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Achene and pollen morphology of the genus *Crepis* L. in Egypt and its systematic significance

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Abstract

In this study, achene, and pollen morphology of *Crepis* L. species in Egypt were investigated by scanning electron microscopy. *Crepis* genus is represented by eight species viz *C. aculeata* (DC.) Boiss., *C. aspera* L., *C.libyca* (Pamp.) Babc. *C. micrantha* Czerep., *C. nigricans* Viv., *C. sancta* (L.) Bormm., *C. senecioide* Del. and *C clausonis* pomel. However, due to occurrence uncertainty regarding *C. clausonis* in Egypt, this study focuses on the remaining seven species. The achenes exhibit morphological diversity, being either homomorphic or dimorphic, with or without a beak, and occasionally winged. Their surface texture varies from smooth to spiculate or scabridulous, with longitudinal ribbing. The pappus is either persistent or deciduous and consists exclusively of setae. Key diagnostic features for differentiating Egyptian *Crepis* species include achene polymorphism, variations in surface sculpturing, shape, beak presence, width, and the minimum and maximum lengths of both peripheral and central achenes, as well as pappus length. The pollen grains of *Crepis* species are characterized as echinolophate and 3-colporate, with apertures located in poral lacunae forming compound structures. The polar area is distinctly defined and covered with echinae. Morphological features such as pollen grain shape and polar area structure hold significant taxonomic value for species delimitation within the genus.This study highlights the importance of achene and pollen traits in distinguishing *Crepis* species in Egypt and provides valuable insights for their taxonomy and classification.

Keywords: Achene characters, *Crepis*, Micro-morphology, pollen characters.

1. Introduction

Crepis genus is a taxonomically problematic, with a lack of perceptive characters (Enke and Gemeinholzer 2008); the genus *Crepis* belongs to subtribe Crepidinae, tribe Lactuceae of the family Asteraceae. Polymorphism is widespread within *Crepis* genus, and many taxonomic features associated with aerial and underground parts vary within a species rather than between closely related species, often leading to unclear species-specific boundaries (Kalmuk, et al 2018). *Crepis* genus comprises about 200 species (Mabberley, 2008), widely distributed in the northern hemisphere and Africa. Eight species were recorded in Egypt *C. micrantha* Czerep, *C. libyca* (Pamp.) Shab., *C. senecioides* Delile, *C. nigricans* Viv., *C. aspera* L., C. *aculeata* (DC.) Boiss and *C. sancta* (L.) Bornm., however, the presence of *C. clausonis* (Pomel) Batt. & Trab. remains uncertain. (Boulos, 2002; 2009).

Both morphology and fruit anatomy provided significant taxonomic information (Karaismailoğlu, 2015). The micromorphological characteristics of the achene viz surface sculpture and the shape of the pappus and its ultrastructure are often used to classify and identify different genera and taxa of the tribe Cichorieae (Pak *et al*., 2001; Zhu *et al*., 2006; Kilian, *et al* 2009; Zhang *et al*., 2013; Karanović *et al*., 2016. However, there are only a few taxonomic studies on the achenes micromorphs of the genus *Crepis*, and these are limited in range and restricted to a limited representative of the genus (Enke, 2009; Karaismailoğlu, 2015; Inceer *et al*., 2016). Therefore, based on the previously studied, a comprehensive investigation of the micromorphological structure of *Crepis* achenes has not yet been conducted.

Pollen morphology is highly significant for the taxonomist; the taxonomic and evolutionary importance of pollen morphology may be at specific, generic and/or higher levels (Davis and Heywood, 1973).

Wortley *et. al.,* (2012) presented a bibliography of one hundred and seventy-two references which indicates the great level of research activity currently taking place in Asteraceae pollen grains morphology.

Pollen grains of Lactuceae were first examined in detail by Wodehouse (1935). Comprehensive surveys of pollen grains in the tribe Lactuceae were carried out by Tomb (1975), Blackmore, (1976), and Skvarla *et al*. (1977). Pollen morphology of some Egyptian species of Lactuceae was studied by Abou El-Naga (1990) and Abou El-Naga and El- Housseini (1995) and Osman (2006). The most comprehensive pollen studies of Crepidinae are those of Blackmore, and Person (1996) and Wang *et al.* (2009). The objectives of this study are to examine the achene micro morphological as well as pollen grain characteristics

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in 7 species of *Crepis* grown in Egypt, and evaluate its taxonomical significance.

2. Material and methods

2.1 We have studied the achene and pollen morphology of the other 7 species, *C. micrantha* Czer., whi Bormm., *C.libyca* (Pamp.) Shab., *C. senecioides* Delile, *C. nigricans* Viv., C. *aspera* L., *C. aculeata* (DC.) Boiss and *C. sancta* (L.) which considered as a synonym of *Lagoseris sancta* in (Tackholm, 1974; Lack, 2007).

The achenes and pollen grains were taken from herbarium specimens, kept in the Cairo University (CAI), Royal Botanic Gardens (K) and British museum natural history (BM) herbaria. Table (1) lists these specimens with locality, date of collection, name of the collectors and the number. Nomenclature followed **(**Boulos 2002; 2009**)**; purification of scientific name followed The *Plant List* https://wfoplantlist.org// and catalogue of life http://www.catalogueoflife.org/col/search/all/key/Crepis+sancta+/fossil/1/match/1

Table 1. Herbarium samples and collectors. s.n. –collecting number is missing. (CAI) = Cairo University herbarium, (K) = Royal Botanic Gardens herbarium and (BM) = The Natural History Museum Herbarium.

Species	Locality	Date of collection	Collector and number	Herbarium
C. micrantha	Mit El Kholi Moumen, Dikirinis, Dakahlia.	21.5.1967	V. Täckholm s.n.	CAI
	Damietta.	1.5.1922	Simpson 1205	K
	Kharga Oasis	17.4,1876	Ascherson 333	K
C. sancta	Wadi El Higaini, N. Galalah	4.4.1924	Simpson 2609	K
	Wady east of (Anthony's) monastery.	23.3.1928	Simpson 5830	K
	Sinai	23.4.1835	W. Schimper 409	K
C. lybica	Daba'a Mariut coast	31.3.1927	Simpson 4650	BM
	Mersa Matruh	1904	Ball 47	K
	Wadi Habs bet. Mersa Matruh and Agiba	24.3.1974	V. Täckholm s.n.	CAI
C. senecioides	Between Matruh and Barrani	11.4.1932	Shabetai 4760	K
	El Khanka,sandy wast ground	26.1.1923	Simpson 1761	K
	Sinai,Wadi Ferieh, Abu Zeitun	24.4.1961	V. Täckholm s.n.	CAI
C. nigricans	Ad Pyramides Egypt	17.2.1834	Wiest 59	BM
	Abou Roash village	27.4.1924	Simpson 2788	K
	Girgeh	23.4.1874	Schweinfurth 49	K
C. aspera	Mariut: Burg El Arab	15.3.1928	G. Täckholm S.n	CAI
	Rafah, near the station	22.3.1928	G. Täckholm s.n.	CAI
	Suez	2.1906	Muschler s.n.	K
C. aculeata	Sinai: Rafah,Bir el Meleha at the coast.	22.3.1928	G. Tackholm s.n.	CAI
	Sinai, in the garden of Deir el Rabba	25.4.1961	V. Tackholm s.n.	CAI
	Catherin, wadi Tih	8.10.1983	El Hadidi s.n.	CAI

2.2 For achenes, five mature achenes as well as the five pappus were mounted on an adhesive surface on the metal holder for SEM; a total of 48 achenes qualitative and quantitative characters were investigated (table 2).

2.3 For pollen grains scanning using electron microscope, a droplet of acetolyzed containing 10 -15 pollen grains in water suspension was pipetted on to photographic film; this film was attached to **S**EM stub. The specimens were examined and photographed with Scanning electron microscope (Jeol JSM-25S) at 25 KV in the Electron Microscope Unit, King's College London. A total of 15 qualitative and quantitative characters were investigated (table 3). Data were statistically analyzed using Past software; one factor analysis of variance (ANOVA) was used to examine differences in the mean values among taxa overall, using SPSS (version 22)

3. Terminology

The terminology of the achene microstructure follows Barthlott (1981; 1984); the pollen terminology follows Wodehouse (1928; 1935).

4. Result and Discussion

4.1. Achene characters

4.1.1. Achenes are homomorphic or 2 (-3) - morphic, beaked or not, occasionally winged, smooth, spiculate or scabridulous, longitudinally ribbed. The pappus is either persistent or deciduous and consists exclusively of setae. Based on Achene similarity, achenes of studied species (Fig 1) were classified into two major classes:

(1). Homomorphic achenes (represented by two species); are beakless, 1.5-2 mm long, subcylindrical, glabrous, furrows minutely tuberculatus with truncate apex and pappus 3.5-4 mm long in *C. micrantha* but they are

beaked, 8-13 mm long, curved, 10 ribbed Antrosely spinulose on the ribs and pappus 7-8 mm long in *C. Libyca*.

(2) Heteromorphic achenes including three sub-groups (A) first sub-group; includes achenes that are beakless (both the peripheral and the central achenes) this sub-group includes only one species; includes only *C. sancta* where the m peripheral arginal achenes are 3-4 x 0.5-1.5 mm, oblong, sulcate or smooth, while the central achenes are 3-4 x 0.5 mm, whitish fusiform, ribbed scabridulous, furrows.

B) Second sub-group; includes two types of achenes; the peripheral achenes are beakless, but the central achenes are beaked this sub-group includes two species; *C. aspera* and *C. aculeate*. Where the peripheral achenes in *C. aspera* are 5-6 x 0.5 mm, beakless, Pale Straw colour, strongly compressed, winged while its central achenes are 4-7 x 0.4 mm, beaked, brown, with Deciduous pappus. Moreover, the outer achenes of *C. aculeate* are 7-8 x 1 mm, slightly compressed, pubescent, yellowish or brownish while its central achenes are 3-3.5 x 0.5 mm, fusiform, glabrous in.

C) The third sub-group comprises two species: *C. senecioides* and *C. nigricans*. which is characterized by having beaked peripheral and central achenes. Moreover, both types of achenes (peripheral and central) in *C. senecioides* are fusiform, with scabridulous surface while the two types of achenes are cylindrical in *C. nigricans.* Furthermore, the peripheral achenes in *C. senecioides* are 1-1.8 x 0.5 mm, compressed, beak 3.5-5.5 mm, while central achenes 3-4 mm, beak 3-4 times as long as the achene body. However, the peripheral achenes in *C. nigricans* are 3-3.5 x 0.2 mm, with coarse beak, beak as long as the body, while the central achenes 5-6 x 0.25 mm, with fine beak, beak 1/3-1/2 the body.

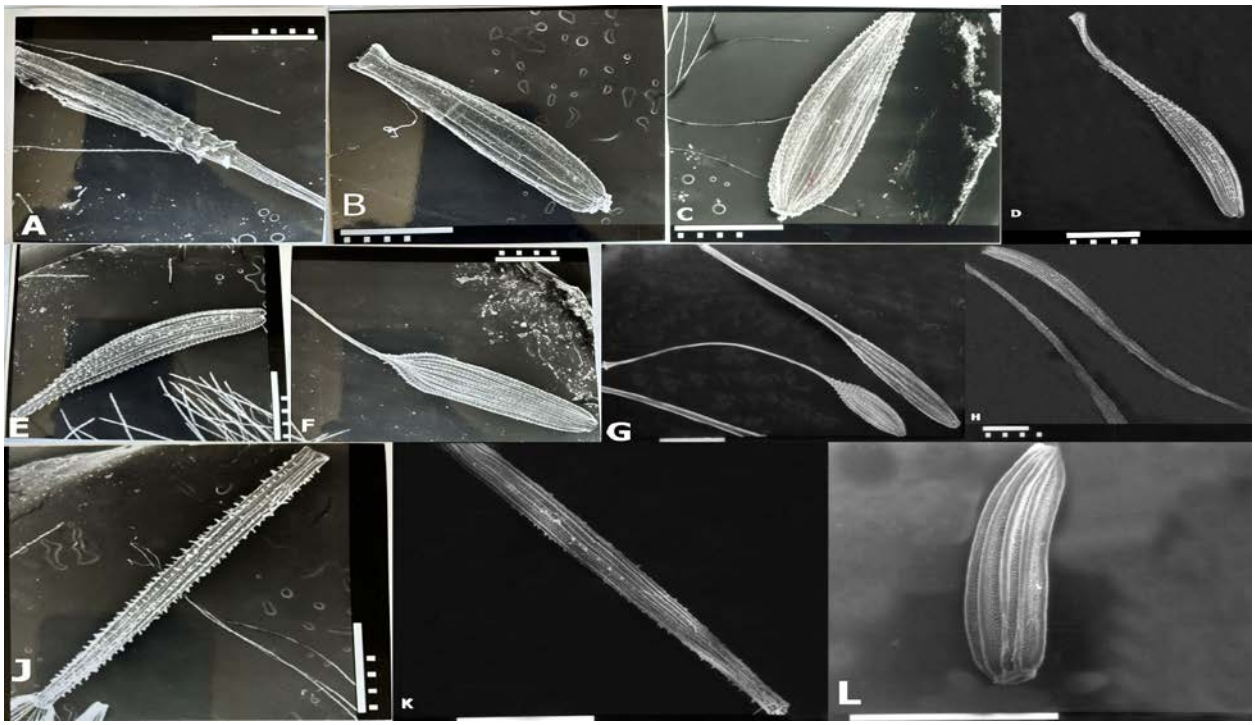

Figure 1. *Crepis* achene; A = *C. aculeata* central achene, B = *C. aculeata* peripheral achene, C= *C. aspera* peripheral achene, D= *C. aspera*, central achene, E = *C. nigricans* central achene, F = *C. nigricans* peripheral achene, G= *C. seneciodes* peripheral achene on top , central achene on down, H = *C. lybica*, J = *C. sancta* central achene , K = *C. sancta* peripheral achene, L= *C. micrantha*, (The scale = 1mm).

3.1.2 A cluster analysis was performed to generate a dendrogram (Fig.2) illustrating the possible relationship between the investigated *Crips* species The analysis was based on Euclidean distance and the paired group method, with achene characteristics and pappus length being the key traits that differentiated the studied species into two main clusters. The first cluster comprised *C. libyca* and was separated from the other species by the maximum length of the peripheral arches. The second cluster included the remaining six species, which were divided into two main subgroups based on peripheral arches dimensions (minimum, maximum length and width): the first group included both *C. micrantha* and *C. sencioides*; the second subgroup included *C. aculeata* in a separate branch and the remaining three species were aggregated in sub-cluster; this agreed with Enke (2008) and Kilian *et al.* (2009) who found that the pappus is generally an important character for distinguishing groups at all taxonomic levels in the tribe Cichorieae. Achene characteristics such as shape, number of ribs and size as well as pappus shape have been shown to be of significant importance for both phylogeny and the taxonomy genus *Crepis*, and this result is in line with the outcomes cited by Babcock (1947a, 1947b) and Enke (2009).

Statistically, significant differences are found in Marginal and central achene max, minimum length and width, pappus maximum and minimum length (*p<0000)*, Table (2).

Results obtained (Table2 and Fig 2) indicate that there are significant differences in the achene surface sculpturing among *Crepis* target species with an agreement with Nursen *et al.* (2018) who reported that the achene

surface sculpturing shows a high taxonomic significance in distinguishing between the 26 for *Crepis* taxa from Turkey. According to Babcock (1947b), peripheral achenes differed morphologically from the central ones, i.e were dimorphic in some species of *Crepis*. (Fig 1). Among the studied taxa, *C. aculata*,*C. aspera*, *C. sancata* , *C.seneciodes* and *C.nigricans* are annual species and have dimorphic achenes in agreement with Imbert (2002) who observed that both annual and biennial species of *Crepis* have dimorphic achenes . The differences in the micro-sculpture between peripheral and central achenes can affect their dispersal ability.

4.1.2. Taxonomic key based on achene micro-characters.

1. Achenes monomorphic ……. 2

1. Achenes dimorphic ……3

2. Achenes short (1.5-2 mm); subcylindrical; ribs glabrous; beakless ……. *C. micrantha*

2. Achenes long (8-13 mm); fusiform ±curved; ribs antrosely spinulose; beaked…….…...*C.lypica*

3. All achenes beakless; achenes Sulcate or smooth ……..…C. *sancta*

3. All (or at least the central) achenes beaked; …….4

4. Peripheral achenes winged; Pale Straw colour, apex not beaked ……..…*C. aspera*

4. Peripheral achenes not winged ……..……5

5. Pappus persistent, Peripheral achenes slightly compressed, pubscent, tapering beakless ……*C.aculeat*

5.Pappus deciduous……. 6

6.a. Peripheral achenes cylindrical, with rugulose coarse beak surface, beaked as long as the body ……..…… ……... *C. nigricans*

6.b. Peripheral achenes compressed Fusiform, with Scabridulous. 10 ribbed surface and capillary beaks. 3.5-5.5 mm …… *C. senecioides*

Table 2. showing achenes micro-morphological characters. One-way ANOVA's were performed separately for each of the achene feature to determine the differences among *Crips*. (*p<0.05*)

Species		*C. micrantha*	*C. libyca*	*Crepis sancta*	*C. senecioides*	*C. nigricans*	*C. aspera*	*C. aculeata*	ANOVA
Achene morphisms		Homomorphic	Homomorphic	Dimorphic	dimorphic	dimorphic	dimorphic	dimorphic	
Peripheral achenes	min length (mm)	1.5	8 including beak	3	1	3.5	5	7	.000
	max length (mm)	2	13 (including beak)	5	1.8	5	6	8	.000
	Width(mm)	0.4	0.8	1	0.5	0.2	0.5	1	.000
	Shape	subcylindrical	fusiform ±curved	Oblong	Fusiform, compressed	cylindrical	Strongly compressed. Winged 10 ribbed	Slightly compressed	
	colour	Tawny brown	brown	whitish	whitish	whitish	Pale Straw colour	Yellowish or brownish	
	Surface	Ribs glabrous, furrows minutely tuberculatus	Antrosely spinulose on the ribs, 10 ribbed	Sulcate or smooth	Scabridulous. 10 ribbed	rugulose coarse beak	Minutely scabridulous	pubescent	
	apex	Truncate beakless	Beaked (4-7 mm)	Truncate, not beaked	Beaked. 3.5-5.5 mm capillary	Beaked as long as the body	Attenuate to a narrow apex not beaked	Tapering beakless	
Central achenes	min length (mm)	-	-	3	3	5	4	3	.000
	max length (mm)	-		4	4	6	7	3.5	.000
	Width (mm)	-		0.5	NA	0.25	0.4	0.5	.000
	shape	-	-	fusiform	fusiform	cylindrical	Fusiform 10 ribbed	fusiform	
	colour	-	-	brown	Dark brown	brown	brown	brown	
	Surface	-	-	Ribbed, Scabridulous furrows tuberculatus	Scabridulous	Fine beak	Scabridulous	Glabrous striate	
	Apex	-	-	Tapering to truncate not beaked	Beak 3-4 times as long as the achene body	Beak1/3-1/2 the body	Beak 2.5-3 mm	Beak as long as the body	
Carpopodium m	character	-	Well developed	Well, developed	-	Well developed	Well developed	Well developed	
	No. of interruptions	-	4	4	-	4	4	2-4	
p p	duration	persistent	persistent	persistent	deciduous	deciduous	Deciduous	persistent	

Species	*C. micrantha*	*C. libyca*	*Crepis sancta*	*C. senecioides*	*C. nigricans*	*C. aspera*	*C. aculeata*	ANOVA
Min length (mm)	3.5	7	3.5	1.5	4	3	4	0.002
max length(mm)	4	8	4	5	N. A	4	5	.000

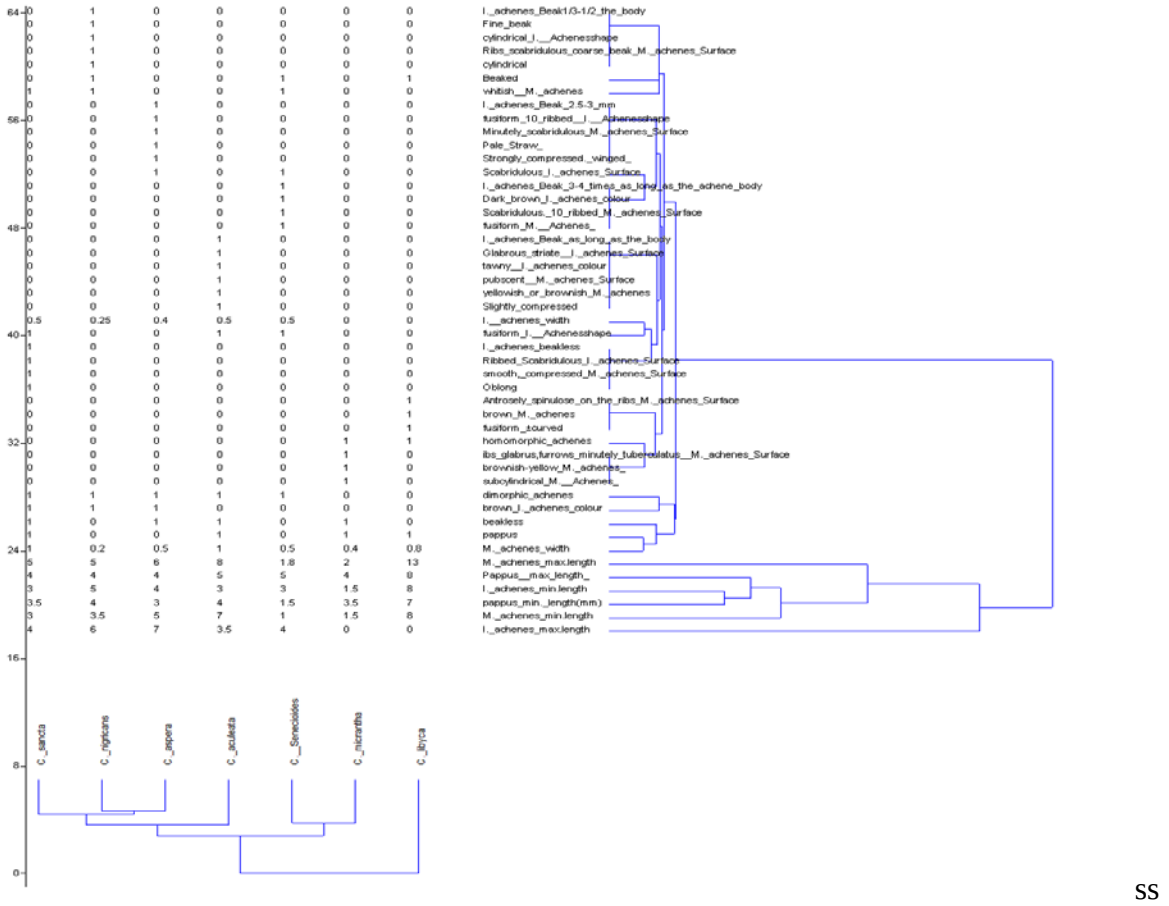

Figure 2. Dendrogram showing the similarity between *Crepis* species using Euclidean distance and the paired group method based on the 48 achene characters

4.2. Pollen grains character

Pollen grains with 15 lacunae (3 poral, 6 paraporal and 6 abporal); 3-colporate, the apertures are situated in poral lacunae flanked in the meridional direction by two abporal lacunae, The poral lacunae are interconnected with the abporal lacunae through interlacunar gaps. The aperture is compound, consisting of:Ectoaperture: A long, broad colpus, divided into three lacunae.

1) Mesoaperture: A slightly elliptical, lolongate porus.
2) Endoaperture: A colpus, lolongate, short, with an acutely tapering end.

The polar area is well-defined and covered with echinae.

4.2.1. ; the polar area is well- defined and provided with echinae. (Table 2; Fig 3)

4.2.2. Based on pollen grain shape, investigated species divided into two main groups*:*

the first is spheroidal included only *C. sancta*; the second one is oblate-spheroidal that included the rest of studied species. The polar area has small concavities as in *C. Lybica*, while it is solid in the other species; this agrees with the finding mentioned by Osman (2006). It was noticed that the pollen size of the studied taxa ranges from 25 x26 µm (in *C. sancta*) to 41 x 48 µm (in *C. aculeata*). However, the Equatorial diameter ranges between 27 ±0.1 µm (in *C. sancta*) to 34.7 ±1.7 µm (in *C. micrantha.*). Furthermore, the number of echinae in polar area ranged between1- as in *C. aculeata* to 10) as in *C. nigricans.* Moreover, the Exine thickness ranged between 4 µm (in *C. micrantha*) to 7 µm) (in *C. aculeata*). In addition, the long axis of polar area extended between 8µm) as in *C. sancta* to 16 µm as in *C. senecioides.* (Table 3)

Palyno-taxonomical features of pollen grains result shows that *C. sancta* pollen grains are the smallest among the studied species, while C. *aculeata* pollen grains are the largest. Pollen grain shape, as well as the polar area, are useful taxonomic features. A cluster analysis was performed to generate a dendrogram (Fig.4), illustrating the possible relationship between the investigated Crips species using Euclidean distance and the paired group method examined Crips micro-morphological characters. The minimum and maximum Polar axis as well as Equatorial diameter were the most important characters

that separated the studied into two main clusters. The first cluster comprised both C. *aculeata* and *C. senecioides.* The second cluster comprised two subclusters with two sub-groups, one of them included only *C. sancata* and the second subgroup comprised the rest of studied species. Those results are compatible with Akyalçin and Altan (2024), where they concluded that both equatorial and Polar axis dimensions were smaller and significantly different between *Crepis* species growing in Türkiye. Statistically significant difference was determined among the examined species, regarding all the analyzed quantitative pollen features ($p < 0.0005$), significant differences were observed in maximum and minimum polar axis, equatorial diameter, and number of echinae in the polar area. However, other features, such as P/E ratio, exine thickness, and exine length, were statistically non-significant ($p > 0.0005$).

Table 3. Showing pollen grain features (the first value indicates mean value ± standard deviation, while values between bracts indicates minimum and maximum values respectively. One-way ANOVA's were performed separately for each of the pollen grain feature to determine the *Crepis* species. ($p<0.05$)

Species	Polar axis (mm)	Equatorial diameter (mm)	P/E	shape	Exine thickness	Echina length	Echina apart	Long axis of polar area (mm)	Number of echinae on polar area (mm)	polar area
C. micrantha	30.3 ±1.5 (28- 35)	34.7 ±1.7 (30-35)	0.87	OS	4 ±0 (4)	2 ±0 (2)	2 ±0 (2)	12.8 ±1.2 (11-14)	5.7 ±1.4 (3-5)	Solid
C. sancta	27 ±0.2 (25-27)	27 ±0.1 (26-27)	1.0	s	4.5 ±0.5 (4-5)	2 ±0 (2)	2.6 ±0.2 (2.5 3)	9.1 ±0.9 (8-11)	5.2 ±1.5 (3-10)	Solid
C. lybica	32.1 ±2.3 (28- 35)	34.2 ±2.6 (28-35)	0.93	OS	5 ±0.5 (4-6)	2 ±0.5 (1-3)	2.25 ±0.2 (2-2.5)	10.3 ±0.8 (9-11)	7.7 ±1.7 (5-10)	concavities
C. senecioides	32.5±1.5 (30-36)	37.4 ±2 (35-43)	0.86	OS	5.5 ±0.5 (5-6)	2.5 ±0.5 (2-3)	2.5 ±0.5 (2-3)	!4.6 ±0.9 (13-16)	6 ±2 (4-7)	Solid
C. nigricans	31.1 ±1.7 (28-34)	34.5 ±1.4 (32-35)	0.90	OS	5 ±0 (5)	2 ±0 (2)	2.3 ±0.2 (2-2.5)	13.2 ±1.5 (12-16)	9.9 ±1.8 (8-10)	Solid
C. aspera	30.6 ±2.3 (26-34)	34.3 ±2.5 (29-35)	0.89	OS	4.6 ±0.7	2.1 ±0.3 (2-3)	2.2 ±0.3 (2-3)	!0.7 ±1.4 (9-14)	6 ±2.3 (3-10)	Solid
C. aculeata	38.3 ±1.8 (34-41)	43.8 ±2.9 (35-48)	0.87	OS	6.2 ±0.6 (5-7)	3 ±0 (3)	2.6 ±0.3 (2-3)	11.2 ±1.2 (11-13)	3 ±1.5 (1-5)	Solid
ANOVA	0.000	0.000	0.594		0.172	0.835	0.956	0.000	0.000	0.000

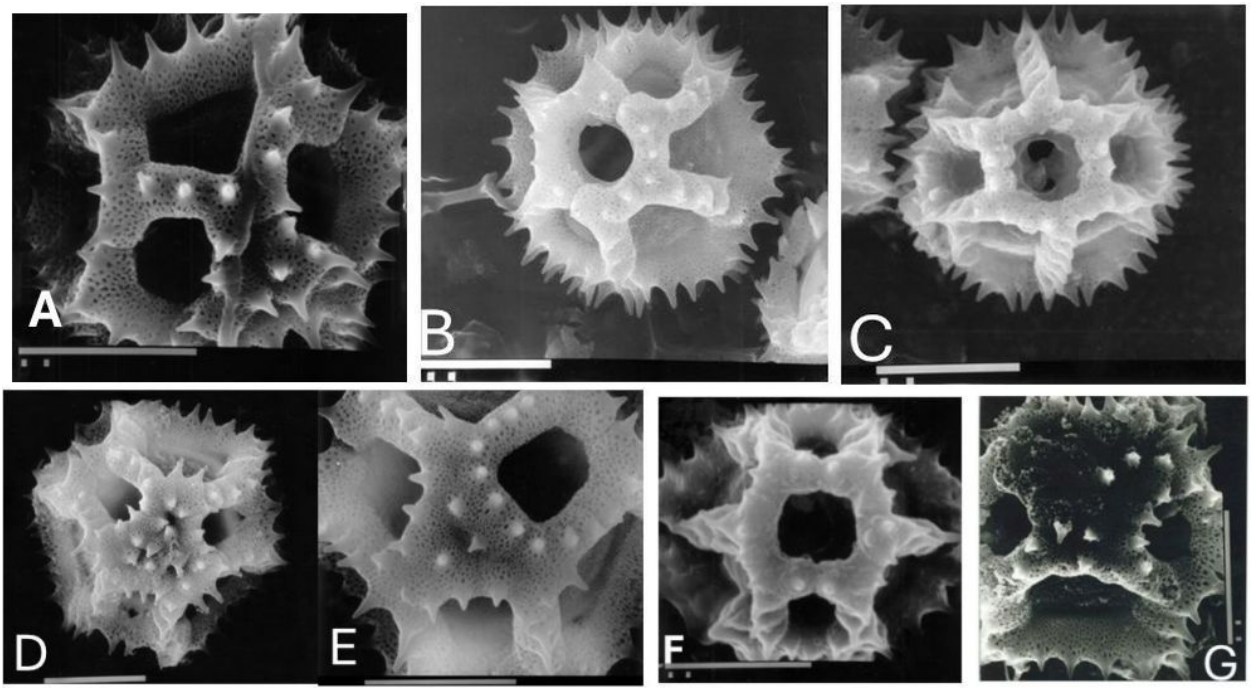

Figure 3. pollen grain of studied *Crepis* species. A = *C. sancta*; B = *C. seneciodes;* C= *C. micrantha*, D= *C. lybica*; E = *C. aculeata*; F = *C. nigricans* and G = *C. aspra* (scale line equal 10 microns)

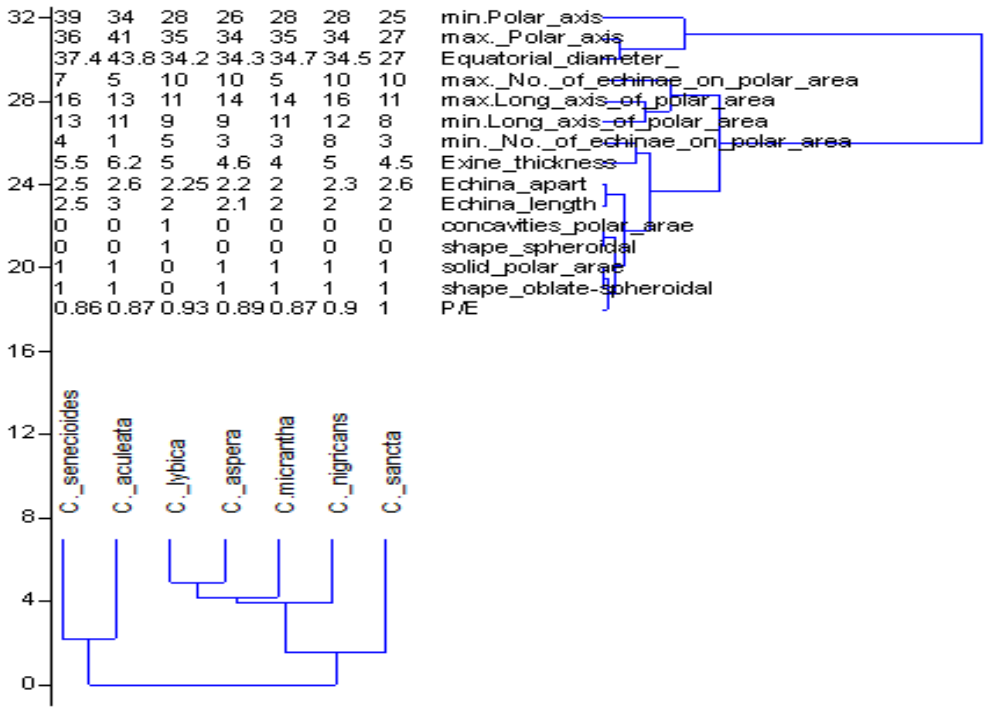

Figure4. Dendrogram showing the similarity between *Crepis* species using Euclidean distance and the paired group method based on 15 pollen characters.

4.2.3. Key to the studied species based on pollen grain.

1 Pollen grains are spheroidal ……..……*C.sancata*

1 Pollen grains non spheroidal ……..…….2

2 The polar area has small concavities…… *C. Lybica*

2 The polar area solid…….3

3 a. Equatorial diameter ranged between (35- 48 µm) ……..... *C.aculeata*

3 b. long axis of polar area ranges between (11-14 µm); polar axis ranges between (28- 35 µm) ……..... *C. micrantha*

3 c. long axis of polar area ranges between (12-16 µm); number of echinae on polar area (8-10) ..... *C. nigricans*

3 d. Equatorial diameters ranged between (35-43 µm), number of echinae on the polar area 4-7 ……. *C. senecioides*

3 f. Equatorial diameters ranged between ((29-35µm), Long axis of polar area ranged between (9-14 µm) …….. *C. aspera*

5. Conclusion

Achene and pollen micro-morphology of seven *Crepis* species grown in Egypt were investigated. The present study documented that achenes morphisms as well as the differences in the achene surface sculpturing, achenes, shape, and beaks are valuable for delimitation the Egyptian *Crepis* species. Also, pollen grain shape, as well as the polar area, are useful taxonomic features.

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