oligocephala*	*Albidae*	Kohkylouy-e-boyerahmad, Sisakht	olg
4	*C. chlorocephala*	*Cousinia*	West Azarbaijan, Khoy	clo
5	*C. hamosa*	*Cousinia*	West azarbaijan, Ziveh	ham
6	*C. microcephala*	*Cousinia*	East Azarbaijan, Sharafkhane	mic
7	*C. seidlitzii*	*Cousinia*	West Azarbaijan, Mahabad	sed
8	*C. tabrisiana*	*Cousinia*	West Azarbaijan, Valadian	tab
9	*C .tenuifolia*	*Cousinia*	West Azarbaijan, Bazargan	ten
10	*C. urumiensis*	*Cousinia*	Kurdestan, Divandareh	urm
11	*C. wilhelminae*	*Cousinia*	West Azarbaijan, Marand	wil
12	*C. aggregata*	*Stenocephalae*	Tehran, Bibishahrbanu	agr
13	*C. alexeenkoana*	*Stenocephalae*	Tehran, Firuzkuh	alx
14	*C. assyriaca*	*Stenocephalae*	Lorestan, Gilavand	asy
15	*C. bijarensis*	*Stenocephalae*	Kurdestan, Dolatabad	bij
16	*C. commutata*	*Stenocephalae*	Mazandaran, Kandovan	com
17	*C. cylindracea*	*Stenocephalae*	Chaharmahal and Bakhtiari, Lordegan	cyl
18	*C. esfandiarii*	*Stenocephalae*	Khorasan, Bojnurd	esf
19	*C. gaubae*	*Stenocephalae*	Alborz, chalus	gub
20	*C. gilanica*	*Stenocephalae*	Gilan, masouleh	gil
21	*C. glaucopsis*	*Stenocephalae*	Tehran, Firuzkuh	glp
22	*C. hypopolia*	*Stenocephalae*	Khorasan, Bojnurd	hyp
23	*C. lucida*	*Stenocephalae*	Lorestan, Azna	luc
24	*C. manouchehrii*	*Stenocephalae*	Esfahan, Ghamsar	man
25	*C. nekarmanica*	*Stenocephalae*	Semnan, Shahrod	nek
26	*C. prasina*	*Stenocephalae*	Gilan, Loushan	prs
27	*C. renominata*	*Stenocephalae*	Khorasan, chenaran	ren
28	*C. stenocephala*	*Stenocephalae*	Kermanshah, Gilane gharb	stn
29	*C. tenuiramula*	*Stenocephalae*	Chaharmahal and Bakhtiari, Borujen	teu
30	*C. thamnodes*	*Stenocephalae*	Esfahan, Ferydonshahr	tam
31	*C. wendelboi*	*Stenocephalae*	Mazandaran, Panjab	wen

Table 2. Morphological characters

R	Character
1	Head number
2	Head length
3	Head wide
4	Head length/ head wide ratio
5	Bract number
6	Floret number
7	Floret length
8	Limb length
9	Tube length
10	Limb length / tube length ratio
11	Stem trichome (0:Glabrous; 1:Low density Arachnoid; 2: Medium density Arachnoid; 3: High density Arachnoid)
12	Leaves color (0: one color; 1: bicolor)
13	Head shape (0:short ovate; 1:ovate; 2:cylindrical)
14	Head trichome (0:Glabrous; 1:Low density; 2: Medium density; 3: High density)
15	Bract shape (0: Imbricate; 1: Erect; 2: Spreading; 3: Curved)
16	Floret color (0: yellow; 1: pink; 2: purple)
17	Anther color (0: yellow; 1: pink)

3. Results and Discussion

UPGMA dendrogram of morphological characters placed representative species of the three sections in separate clusters (Figure 2). Species of the section *Cousinia* were placed in two separate sub-clusters. *C. tabrisiana*, *C. microcephala*, *C. seidlitzii* and *C. urumiensis* showed a higher degree of morphological similarity and formed the first sub-cluster, while the other species studied from this section formed the second sub-cluster.

The species studied from section *Stenocephalae* formed three sub-clusters. *C. bijarensis*, *C. prasina*, *C. assyriaca*, *C. thamnodes*, *C. tenuiramula* and *C. gaubae* comprised the firs sub-cluster. The members of this sub-cluster also showed some similarity to the species of section *Cousinia* and were placed close to them.

C. stenocephalae, *C. renominata*, *C. esfandiarii*, *C. hypopolia*, *C. gilanica*, *C. commutata* and *C. wendelboi* of section *Stenocephalae*, formed the second sub-cluster, while the other studied species of this section formed the third sub-cluster.

Three studied species of the section *Albidae* were placed in a separate cluster, with *C. oligocephala* and *C. bachtiarica* showing a higher degree of morphological similarity.

PCA plot of morphological characters (Figure 3) produced very similar groupings of the studied species, separating section *Albidae* from the other sections. PCA plot also separated the studied species of section *Stenocephalae* into three groups supporting the UPGMA dendrogram result. However, the PCA plot showed some degree of morphological overlap between some species of sections *Cousinia* and *Stenocephalae*.

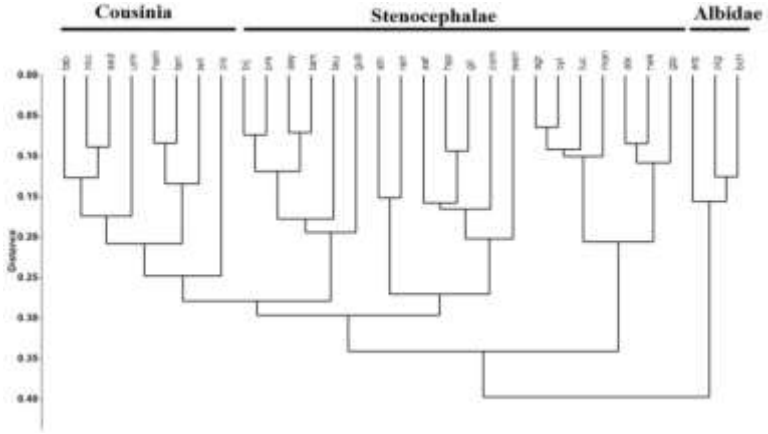


Figure 2. UPGMA tree of morphological data three section of *Cousinia* studied

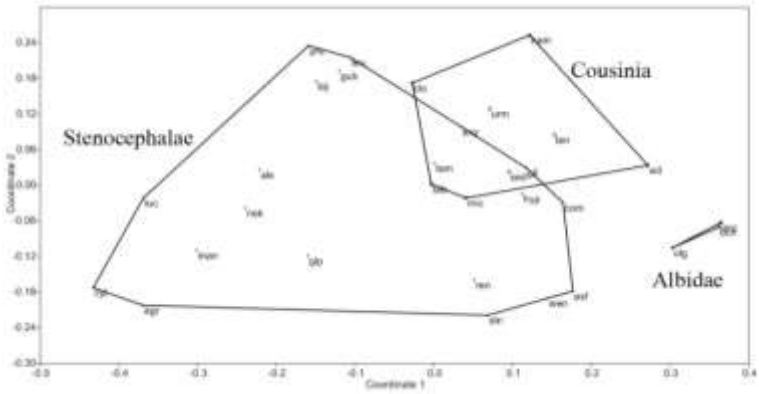


Figure 3. PCA plot of morphological data of three section of *Cousinia* studied

Table 3. Micro-morphological characters

1	2	3	4	5	6	7	8	9	10	11	12
Sect. *Albidae*											
C. *bachtiarica*	110	24(28.17±2.11) 33	16.5(19.97±2.24) 28	1.41	9(13.87±1.75) 18.5	28.5(53.11±11.35) 75.5	16.6(24.85±4.83) 35.3	2.13	1	2	2
C. eriorhiza	210	24.6(28.8±2.74) 36.1	17.83(19.62±1.7) 23.9	1.46	8.7(10.8±1.12) 13.8	24.4(30.89±10.06) 49.1	12.9(18.8±3.99) 27.21	1.64	1	2	2
C. *oligocephala*	145	22(30.9±3.45) 36	17.5(19.32±1.22) 22.3	1.59	11(14.65±2.24) 19	37(59.29±15.02) 87.7	13.5(22.67±4.77) 30	2.61	1	2	2
Sect. *Cousinia*											
C. chlorocephala	217.64	20(26.99±2.43) 31.5	16.2(20.74±2.98) 27	1.30	7(14.9±1.99) 16.3	24(39.87±15.81) 77.8	8.9(14.82±3.79) 24.6	2.68	0	2	2
C. hamosa	207.5	22(26.76±2.77) 33.5	19(23.95±2.57) 29	1.11	6.3(11.25±1.56) 14.2	16.67(35.22±9.2) 51.8	9.1(15.12±3.07) 21.6	2.32	1	0	1
C. microcephala	218.82	20.5(29.97±3.83) 36.7	12.3(22.85±2.98) 27	1.31	6.7(11.46±2.37) 15.9	18(34.01±7.62) 49.4	9.9(15.91±3.21) 21	2.13	1	0	1
C. seidlitzii	210	20(24.96±3.11) 33	14.9(19.74±2.55) 24.5	1.26	6.5(10.14±1.81) 12.5	16.55(35.52±9.82) 53.7	9(15.26±3.4) 22.4	2.32	1	0	1
C. tabrisiana	231.17	16.2(27.08±4.41) 35.7	16.2(22.03±2.76) 29.7	1.22	6.7(12.26±2.06) 15.3	18(37.71±11.20) 67	14(16.79±3.52) 24.5	2.24	1	0	2
C .tenuifolia	290	24.6(30.25±2.91) 35.6	20(25.57±2.65) 32	1.18	7.5(11.41±1.89) 16	16.2(37.41±9.54) 52	9.7(16.2±3.45) 22.5	2.3	1	0	1
C. urumiensis	189.41	22.2(29.46±3.80) 37	19.9(24.43±2.31) 28.5	1.20	9.6(12.45±1.56) 15.2	16(33.62±8.21) 52	8.9(14.74±3.66) 21	2.28	1	0	1
C. wilhelminae	205	24(27.10±3.64) 33.5	15(18.64±2.42) 24.5	1.45	9.5(13.48±2.48) 18	22(34.80±9.26) 60	12.7(17.77±4.35) 28.5	1.95	1	1	2
Sect. *Stenocephalae*											
C. aggregata	182	19(29.95±3.88) 36.7	15.8(22.51±2.58) 28.7	1.33	6(10.63±2.08) 14.3	24.3(47.9±10.78) 60.5	12.5(21.13±6.00) 40.2	2.26	1	1	2
C. Alexeenkoana	124.95	25.6(34.37±4.44) 45	17.5(25.44±4.18) 35	1.35	13.8(16.16±2.20) 19.5	23.1(44.95±9.28) 68.6	14.3(20.5±4.86) 30.1	2.19	1	1	1
C. assyriaca	219.3	16.3(25.65±3.15) 31.5	15(20.42±2.61) 24.5	1.25	5(13.5±2.37)15.2	24(39.94±10.04) 63	9.6(21.95±4.74) 29.9	1.81	0	2	1
C. bijarensis	217.1	24(27.46± 3.23) 39.6	17.1(20.7±2.31) 26.6	1.32	11(14.1±1.99) 19.3	22(39.74±11.83) 64.5	15.1(22.95±4.87) 34.7	1.73	1	2	2
C. commutata	132.6	22(26.99±3.45) 34	16(19.75±2.80)25.4	1.36	7.5(10.92±1.66) 14	18(47.5±9.99) 68.3	8.6(15.58±4.83) 32	3.02	1	1	1
C. cylindracea	183.6	24.7(31.15±3.30) 36	19.5(23.47±2.11) 28	1.32	7(11.22±2.37) 18.6	24(48.41±13.26) 75.6	11.3(19.10±4.24) 35	2.53	1	2	1
C. Esfandiarii	149.3	17(25.7±4.88) 29	14(18.51±4.88) 29	1.38	6(13.98±2.43) 12.5	25.1(41.2±9.17) 60.56	8.2(16.7±4.9) 31.8	2.45	1	0	0
C. Gaubae	394.1	15(23.33±3.69) 31	13(17.97±2.51) 23.4	1.29	6(11.3±2.43) 14.8	16(22.33±3.22) 29.7	11(16.92±3.56) 23	1.32	1	1	2
C. gilanica	172.2	24.1(31.4±2.98) 35.9	17(22±2.7) 26.6	1.42	7.3(12.87±2.2) 15.1	25.3(38.57±10) 64.2	15.2(20.42±4.1) 30	1.93	1	1	1
C. glaucopsis	163.5	24.9(32.8±3.34) 36.7	19.7(24.1±2.2) 27.8	1.37	12.3(16.1±2.44)18.9	26(44.2±9.16) 61.3	11.4(19.55±4.65) 37.2	2.26	1	2	1
C. hypopolia	157.6	21.2(26.2±2.94) 40	17.3(19.14±3.15) 30	1.37	12(16.1±1.80) 20	18(41.89±11.77) 66	6.5(17.1±6.50) 36.7	2.44	0	1	0
C. lucida	179.5	24.1(30.90±3.55) 39	21(25.1±2.24) 30.7	1.22	6.8(11.02±2.44) 16	22.2(48.08±13.78) 72	11.6(21.98±5.66) 37	2.18	1	1	0
C. Manouchehrii	133.8	25(32.93±4.50) 41	16(22.19±2.64) 27	1.48	10.5(13.53±1.63) 17	29(58.39±16.71) 93.5	9.5(18.01±5.31) 27	3.24	1	2	1
C. nekarmanica	109.9	16.1(31.43±5.72) 40	16(23.34±4.09) 30.1	1.34	10.1(15.69±3.14) 22	32(46.1±9.87) 72.5	14(21.1±6.84) 40	2.19	1	2	1
C. prasina	118.82	28.1(34.79±3.01) 40.3	20(22.56±2.14) 29	1.54	8(14.46±3.43) 19.8	12.5(43.42±10.36) 60	9.8(18.49±5.26) 35	2.34	1	2	1
C. renominata	122.35	19.5(26.79±2.96) 31.3	10.2(21.15±3.54) 31	1.26	9(11.86±2.20) 18	14.5(34.67±7.79) 49	9.5(16.46±6.87) 34	2.1	0	2	1
C. Stenocephala	273.9	19(24.09±2.99) 31	14.5(19.1±2.38) 24.5	1.26	4(9.08±1.39) 11.2	19.7(35.22±10.28) 52	11.5(17.76±6.42) 32.4	1.98	1	0	1
C. tenuiramula	73.71	26(31.92±2.55) 36	21(23.9±2.18) 29.5	1.33	10(13.52±1.80) 16	22(49.87±14.11) 78.5	9.4(18.38±3.99) 26	2.71	1	1	1
C. thamnodes	160.29	23(28.68±3.16) 34.5	19(21.22±1.67) 25	1.35	6(8.24±1.68) 11.5	21(49.38±13.34) 79	13(17.70±4.98) 31	2.78	1	2	2
C. wendelboi	178.5	20.5(29.32±3.15) 36.4	18.8(24.9±3.14) 31	1.17	7.3(13.91±2.19) 17	14(35.07±11.80) 65.5	8(11.93±2.64) 20	2.94	0	1	1

Column1: Species; Column2: Stomata density (n/mm^2); Column 3: Stomata cells length; Column 4: Stomata cells wide; Column 5: Stomata cells length/ wide ratio; Column 6: Stomata pore length; Column 7: Epidermal cells length; Column 8: Epidermal cell wide; Column 9: Epidermal cell length/wide ratio; Column 10: Epidermal wall shape (Puzzle shape:0 & Curve shape:1); Column 11: Distinction of epidermal cells (0: Low distinct, 1: Distinct & 2: High Distinct); Column 12: Deep of stomata (0: On surface, 1: Semi depressed & 2: Depressed)

C. tabrisiana, C. seidlitzii and *C. microcephala* of section *Cousinia* and *C. assyriaca, C. gilanica* and *C. thamnodes* of section *Stenocephalae* showed similar morphological characters like: presence of bicolor leaves, the number of head, the density of head trichome, the floret number and length. These results clearly showed difficulties in the taxonomy of the group and a lack of clear-cut separation of the studied species.

PCA analysis of morphological characters revealed that the first three PCA components comprised about 72% of the total variation (Table 4). Morphological characters like trichome cover, present leaves bicolor, head number, head wide, head length/wide ratio, head shape, floret number, and floret length had the highest value of correlation with these components and are the most variable morphological characters among the studied species. The Combination of these characteristics separated *Cousinia* species of the studied sections.

Table 4. PCA Components based on Morphological data of taxa (ANOVA: P<0.05)

Morphological Characters	Eigenvalue	%Variance
Head Number	0.0451	41.595
Head Length	0.0187	18.121
Head Width	0.0126	12.313
Head Length/Width Ratio	0.0078	7.2922
Involucral Bracts Number	0.0048	4.6782
Florets Number	0.0038	4.4830
Florets Length	0.0027	3.058
Corolla Limb Length	0.00124	1.978
Corolla Tube Length	0.00110	1.4325
Limb Length / Tube Length Ratio	0.00079	0.932

3. 1. Micro-morphometry of leaf

PCA analysis of Micro-morphological characters revealed that the first three PCA components comprised about 86.8% of the total variation (Table 5). PCA plot of species relationship based on leaf micro-morphological character is presented in Figure 4 This plot also separates the species of the studied sections from each other and is in good agreement with morphological results.

C. chlorocephala differed from the other species of the section *Cousinia* and had a maximum Head length/width ratio and absence of trichome on the stem.

Table 5. PCA Components based on Micromorphological data of taxa (ANOVA: P<0.05)

Micromorphological Characters	Eigenvalue	%Variance
Epidermal Cell Length	0.0183	50.825
Epidermal Cell Width	0.0074	20.781
Epidermal Cell Length/ Width Ratio	0.0055	15.259
Stomata Length/Width Ratio	0.0035	6.7621
Stomata Length	0.0011	1.6701
Stomata Width	0.0008	1.3732
Epidermal Cell Shape	0.0004	0.9831
Density of Stomata	0.0001	0.4781

This species differed greatly in its micro-morphological characters too by having puzzle-shaped epidermal cell wall (curved shape in other species of this section), the largest stomata pore size, and the longest epidermal cell length.

In both morphological and micro-morphological analyses, *C. chlorocephala* was separated from the other species of section *Cousinia*. PCA plot based on combined morphology and leaf micro-morphology (Figure 5 and 6) separated *C. chlorocephala* far from other species of the section *Cousinia*.

The presence of characters like head length/ head-to-width ratio, limb/tube of floret ratio, epidermal cell length, and epidermal cell wall shape, bring *C. chlorocephala* closer to the species of section *Stenocephalae*.

Section *Stenocephalae* is one of the largest sections of the genus *Cousinia.* Seven species of this section showed affinity to each other and formed a separate cluster based on micro morphological and morphological characters.

Cousinia aggregata, C. alexeenkoana, C. cylindracea, C. glaucopsis, C. lucida, C. manouchehrii, and C. nekarmanica

showed similarity in characters: head bracts shape, epidermal wall shape, stomatal cell length and wide (Largest stomata). Moreover, they are different from the other species of the section *Stenocephalae* by characters: head number, head shape, and head length/width ratio (maximum size).

The palynological study also showed that these species differed from the other species of this section (Saber *et al*., 2009). It seems this species formed a subsection in section *Stenocephalae*. SEM micrograph of abaxial surface of Cousinia leaves species are shown in pictures 1-31.

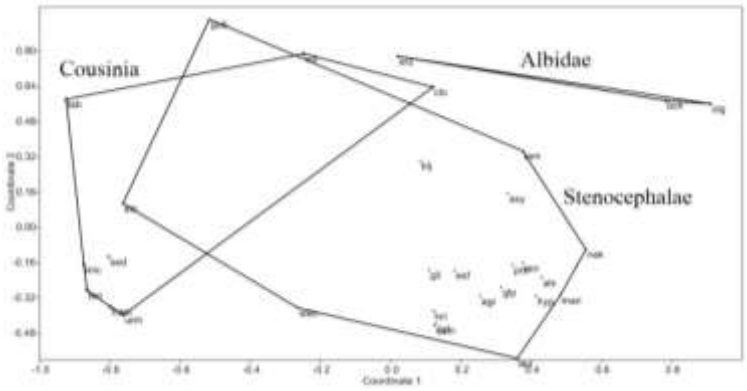


Figure 4. PCA plot of micro-morphological data of three section of *Cousinia* studied

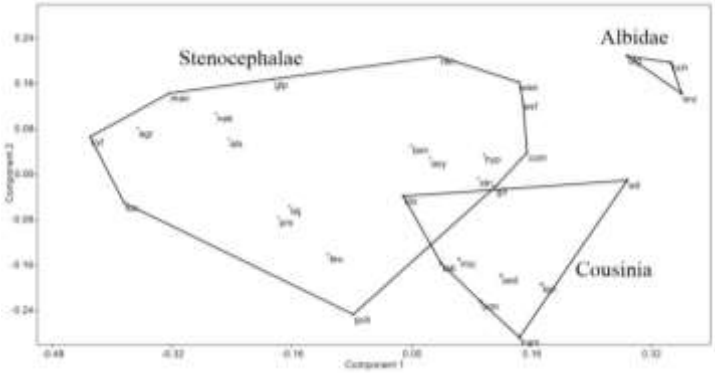


Figure 5. PCA plot of micro-morphological and morphological characters in three sections of *Cousinia* studied

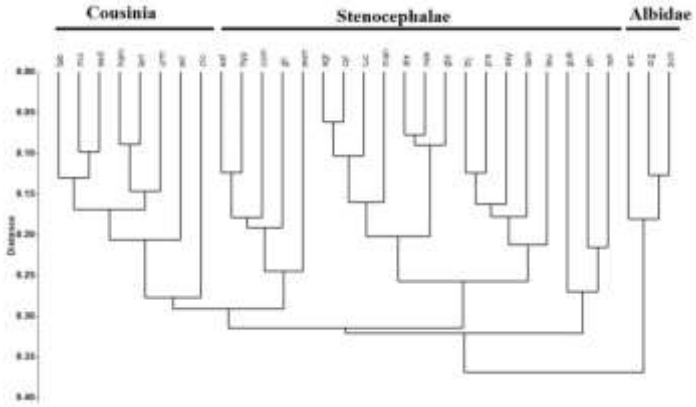
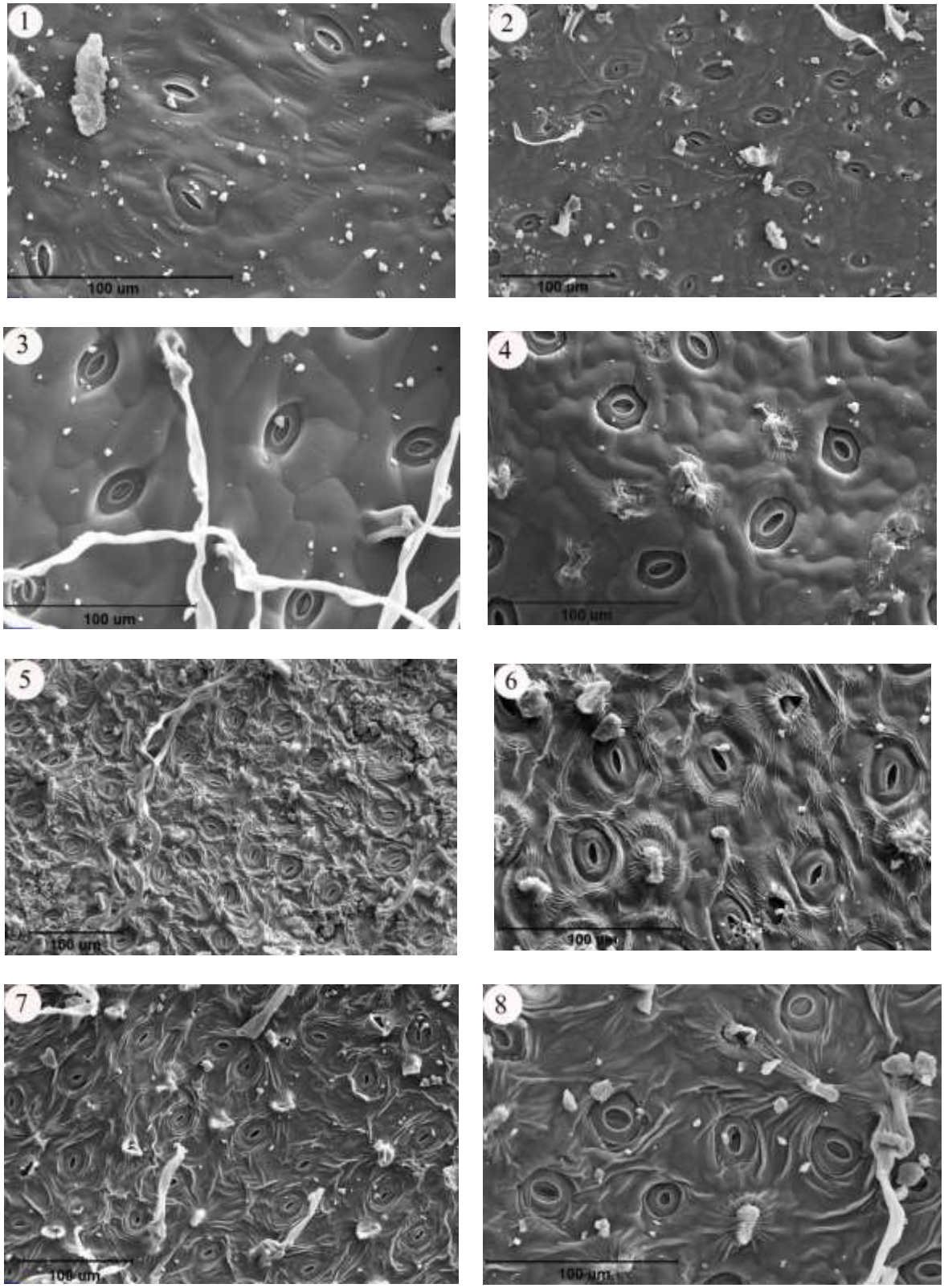
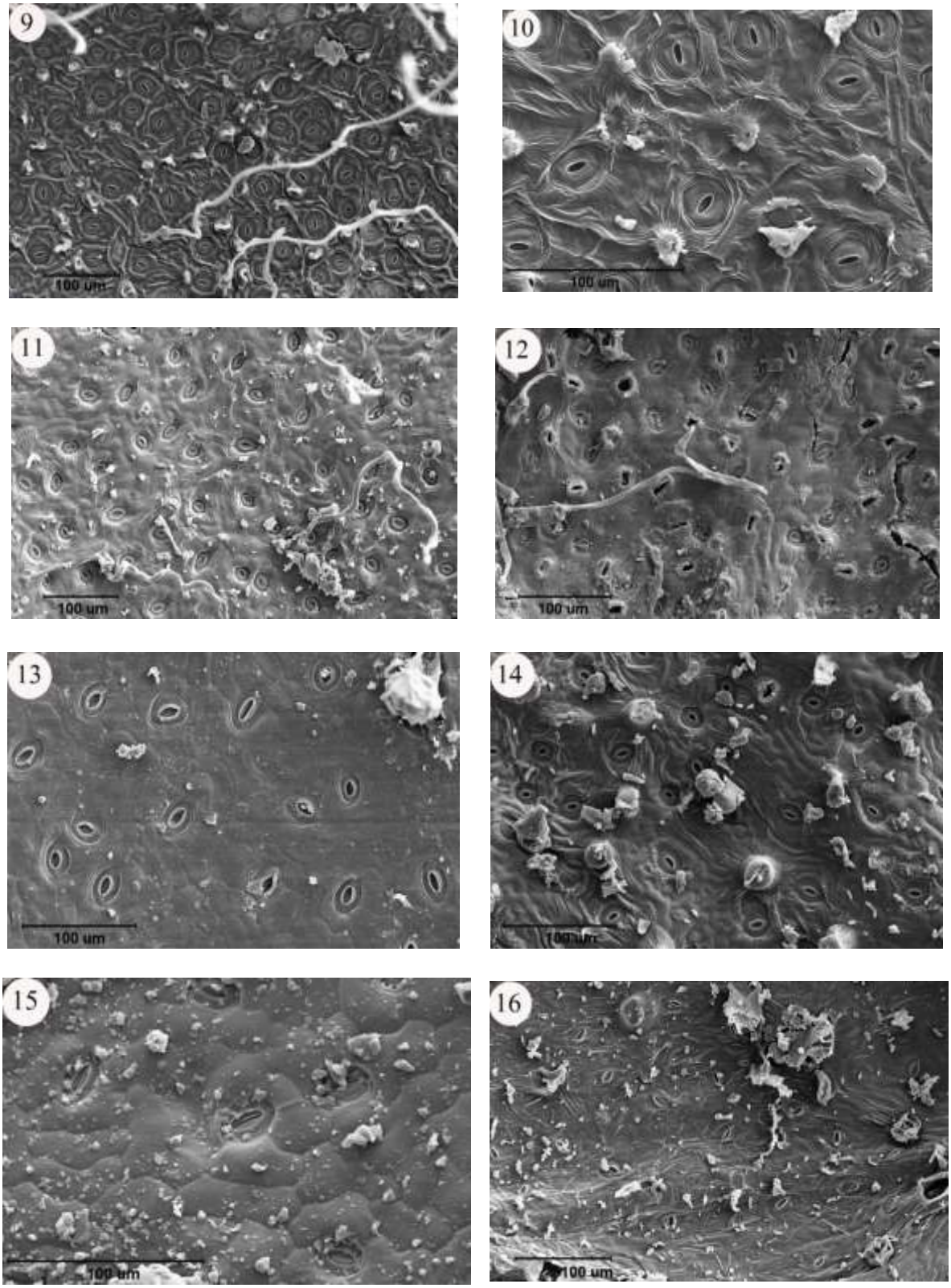


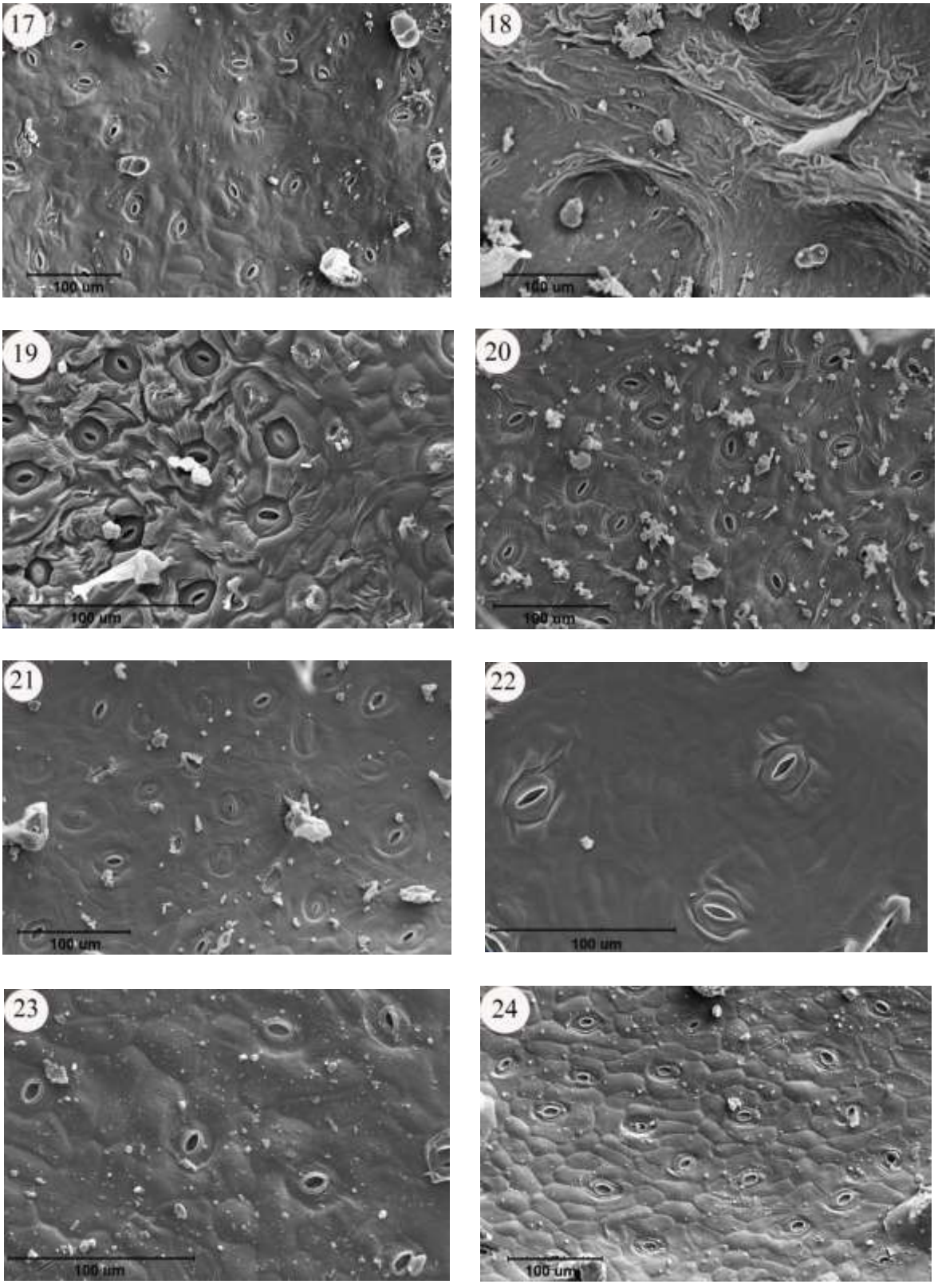
Figure 6. UPGMA tree of micro-morphological and morphological characters in three sections of *Cousinia* studied



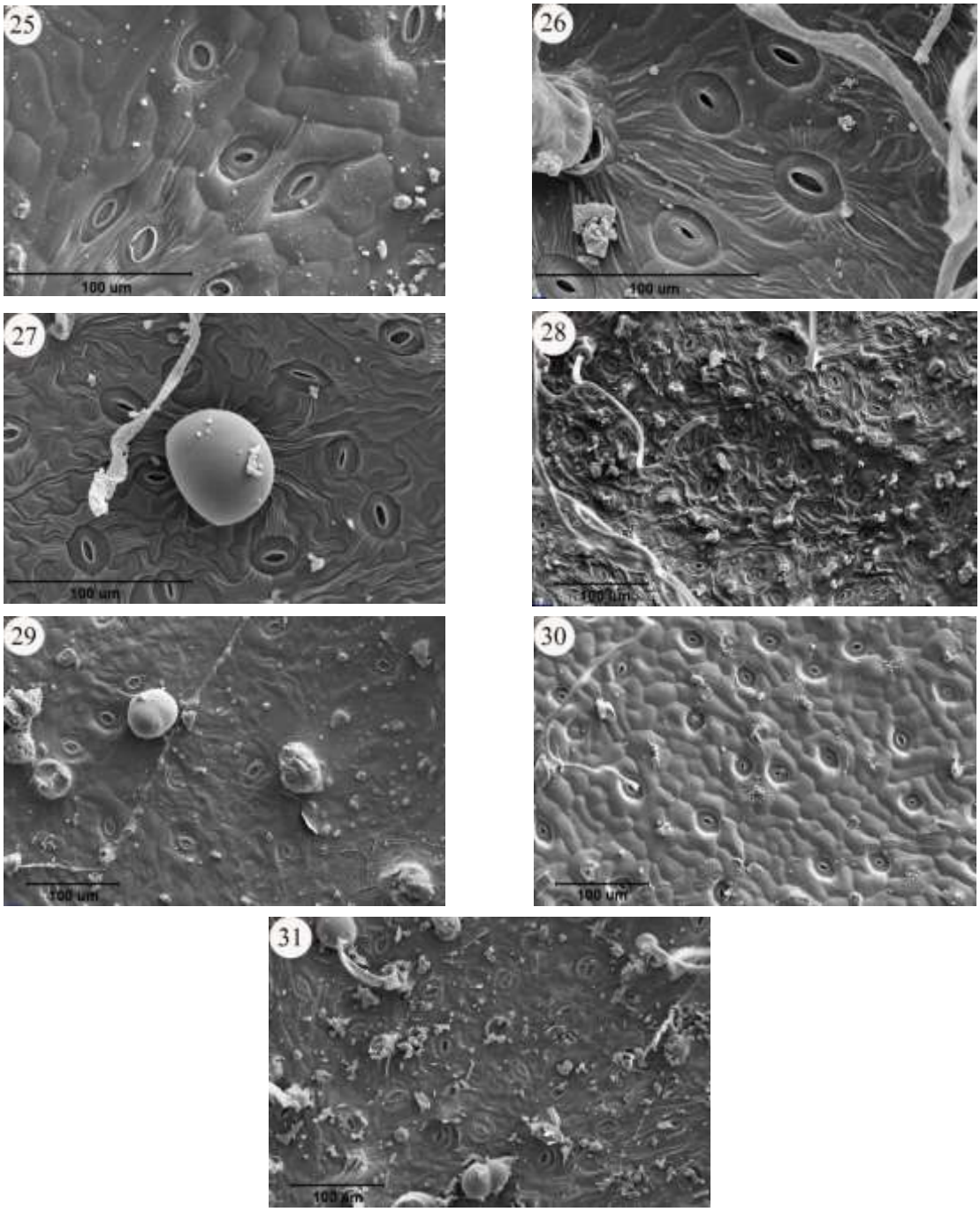
Pics. 1-8. SEM micrograph of abaxial surface of *Cousinia* leaves species. (1) *C. bachtiarica*, (2) *C. eriorhiza*, (3) *C. oligocephala*, (4) *C. chlorocephala*, (5) *C. hamosa*, (6) *C. microcephala*, (7) *C. seidlitzii*, (8) *C. tabrisiana*. Scale bare = 100 μm



Pics. 9-16. SEM micrograph of abaxial surface of *Cousinia* leaves species. (9) *C. tenuifolia*, (10) *C. urumiensis*, (11) *C. wilhelminae*, (12) *C. aggregata*, (13) *C. alexeenkoana*, (14) *C. assyriaca*, (15) *C. bijarensis*, (16) *C. commutata*. Scale bare = 100 µm



Pics. 17-24. SEM micrograph of abaxial surface of *Cousinia* leaves species. (17) *C. cylindracea*, (18) *C. esfandiarii*, (19) *C. gaubae*, (20) *C. gilanica*, (21) *C. glaucopsis*, (22) *C. hypopolia*, (23) *C. lucida*, (24) *C. manouchehrii*. Scale bare = 100 µm



Pics. 25-31. SEM micrograph of abaxial surface of *Cousinia* leaves species. (25) *C. nekarmanica*, (26) *C. prasina*, (27) *C. renominata*, (28) *C. stenocephalae*, (29) *C. tenuiramula*, (30) *C. thamnodes*, (31) *C. wendelboi*. Scale bare = 100 μm

4. Conclusion

The current study demonstrates that employing both micro-morphological and morphological characters for analysis yields a more accurate representation of species relationships compared to relying solely on morphological data analysis. The findings suggest that, based on the results indicating species relationships using the complete set of characteristics within this taxon, there is a need for revisiting the classification. Moreover, to enhance the level of certainty, further molecular studies in this field are required.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

Data Availability

Data will be made available on request.

References

Adedeji, O., & Jewoola, O.A. (2008). Importance of leaf epidermal characters in the Asteraceae family. Not. Bot. Hort. Agrobot. Cluj, 36 (2), 7-16.

Attar, F. (2011). A taxonomic revision of *Cousinia* Cass., sect. Albidae Bunge (Asteraceae) in flora of Iran. *Iranian Journal of Botany*, 17 (2), 150-157.

Chen, J., Sun, H., & Yang, Y. (2008). Comparative morphology of leaf epidermis of Salix (Salicaceae) with special emphasis on sections Lindleyanae and Retusae. *Botanical Journal of the Linnean Society*, 157, 311-322.

Djamali, M., Baumel, A., Brewer, S., Jackson, S.T., Kadereit, J. W., López –Vinyallonga, S., Mehregan, I., Shabanian, E., & Simakova, A. (2012). Ecological implications of *Cousinia* Cass. (Asteraceae). persistence through the last two glacial-interglacial cycles in the continental Middle East for the Irano-Turanian flora. *Elsevier. Review of Palaeobotany and Palynology*, 172, 10-20.

Djavadi, S.B. 2012. NEW chromosome counts in *Cousinia* (Asteraceae, Cardueae) sections Cynaroideae anf Stenocephalaeae from Iran. - Iranian Journal of Botany. 18(1): 86-93.

Frodin, D.G. 2004. History and concepts of big plant genera. - Taxon 53 (3): 753-776.

Ganeva, T., & Uzunova, K. (2010). Comparative leaf epidermis study in species of genus *Malus* Mill. (Rosaceae). *Botanica Serbica*, 34 (1), 45-49.

Ghahreman, A., & Attar, F. (1999). Biodiversity of plant species in Iran, vol. 1. Tehran Univ. Press.

Ghahremaninejad, F., Khalili, Z., Maassoumi, A., Mirzaie, H., & Riahi, M. (2012). Leaf epidermal features of *Salix* species (Salicaceae) and their systematic significance. *American Journal of Botany*, 99(4), 769-777.

Knapp, H.D. (1987). On the distribution of genus *Cousinia* (Compositae). *Plant Systematic and Evolution*, 155, 15-25.

López-Vinyallonga, S., Susanna, A., & Garcia-Jacas, N. (2010). Chromosome counts in the genera *Cousinia*, *Olgaea* and *Syreitschikovia* (Compositae). *Folia Geobotanica*, 45, 201-214.

Podani, J. (2000). Introduction to the Exploration of Multivariate Data English translation, Backhuyes publisher, Leide, 407pp.

Rechinger, K.H. (1986). *Cousinia:* morphology, taxonomy, distribution and phytogeographical implication. Proceedings of the Royal Society. Edinburgh. Section B. Biological Sciences. 89:, 45-58.

Saber, A., Attar, F., & Djavadi, S.B. (2009). Studies of pollen grains in the Sect. Stenocephalae (Cousinia Cass.-Asteraceae) in Iran. *Iranian Journal of Botany*, 15 (1), 114-128.

Sheidai, M., Mehdigholi, K., Ghahreman, A., & Attar, F. (2006). Cytogenetic study of the genus *Cousinia* (Asteraceae, section Serratuloideae) in Iran. *Genetic and Molecular Biology*, 29, 117-121.

Susanna, A., & Garcia-Jacas, N. (2007). Tribe Cardueae Cass. (1819) in: Kadereit, J. W. & Jeffrey, C. (volume ed.), The Families and Genera of Vascular Plants. *Berlin, Heidelberg: Springer Verlag*, 8, 123-147.

Susanna, A., Garcia-Jacas, N., Vilatersana, R., Garnatje, T., Vallès, J., & Ghaffari, S.M. 2003. New chromosome counts in the genus *Cousinia* and the related genus Schmalhausenia (Asteraceae, Cardueae). *Botanical Journal of the Linnean Society*, 143, 411-418.

Taher, S., & Rajput, M. (2009). S.E.M. structure distribution and taxonomic significance of foliar stomata in *Sibbaldia* L. species (Rosaceae). *Pakistan Journal of Botany*, 41(5), 2137-2143.

Tscherneva, O.V. (1962). *Cousinia* Cass.Pp.108-357 in: shishkin, B.K. (ed.), Flora of the USSR, Vol. 27. Leningrad: Akademiya Nauk.

Wissemann, V. (2000). Epicuticular wax morphology and the taxonomy of *Rosa* (section Caninae, subsection Rubiginosae). *Plant Systematic and Evolution*,221,107-112.