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Original Research Article

The Study of the Pollen Grains of Various Species Belonging to the Genus *Cousinia* Cass. (Asteraceae) in Iraq

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Abstract: The genus *Cuosinia* Cass. is among the most common genera within the Asteraceae family. This genus contains an approximate range of 500 to 600 taxa. Pollen morphology analysis has proven to be a significant tool in the categorization of the Asteraceae, in addition to helping in the classification of specific genera and species under the family. An extensive examination of the pollen morphological features of six species belonging to the *Cuosinia* genus was carried out using both light and scanning electron microscopy. The pollen features that are beneficial for distinguishing various species of *Cousinia* based on characteristics instance form, dimension of the polar diameter and equatorial diameter, polar to equatorial axis ratio (P/E), exine thickness, sculpturing, and quantity of verrucae. Examination using light microscopy revealed that pollen grains were monads, tricolporate, isopolar, prolate-spheroidal, prolate, Sub-prolate. Using scanning electron microscopy, we categorized the pollen grains into three groups based on their exine ornamentation: group I (densely verrucate), group II (verrucate), and group III (loosely verrucate). Micrographs illustrating the morphology of pollen grains are accompanied by comprehensive descriptions of their structural characteristics. The pollen traits, such as morphology, exine thickness, and surfaces sculpturing, were found to be highly useful for classifying purposes.

Keywords: *Cousinia*, Asteraceae, Pollen grains, Palynology, Iraq.

INTRODUCTION

The genus *Cousinia* Cass., with over 700 species, forms one of the more diverse genera within tribe Cardueae of Asteraceae, the regions of middle and south-west Asia (Rechenger, 1972; Attar & Ghahreman, 2006; Mehregan and Assadi, 2016; Mineaifar *et al*., 2016; Rastegar *et al*., 2018). *Cousinia* exhibits the largest distribution and diversity mostly in the high-altitude regions of Iran, Afghanistan, and Turkmenistan. There exist more than 420 species, and of these 377 are classified considered endemic (Rechenger, 1986). The genus *Cousinia* ranks as the 50th biggest genus in regard to the number of species amongst flowering plants, with an approximate representation of 650 species (Frodin, 2004; Ulukus and Tugay, 2020). Genus *Cousinia* exists throughout West and Middle Asia. It exhibits the usual features of the Irano-Turanian phytogeographical distract and also demonstrates a high rate of endemism (Djemali *et al.*, 2012). In Iraq, there are currently over 24 species belonging to the *Cousinia* genus (Ghazanfer *et al.*, 2019). Pollen features are valuable indicators in the context of current molecular theories of relationships and can be utilized to reinforce species relationships or offer distinguishing features for various groups, especially in the Asteraceae family (Wartley *et al*., 2007). Palynology offers a distinct set of tools for examining species connections and taxonomy, in contrast to traditional studies based on morphology. It also provides data that is less impacted by ecological specializing of the plant, which can result in significant morphological distinctions on a wider scale (Mineaifar *et al.*, 2016). Adding pollen features might also help get around the problems that come up when morphological traits are all the same, which would make the results more accurate (Scotland *et al.*, 2003). Nevertheless, the examination of pollen structure can be valuable in defining important species, and lately there have been substantial investigations in the field of palynology (Susanna *et al.*, 2007; Zafar *et al.*, 2007; Khanbeygi *et al*., 2011; Djemali *et al.*, 2012; Dehgheni *et al.*, 2017). Hence, the primary objective of this study is to recognize and describe species belonging to the *Cousinia* genus by analyzing the structure of their pollen. Additionally, the study aims to

examine the relations between these species by analyzing their palynological characteristics and to explore the differentiation of species by studying the morphology of their pollen grains.

MATERIAL AND METHODS

The present study analyzed the pollen grains of *Cousinia* species, which were obtained from fresh samples gathered throughout field trips in various regions of central and northern Iraq. The sample collection period spanned one year, from 2021 to 2022 (Table 1). Multiple publications were employed to classify the plant specimens being examined (Post, 1933; Davis, 1975; AL-Rawi, 1964; Ghazanfer *et al.*, 2019). The specimens were conserved using a solution of ethyl alcohol at a concentration of 70%. Erdtman (1952) employed a sample preparing technique. An efficient method for preparing fresh pollen grains for examination under a Light Microscope and Scanning Electron Microscope is as follows: Select mature and unopened florets from multiple flower heads, place them on a glass slide, and apply some drops of acetolysis fluid (a mixture of 99% acetic anhydride and 95-97% sulfuric acid in a ratio of nine to one). The florets were dissected using two dissecting needles, and certain components, including the petals and pistil, were eliminated. Additionally, the stamina tube was taken away from the florets. Subsequently, the anthers were cracked open to collect the pollen grains. Following this, the components of the anther were eliminated, and the chosen pollen grains were subsequently moved onto fresh drops of glycerin on fresh glass slides. These glass slides were later imaged using a Light Microscope, and the cover slide was set on top. Pollen grains may also be deposited to Scanning Electron Microscope (SEM) stubs utilizing a technique called sputter coating with gold and then imaged under the SEM. Each slide was scrutinized using the light microscope, and precise measurements were obtained for a sample of (20-25) pollen grains. The ocular micrometer was used for measuring the length of the equatorial axis, polar axis, number of apertures, surface sculpturing, wall, diameter of the germination aperture, thickness of the grain, and length of the groove (colpus) of each pollen grain. The digital camera on the microscope, equipped with an oil immersion lens, captured these measurements. The description of pollen was based on the terminology established by (Erdtman, 1952; Halbritter *et al.*, 2018).

Table 1: Listing of species and locations under investigation

Species	Location	Elevation (m)
Cousinia aintabensis Boiss. & Hausskn.	Erbil, Shaqlawa and the road side between mosul and Dohuk.	347- 1033
Cousinia kopi-karadaghensis Rech. Fil.	Qara-Dagh mountain, kupi-Qara-dagh.	1534-1842
Cousinia macrolepis Boiss. & Hausskn.	Haibat Sultan Dagh, Pira-Magrun and the road side between Pira-Magrun and Dokan	708-1465
Cousinia odontolepis DC.	Amadiya, Zawita and Al-Sulaymaniyah	800-1400
Cousinia pergamacea Boiss. & Hausskn.	Penjwin and the road side between Penjwin and Said Sadaq.	1620-2037
Cousinia stenocephala Boiss.	Altun Kupri, Qara-Hanjer and the road side between Dokan and Tasloja.	300-877

RESULTS AND DISCUSSION

The characteristics of pollen grains of *Cousinia* species under study were delineated using both scanning electron microscopy and light microscopy (Figures 1, 2 and Table 2 & 3). The pollen grains of all the *Cousinia* species examined in this study were isopolar, monads, and tricolporate, consistent with prior palynological investigations (Schtepa, 1976; Susanna *et al.*, 2003; Khanbeygi *et al.*, 2011; Atazadeh *et al.*, 2020). The pollen grains of all *Cousinia* species examined were of considerable size, making them unsuitable as a distinguishing characteristic for species separation. The mean equatorial diameter was from 41 ± 1.39 µm in *C. pergamacea* to 49 ± 1.93 µm in *C. stenocephala*, whereas an average, the polar axis length varied from 51 ± 1.09 µm in *C. kopi-karadaghensis* to 59 ± 3.48 µm in *C. macrolepis*. The polar-to-equatorial diameter ratio varied from 1.06 in *C. stenocephala* to 1.40 in *C. macrolepis* (Table 2). The findings regarding pollen size are generally inconsistent with the taxonomic approach described by (Khanbeygi *et al.*, 2011), but align with the results reported by (Susanna *et al.* 2003; Atazadeh *et al.*, 2020). The pollen grains of the investigated *Cousinia* species have a morphology shape that can be described as prolate-spheroidal (P/E 1.01-1.14) in the species *C. kopi-karadaghensism*, *C. odontolepis*, and *C. stenocephala*. while, the species *C. aintabensis* stands out with a unique subprolate form (P/E 1.15-1.133). whereas, the species *C. macrolepis* and *C. pergamacea* possess prolate grains (P/E 1.34-2), as defined by (Moore *et al.*, 1991). These results align with the conclusions drawn from previous palynological studies on the genus *Cousinia* conducted by (Kuprianova and Tscherneva, 1982; Saber *et al.*, 2009). However, it differs from the taxonomic criteria outlined by (Atazadeh *et al.*, 2020). Saber *et al.* (2009) found that all taxa belonging to the subtribe Carduinae, with the exception of *Cousinia*, possess nearly one type of pollen shape. Meo & Khan (2003) examined the pollen features of *Cousinia minuta* They found that the pollen grains were semi-angular when viewed from the poles and had a prolate to spherical shape when viewed from the equatorial view.

Table 2: The dimension and form of pollen from various species of *Cousinia* were studied

Species	Polar axis µm	Equatorial axis µm	P/E	Shape
C. aintabensis	43 (55 ±2.34) 58	33 (43 ± 1.23) 49	1.27	Sub-Prolate
C. kopi-karadaghensis	39 (51 ± 1.09) 55	35 (46 ± 1.68) 51	1.10	Prolate-Spheroidal
C. macrolepis	46 (59 ± 3.48) 63	31 (42 ± 1.41) 47	1.40	Prolate
C. odontolepis	37 (53 ± 2.84) 57	36 (47 ± 1.87) 53	1.12	Prolate-Spheroidal
C. pergamacea	42 (57 ± 1.73) 61	32 (41 ± 1.39) 47	1.39	Prolate
C. stenocephala	41 (52 ± 2.49) 59	38 (49 ± 1.93) 55	1.06	Prolate-Spheroidal

Based on the assessment of the exine thickness, it is evident that there are variations in the thickness of the outer layer (exine) among the species under study. The species *C. kopi-karadaghensis* exhibited the lowest average exine thickness, measuring at 4.62±0.91 µm. On the other hand, the species *C. odontolepis* had the highest average exine thickness, measuring 7.22±0.86 µm (Table 3). These findings are in agreement with (Zafar *et al*., 2007). The obtained result aligns perfectly with the findings given by (Lopez-Vinyallonga *et al*., 2009). Regarding the average length of colpus, the study of the investigated species revealed that species *C. macrolepis* had the lowest average colpus length 38.93 ± 4.67 µm, whereas species *C. stenocephala* had the highest average colpus length of 46.83 ± 2.97 µm. The remaining species exhibited overlapping colpus lengths (Table 3). These findings are in agreement with (Saber *et al*., 2009).

Table 3: The pollen apertures and Exine data of pollen grains from several species of *Cousinia* under study

Species	Exine thickness (µm)	Pore width (µm)	Colpus length (µm)	Surface sculpturing
C. aintabensis	4.23 (5.32±1.21) 6.73	3.89 (5.51±1.28) 7.69	30.21 (39.71 ± 3.73) 46.37	Verrucate
C. kopi-karadaghensis	3.47 (4.62±0.91) 5.28	4.69 (6.97±1.63) 10.35	32.57 (43.64 ± 2.88) 48.52	Loosly Verrucate
C. macrolepis	4.68 (5.13±0.68) 6.21	4.13 (6.19±1.20) 8.51	30.89 (38.93 ± 4.67) 45.46	Very Verrucate
C. odontolepis	5.85 (7.22±0.86) 8.03	3.17 (4.12±0.76) 5.39	34.61 (44.57 ± 2.54) 50.72	Very Verrucate
C. pergamacea	3.69 (4.72±0.54) 5.41	4.51 (5.42±1.16) 7.48	31.10 (40.15 ± 3.41) 46.81	Verrucate
C. stenocephala	4.39 (5.28±0.96) 6.48	4.20 (6.11±1.31) 8.44	35.73 (46.83 ± 2.97) 53.59	Loosly Verrucate

Regarding pore width, species *C. odontolepis* exhibited the smallest average pore width of 4.12±0.76 µm, whereas species *C. kopi-karadaghensis* had the largest average pore width of 6.97±1.63 µm (Table 3). Wagenitz (1976) proposed that the Asteraceae family exhibits progressive traits in terms of pore width, colpus length, and exine thickness. The exines sculpturing of the species *C. macrolepis* and *C. odontolepis*, which have more than 35 verrucae, are classified as densely verrucate. On the other hand, *C. aintabensis* and *C. pergamacea*, which have 10-30 verrucae, are identified as verrucate. The species *C. kopi-karadaghensis* and *C. stenocephala* are characterized as having less than 10 verrucae and are loosely verrucate. Three distinct groups were identified within the studied species *Cousinia*, based on the morphology and abundance of verrucae upon the exine surfaces of the pollen. The grouping of species based on the structure and form of pollen is generally inconsistent regarding the taxonomic concept offered by (Attar and Djavadi, 2009), but aligns with the findings presented by (Susanna *et al*., 2003). In their study, Saber et al. (2009) classified species of *Cousinia* from *Stenocephala* Bunge section into two groups based on their abundance of verrucae. Group 1 included species with cylinder-like, clustered heads and more than 25 verrucae. Group 2 included species with solitary or 2-3 oval heads and 3-25 verrucae. This categorization was in line with their broader morphological findings. Nevertheless, these characteristics were deemed unsuitable or impractical for taxonomical classifying levels in the present investigation.

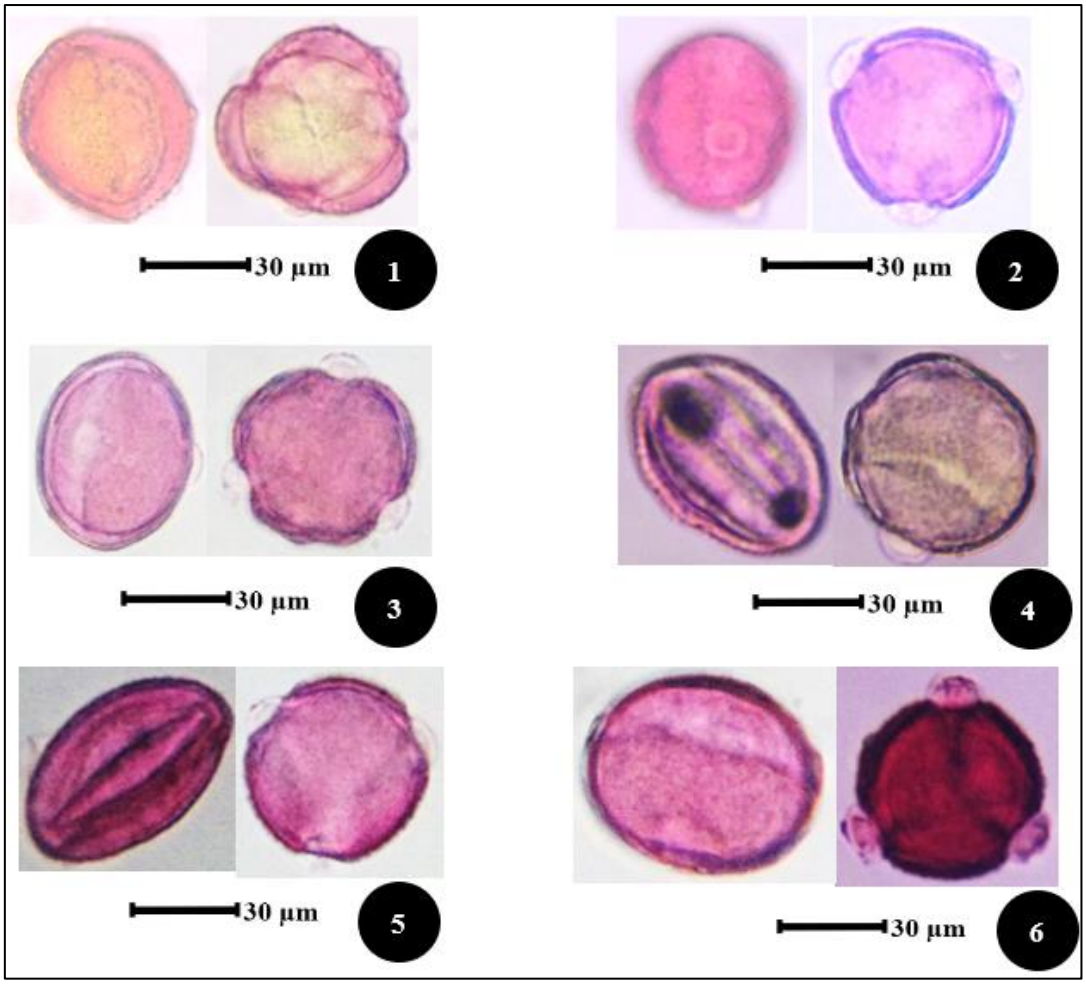

Figure 1: The pollen grains (light microscopy) of several species of *Cousinia* were examined. 1. *Cousinia aintabensis* 2. *Cousinia kopi-karadaghensis* 3. *Cousinia macrolepis* 4. *Cousinia odontolepis* 5. *Cousinia pergamacea* 6. *Cousinia stenocephala*.

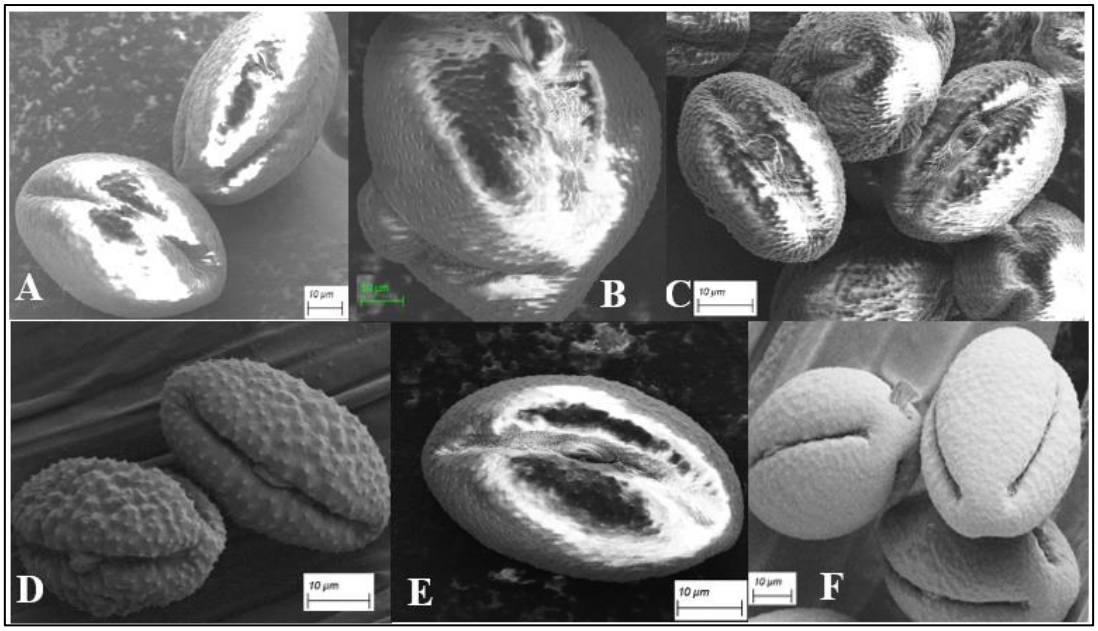

Figure 2: Micrographs obtained by scanning electron microscopy illustrate the distinctive characteristics of pollen in various species of *Cousinia*. A. *Cousinia aintabensis* B. *Cousinia kopi-karadaghensis* C. *Cousinia macrolepis* D. *Cousinia odontolepis* E. *Cousinia pergamacea* F. *Cousinia stenocephala*.

CONCLUSION

The current study shows that pollen characteristics, including form, surface sculpturing, exine thickness, and colpus length, are valuable traits for classification purposes. Additional investigations in morphology, karyology, palynology, and molecular biology will be helpful in resolving taxonomic issues pertaining to *Cousinia*.

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