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USE OF POLLEN MORPHOLOGY TRAITS FOR IDENTIFYING SPECIES OF CENTAUREA L. ASTERACEAE IN KURDISTAN-IRAQ

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ABSTRACT:
The polleniferous material of *Centaurea taxa* grow in Kurdistan-Iraq was collected. Samples were tested using scanning electron microscopy (SEM) and light microscopy (LM). In the polar view, pollen grain outlines are mostly triangular or triangular-circular. In equatorial view, the elliptic shape is common, while circulars or semicircular shapes are rare. The exine sculpture is scabrate, and the tectum is perforate. Number of sculpture microspinules/ μm^2, and their heights together with the number of wall perforations provide significant characters for taxa delimitation. Pollen grains of all species are of medium size, very few of some species pollen are larger or smaller in size. Species producing subprolate pollen shape is common, while oblate spheroidal and Prolate spheroidal shapes are less common. The principal component analysis and the discriminant analysis can recognize 65.478% and 87.54% of the total variability respectively; moreover, the discriminant function can correctly classify 125 or 78.125% out of 160 observations for pollen traits.

KEYWORDS: *Centaurea* L., Microspinules, Suboblate, Prolate, *Centaurea regia*.

1. INTRODUCTION

Centaurea L. is one of the largest genera of Asteraceae with 250-700 species of herbaceous annual, biennial and perennial plants (Bancheva et al., 2014; Erkara et al., 2012; Shabestari et al., 2013; Susanna & Garcia-Jacas, 2007; Tasar et al., 2018). Species of the genus are found in the northern Hemisphere, mostly in the Eastern part of the Hemisphere; the Middle east and nearby areas is species-rich. Distribution is predominantly of old world ranges (Bancheva et al., 2014; Bremer, 1994; Dittrich, 1977).
The laboratories dealing with palynological traits increased the diversity of pollen grains and using their results in plant taxonomy. Such laboratories increased dramatically our knowledge on morphological diversity of pollen grains and spores than previously, but their influence on plant taxonomy is still less than it should have done. Some of the reasons why the taxonomic impact of palynology has been less than it could have been are identified. Firstly, studies of pollen have not always been integrated with the results obtained from other kinds of studies (Blackmore & Barnes, 1991). Secondly, modern taxonomic methods of character analysis have often not been used. Therefore, palynology has tended to be left behind while systematics has moved on. According to Blackmore (1996), there are two more factors that tend to reduce the impact of palynology on systematic: the complex terminology of the subject (Davis & Heywood, 1963) and the extreme complexity of circumscribing comparative characters based on pollen grains morphology.
The knowledge of pollen morphology can be used as an instrument of multiple scientific researches in systematic botany, paleobotany, paleoecology, pollen analysis, aeropalynology, criminology, allergy, stratigraphic correlation of oil-bearing rocks and coal fields, drugs in the field of medicopalynology and improvement of honey in the field of mellittopalynology and copropalynology (Meo & Khan, 2003).

Palynology is one of the most widely used research tools in quaternary studies (Edwards, 1983). It plays an important role in plant systematic studies. Moreover, as indicated by (Edwards & Macdonald, 1991; Prentice, 1988) data derived from pollen studies can be used to provide an indication as to the response of natural vegetation to human impacts through history, and to climatic and environmental changes. Huntley (1990) recorded data for pollen strengthen predictions of how vegetation is likely to respond to future climatic conditions, thereby providing an indication of the future agricultural and silvicultural potential of various regions.
Pollen morphology provides significant evidences to support the separation of the taxa at different levels of hierarchy. For example pollen provides the primary basis for recognition of subfamilies in the Labiatae, but at the tribal level only the Ajugeae has distinctive pollen. Several genera, notably *Collinsonia*, *Salvia*, *Teucrium*, and *Trichostema*, have pollen that is very different from other genera. At the infrageneric level, pollen provides valuable taxonomic characters in several genera, notably *Hyptis, Monardella, Salvia, Stachys, Teucrium*, and *Trichostema* (Binzat et al., 2014).
Pollen morphology of 19 taxa of the *Centaurea* in Iran was investigated by light and scanning electron microscope (SEM). Examination showed pollen grains to be tricolporate, isopolar, radially symmetrical, oblate spheroidal, prolate spheroidal, subprolate, tectum perforate and scabrate. Based on the exine ornamentation, two types of pollen grains were recognized: 1. dense acute spinules and 2. sparse spinules (Shabestari et al., 2013).
Some endemic Turkish species (*Centaurea antalyense* H. Duman & A. Duran, *C hierapolitana* Boiss., Heimerl, *C. lycia* Boiss., *C. tossiensis Freyn* and *C. wagenitzii* Hub.-Mor) were studied under light and electron microscopy (SEM), investigations showed that the pollen grains are more or less

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spheroidal-subprolate, tricolporate and the exine sculpture is tecate-scabrate (Erkara et al., 2012).
The aim of this study in Kurdistan-Iraq is to provide additional knowledge about the pollen morphology of the taxa under investigation and to determine the extent to which the data can be used as taxonomic character in discriminating between taxa.

2. MATERIALS AND METHODS

Flowering buds in the stage just before anthesis were obtained from living plants in the field, of all *Centaurea* species growing widely in Kurdistan region. Plant samples were dried in the laboratory conditions, and kept at 4Cº for subsequent use. Preparation of the slides of pollen grains was mounted in methyl green glycerine jelly; large chunks of pollen grains may be separated if necessary by stirring (1g methyl green dissolved in 100 ml alcohol 95%) (Radford et al., 1974).
The slides were observed by light microscope and photographed using digital Sony camera (18.2 mega-pixels). Morphological observations, including polar length (P) and equatorial diameter (E), P/E ratio, number of apertures, mesocolpium in the equatorial view, apocolpium, colpus length, exine thickness, apertural shape, mean, range and standard deviation for each character was based on 30 observations for each species, Size classes of (Erdtman, 1945) were assessed, while shape classes of (Erdtman, 1952) were followed. The pollen terminology was followed by Punt et al., (2007) and Erdtman, (1971). The samples were examined and photographed by using scanning electron microscope model TESCAN MIRA 3.

3. RESULTS AND DISCUSSION

The pollen grains are isopolar, radially symmetrical, tricolporate, monad, Characters of the studied pollen grains of 16 *Centaurea* species using LM and SEM are listed in Table (3) and shown in figures (3-10).
In the polar view, pollen grain outlines are mostly triangular or triangular-circular and the colpi are acute at both ends. In equatorial view, the elliptic shape is abundant, no triangular are present, while circulars or semicircular are fewer. Tetrangular pollen shapes are only observed in polar view of both *C. persica* and *C. tomentella*. In all species examined, the ectoaperture is bigger than the endoaperture. The exine is consisting of two layers, well visible in LM.
Under LM and SEM, the exine sculpture is scabrate, psilate towards the colpi and the tectum is perforate, respectively (Figures 3-10). Pollen grains sculpture is spinulose (echinute) with spines or microspinules. Scanning electron micrograph of a pollen grain of *C. aggregata*, *C. behen*, *C. carduncul*us, and *C. tomentella* showing colpi with lalongate ora (Figures 3, 4, 5, 10), although a common structure, but is useful for identification of number of Iraqi *Centaurea* species.
According to number of spines or microspinules the following groups can be formed Table 5:
1. Low density microspinules (spinules), ≤1/ µm²: *C. solstitialis, C. cyanus, C. gudrunensis, C. balsamita, .C. bruguierana*.
2. Medium density microspinules, > 1 ≤ 2/µm²: *C. hyalolepis, C. carduncul*us, *C. behen, C. rigida, C. persica, C. regia.*
3. High density microspinules > 2/ µm²: *C. iberica, C. virgata, C. aggregata, C. gigantea, C. tomentella.*
Based on the number of perforation in the pollen grain tectum, the following groups may be formed:
1. Low number of wall perforations, < 4 /µm²: *C. iberica, C. solstitialis, C. hyalolepis, C. carduncul*us, *C. rigida, C. tomentella, C. persica.*
2. Medium number of wall perforations, ≥ 4 to < 4.5/µm²: C. regia, *C. gudrunensis, C. balsamita, C. bruguierana*.
3. High number of wall perforations, ≥4.5/ µm²: *C. cyanus, C. behen, C. gigantea, C. virgata, C. aggregata.*
Based on the height of microspinules (spinules), three types are observed:
1. Very short microspinules < 0.300 µm: *C. cyanus, C. persica.*
2. Medium long microspinules, ≥ 0.300 µm to < 0.500µm: *C. iberica, C. hyalolepis, C. regia, C. carduncul*us, *C. behen, C. gudrunensis, C. rigida, C. gigantean, C. tomentella, C. bruguierana. C. virgata, C. balsamita, C. balsamita.*
3. Very long microspinules, < 0.500 µm: *C. aggregata*
Using Anova (Table 3) for testing pollen grain traits proved to be highly significant under 1% level of probability.
Dimensions of the polar (P) axis ranges from 22.183 µm (*C. aggregata*) to 58.483 µm long (*C. gigantea*), with the overall average of 35.949 µm, while dimensions of the aquatorial (E) axis is found to range from 19.090 µm (*C. bruguierana*) to 59.964 µm (*C. persica*) with the average of 32.535 µm. The polar length/the equatorial diameter ranges between 0.473 (*C. persica*) to 1.733 (*C. behen*). The average is 1.141. In case of the apoculpium, the minimum value is 26.989 µm (*C. virgata*) to 57.228 (*C. carduncul*us), with average of 39.400 µm. The mesoculpium varies between 6.619 µm (*C. gudrunensis*) and 27.480 µm (*C. persica*) with the average of 15.357 µm. The colpus length is found to be 15.457µm (*C. rigida*) to 35.611µm (*C. persica*) and the average of 24.197 µm. the minimum exine thickness is 2.769 µm (*C. aggregata*), while the maximum is 4.697 µm (*C. balsamita*) with the average of 3.377 µm.
According to (Erdtman, 1945), about 98 % of all investigated species, are medium sized, very little number of the total pollen grains (1.25%) of species *C. cyanus, C. carduncul*us, *C. behen, and C. gigantea* are found to contain some large-sized pollen, and only 0.312% of *C. virgata* pollen grains for (Erdtman, 1945) are considered small.
According to pollen shapes of (Erdtman, 1971) all *Centaurea* species of Kurdistan region could be divided into the following groups:

1. Subprolate:

C. iberica	P/E = 1.290
*C. carduncul*us	P/E = 1.301
C. behen	P/E = 1.299
C. rigida	P/E = 1.175
C. tomentella	P/E = 1.146
C. bruguierana	P/E = 1.209
C. aggregata	P/E = 1.226

2. Oblate spheroidal:

C. solstitialis	P/E = 0.953
C. hyalolepis	P/E = 1.005
C. cyanus	P/E = 0.901
C. regia	P/E = 0.970

3. Prolate spheroidal:

C. gudrunensis	P/E =1.143
C. balsamita	P/E = 1.086
C. virgata	P/E = 1.053

4. Suboblate:

C. persica	P/E = 0.878

5. prolate:

C. gigantea	P/E = 1.423

Morphological characters of our *Centaurea* species, regarding grain shape and exine pores and sculpture are in concomitant with results obtained by (Shabestari et al., 2013) on the pollen grain of species grows in Iran and some of those local endemic species investigated by (Erkara et al., 2012) in Turkey.

Use of multivariate analysis for distinguishing studied *Centaurea* species

Principal component analysis

Two components accounting for 65.477% of the variability in the Original data are extracted. The projection of the 7 studied pollen grain characters (Table 1) onto the plane defined by the 1-2 component planes show that the first principal component is influenced strongly by equatorial diameter and apocolpium length, while the second principle component is highly influenced by P/E ratio. Therefore the mentioned pollen characters are expected to determine the species.

Table 1. Principal Components Analysis, components extracted rising 160 complete cases for 7 variables

Component number	Eigen value	Percent of variance	Cumulative Percentage
1	3.04282	43.469	43.469
2	1.54061	22.009	65.478
3	0.949065	13.558	79.036
4	0.721919	10.313	89.349
5	0.503371	7.191	96.540
6	0.209464	2.992	99.532
7	0.032748	0.468	100.000

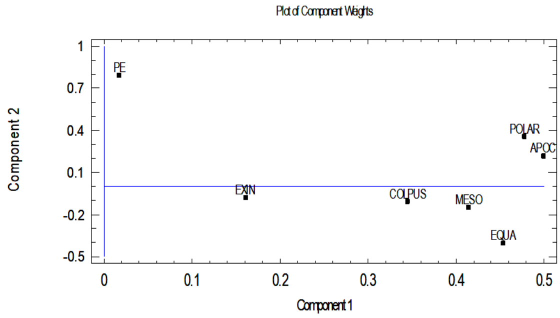

Figure 1. Principal component analyses for 7 pollen grain characters.

The first two discriminant functions (in the table 2) below account for 87.54% of the total variability, when pollen grains classified using discriminant functions, it appears that large number of pollen is assigned correctly to their original taxa. Results indicate that amongst the 160 observations used to fit the model, 125 or 78.125% were correctly classified. The projection of pollen characters onto the plane defined by the 1-2 discriminant functions indicates clear distinction of species (Figure 2).

Table 2. Discriminant analysis, components extracted rising 160 complete cases for 7 variables

Discriminate function	Eigen value	Relative percentage	Canonical correlation
1	16.0343	76.12	0.9702
2	2.40589	11.42	0.84047
3	1.32664	6.3	0.75511
4	0.633229	3.01	0.62267
5	0.324592	1.54	0.49503
6	0.313029	1.49	0.48826
7	0.027660 3	0.13	0.16406

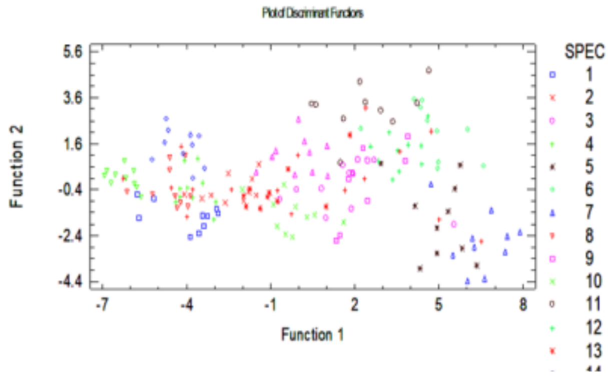

Figure 2. Discriminant analysis for identifying species.

Keys: 1. *Centaurea aggregata*, 2. *Centaurea balsamita*, 3. *Centaurea behen*, 4. *Centaurea bruguierana*, 5. *Centaurea cardunculus*, 6. *Centaurea. cyanus*, 7. *Centaurea gigantea*, 8. *Centaurea gudrunensis*, 9. *Centaurea. hyalolepis*, 10. *Centaurea. iberica*, 11. *Centaurea. persica*, 12. *Centaurea regia*, 13. *Centaurea rigida*, 14. *Centaurea solstitialis*, 15. *Centaurea tomentella*, 16. *Centaurea virgata.*

From the overall data, it is concluded:

1. Pollen grains of all species are of medium size but very small ratio of certain species (*C. cyanus, C. cardunculus, C. behen, C. gigantea*) contain large pollens, while only pollens of *C. virgata* contain very little number of small pollens.
2. Pollen grain outline shape in polar view of all species is mostly triangular, subtriangular, very rarely circular or semicircular. In the equatorial view, pollens are mostly more or less elliptic, seldom circular or semicircular.
3. High ratio of tetra-aperturate pollen grains in the polar view is present among pollen grains of both *C. persica* and *C. tomentella.*
4. Among pollen grain characters the shape is very useful for separating taxa. Species possessing Subprolate shape is more common, Oblate spheroidal and Prolate spheroidal shapes are coming next. The Suboblate shape (P/E = 0.86) in C. persica and prolate shape (P/E = 1.423) in C. gigantea are very exceptional shapes among pollen grains of Centaurea species of Kurdistan-region.
5. Principal component analysis and discriminant analysis can recognize 65.478% and 87.54% of the total variability respectively, moreover, amongst the 160 observations used to fit the discriminant function, 125 or 78.125% are correctly classified, thus can help prediction of taxa.
6. In the pollen sculpture Microspinules height (µm) and number/µm² characterized to have numbers of perforations/ µm² and this character helpful to diagnostic characters for distinguishing our local *Centaurea* species.

Table (3). Pollen grain characters (µm)

Species		Polar view	Equatorial view	Polar view/ Equatorial view	Apoculpium	Mesoculpium	Colpus length	Exine Thickness
Centaurea iberica	Mean	36.365	28.745	1.290	40.684	13.831	22.751	3.159
	Min	31.560	26.092	1.107	35.485	8.886	20.505	2.853
	Max	39.915	31.839	1.441	44.183	19.148	24.753	3.834
C. solstitialis	Mean	28.619	30.169	0.953	32.236	14.535	21.790	3.282
	Min	23.542	23.669	0.716	28.263	11.156	17.458	2.953
	Max	32.559	34.595	1.248	36.260	19.387	25.979	3.665
C. hyalolepis	Mean	39.489	39.821	1.005	44.076	14.002	22.459	3.200
	Min	29.469	27.900	0.756	39.572	10.203	19.374	2.905
	Max	46.570	44.547	1.404	47.815	18.416	24.984	3.552
C. cyanus	Mean	42.786	43.978	0.901	45.789	18.532	29.315	3.417
	Min	30.407	38.890	0.685	36.864	15.546	26.134	3.066
	Max	51.525	48.409	1.062	54.509	20.226	34.370	3.966
C. regia	Mean	38.953	40.006	0.970	46.677	20.040	25.941	3.556
	Min	35.442	37.332	0.810	41.915	14.686	23.526	3.148
	Max	42.741	43.732	1.080	54.158	24.466	30.219	4.355
C. cardunculus	Mean	44.006	34.059	1.301	51.767	18.822	24.410	3.703
	Min	37.158	26.329	1.076	39.658	12.990	19.318	3.279
	Max	50.758	38.740	1.616	57.228	24.227	30.893	4.567
C. behen	Mean	39.903	30.663	1.299	41.979	12.527	29.864	3.141
	Min	33.771	26.626	1.012	33.467	8.448	25.198	2.853
	Max	51.279	36.704	1.733	48.356	18.729	34.686	3.365
C. gudrunensis	Mean	29.499	25.942	1.143	32.738	10.341	22.571	3.410
	Min	25.160	21.491	0.891	30.523	6.619	20.645	3.066
	Max	33.532	30.542	1.443	36.143	13.455	27.707	3.822
C. balsamita	Mean	32.097	30.200	1.086	35.900	11.237	22.103	3.697
	Min	29.369	25.244	0.924	28.804	8.995	19.518	3.066
	Max	35.394	35.196	1.302	37.698	13.270	24.785	4.697
C. rigida	Mean	35.113	30.103	1.175	32.097	16.079	21.436	3.277
	Min	32.915	27.024	0.868	29.369	13.234	15.457	2.953
	Max	37.345	42.395	1.349	35.394	18.402	24.504	3.907
C. persica	Mean	35.929	41.418	0.878	41.329	21.274	27.800	3.224
	Min	28.357	25.841	0.473	34.802	16.116	24.209	3.066
	Max	43.745	59.964	1.518	46.305	27.480	35.611	3.758
C. gigantea	Mean	49.463	34.748	1.432	53.447	20.952	23.67	3.342
	Min	36.914	32.009	0.988	49.235	17.494	18.599	2.905
	Max	58.483	38.398	1.678	56.229	24.408	29.022	3.719
C. tomentella	Mean	36.188	31.209	1.146	39.023	15.120	30.318	3.233
	Min	32.742	28.503	1.015	37.026	6.299	24.611	2.853
	Max	39.212	34.601	1.323	40.695	20.119	34.631	3.809
C. bruguierana	Mean	31.521	26.262	1.209	32.304	14.559	20.153	3.135
	Min	27.346	19.090	0.920	30.157	12.592	17.207	2.905
	Max	38.251	32.343	1.709	34.378	16.499	23.764	3.348
C. virgata	Mean	26.141	24.845	1.053	29.563	11.962	20.681	3.233
	Min	24.096	23.312	0.947	26.989	9.691	16.952	2.853
	Max	28.224	26.863	1.133	31.998	13.899	24.514	3.552
C. aggregata	Mean	31.104	25.363	1.226	36.356	12.541	19.368	3.109
	Min	22.183	23.708	0.881	33.884	8.765	16.977	2.769
	Max	38.853	28.464	1.639	39.193	15.423	20.720	3.365

Table 4. Analysis of variance for pollen grain characters (µm)

Source of variation	Degree of freedom	Mean Square	F-value	P. value
Equatorial diameter (Equa) Error	15 227	428.463 8.69934	49.25	0
Polar length (Polar) Error	15 265	690.909 9.71752	71.1	0
Apocolpium (Apoc) length Error	15 277	946.047 6.84213	138.27	0
Mesocolpium (Meso) length Error	15 214	191.12 6.78252	28.18	0
Colpus length Error	15 145	125.639 6.44935	19.48	0
Exine thickness (Exin) Error	15 144	0.34214 0.08660 17	3.95	0
PE ratio Error	15 210	0.3531 0.0217	16.26	0

Table 5. Average microspinule number and height and perforations number for the pollen grain sculpture

Species	Number of micospinules/µm²	Number of perforations/µm²	Microspinule height/µm
Centaurea iberica	2.5	3	0.396
C. solstitialis	0.75	3.5	0.468
C. hyalolepis	1.4	2.5	0.338
C. cyanus	0.3	7	0.266
C. regia	1.75	4.3	0.333
C. cardunculus	1.5	3.5	0.367
C. behen	2	7	0.35
C. gudrunensis	0.7	4	0.312
C. balsamita	1	4	0.459
C. rigida	1.85	3.7	0.331
C. persica	1.5	2	0.270
C. gigantean	3.5	7.5	0.336
C. tomentella	3	3	0.372
C. bruguierana	0.4	4	0.392
C. virgata	2.5	4.5	0.456
C. aggregata	2.5	5	0.867

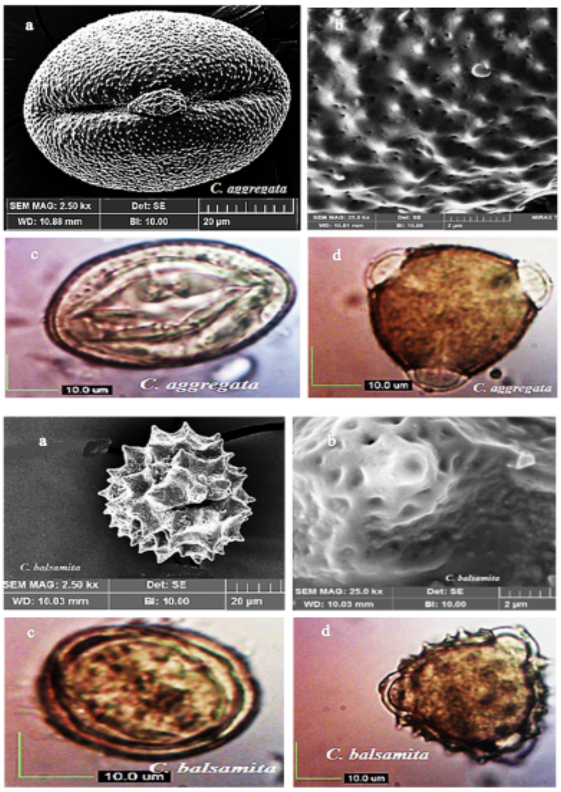

Figure 3. *Centaurea aggregata,* SEM: (a) equatorial view, (b) exine sculpture. LM: (c) equatorial view, (d) polar view. *Centaurea balsamita*, SEM: (a) equatorial view, (b) exine sculpture. LM: (c) equatorial view, (d) polar view.

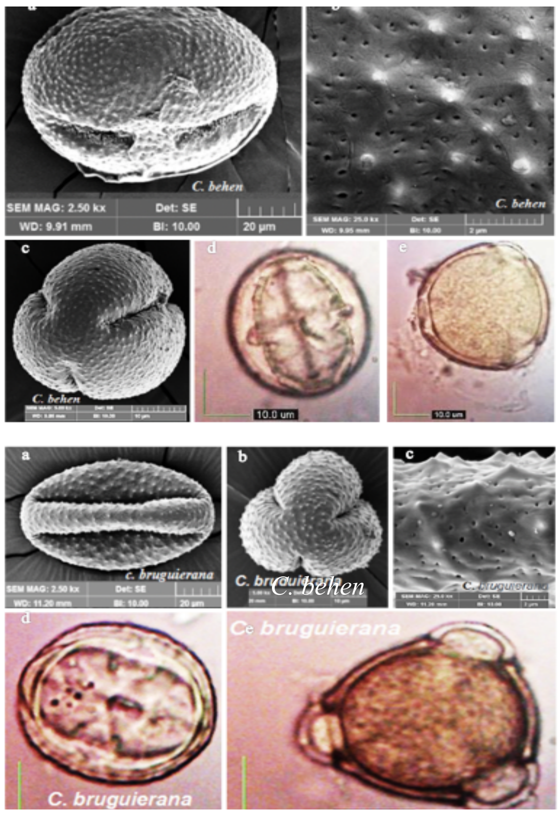

Figure (4). *Centaurea behen,* SEM: (a) equatorial view, (b) exine sculpture, (c) Polar view. LM: (d) equatorial view, (e) polar view. *C. bruguierana*, SEM: (a) equatorial view, (b) polar view (c). exine sculpture. LM: (d) equatorial view, (c) polar view.

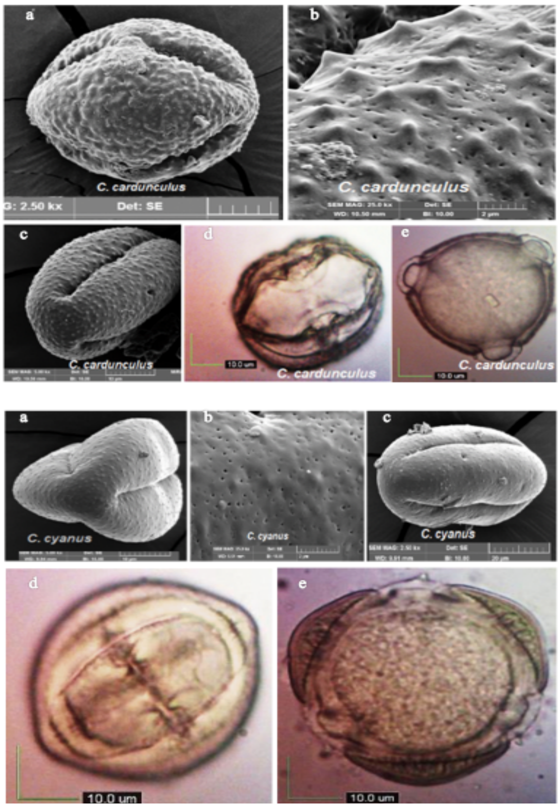

Figure 5. *Centaurea carduncululus,* SEM: (a) equatorial view, (b) exine sculpture, (c). Polar view. LM: (d) equatorial view, (e) polar view. *C. cyanus,* SEM: *(a) equatorial view, (b) exine sculpture.*(c) polar view *LM: (d) equatorial view,* (c) polar view

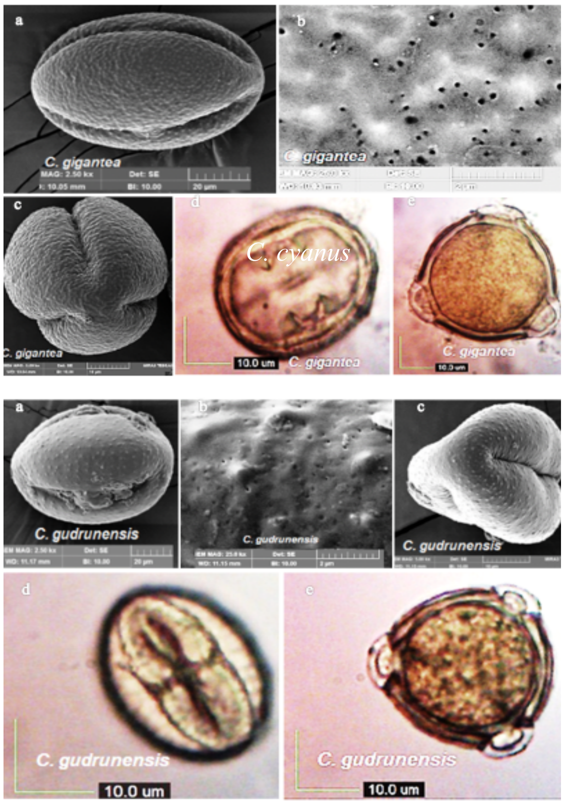

Figure 6. *Centaurea gigantea*, SEM: (a) equatorial view, (b) exine sculpture, (c). Polar view. LM: (d) equatorial view, (e) polar view. *C. gudrunensis* SEM: (a) equatorial view, (b) exine sculpture, (c) polar view LM: (d) equatorial view, (c) polar view

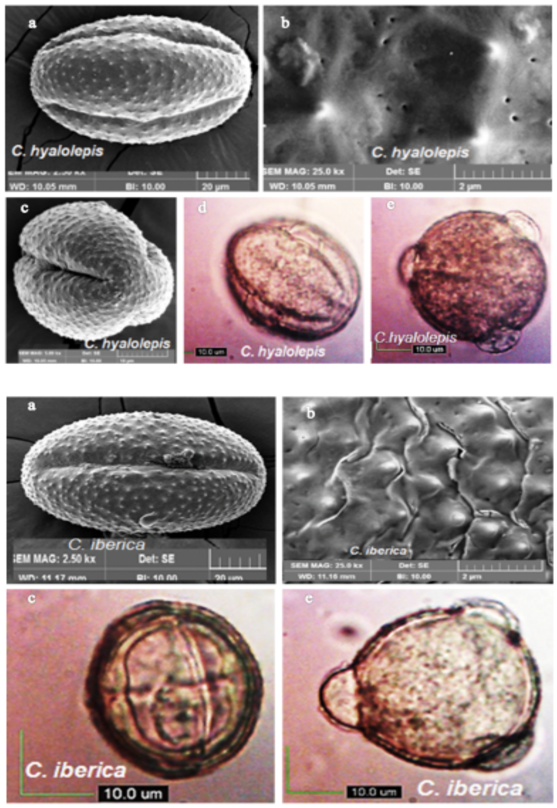

Figure 7. *Centaurea hyalolepis,* SEM: (a) equatorial view, (b) exine sculpture, (c). Polar view. LM: (d) equatorial view, (e) polar view. *C. iberica,* SEM: (a) equatorial view, (b) exine sculpture, LM: (c) equatorial view, (c) polar view.

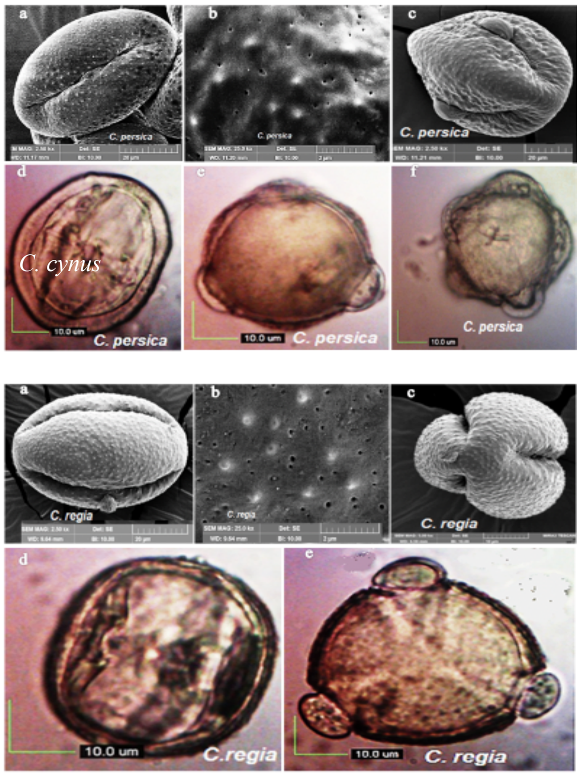

Figure 8. *Centaure persica,* SEM: (a) equatorial view, (b) exine sculpture, (c). Polar view. LM: (d) equatorial view, (e) polar view, (f) polar view (five apertures). *C. regia,* SEM: (a) equatorial view, (b) exine sculpture, (c) polar view. LM: (d) equatorial view, (e) polar view.

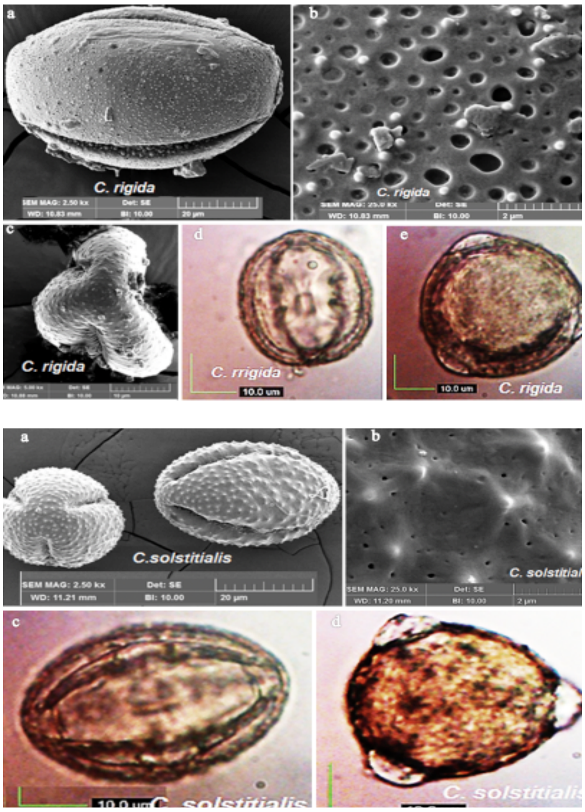

Figure 9. *Centaurea rigida,* SEM: (a) equatorial view, (b) exine sculpture, (c). Polar view. LM: (d) equatorial view, (e) polar view. *C. solstitialis,* SEM: (a) polar view, (b) equatorial view (c) exine sculpture, LM: (d) equatorial view, (e) polar view**.**

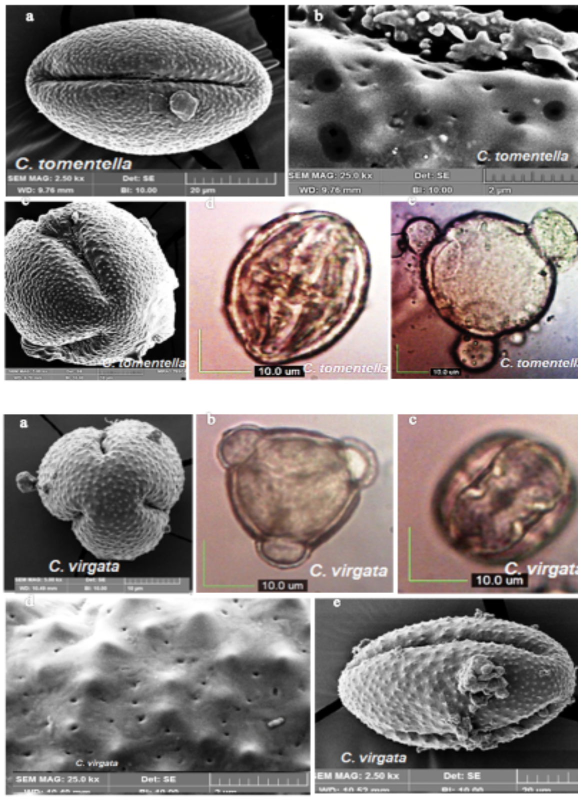

Figure (10). *Centaurea tomentella,* SEM: (a) equatorial view, (b) exine sculpture, (c). Polar view. LM: (d) equatorial view, (e) polar view. *C. virgata,* SEM: (e) equatorial view, (d) exine sculpture, (a) polar view. LM: (c) equatorial view, (b) polar view.

4. CONCLUSION

Among pollen grain characters the shape is very useful for separating taxa. Species possessing subprolate shape is more common, Oblate spheroidal and prolate spheroidal shapes are coming next. The Suboblate shape (P/E = 0.86) in C. persica and prolate (P/E = 1.423) in C. gigantea are very Exceptional shapes among pollen grains of Centaurea species. Principal component analysis and discriminant analysis can recognize 65.478% and 87.54% of the total variability respectively. In the pollen sculpture Microspinules height (µm) and number/µm², moreover the number of perforations/ µm² is very useful diagnostic characters for distinguishing *Centaurea* species.

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