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Pollen Micromorphological Study of Ten Genera of Brassicaceae in West Iraq Desert

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Abstract. This research included a micromorphological study of pollen grains of 11 taxa belonging to 10 plant genera of the Cruciferae family (Brassicaceae) distributed in Western Desert District of Iraq. The pollen grains of all species were monads, homopolar, regular, in addition to being tricolpate. They differed in shape, ranging from prolate, subprolate and Subprolate-subspheroidal. The polar dimension also differed greatly among species, which ranged between 25.2 μm . as an average in *Morettia philaeana* to 37.3 μm . as an average in *Zilla spinosa*. Thus, the equatorial dimension ranged between 21.4-30.1 μm . in the two species *Leptaleum filifolium* and *Rapistrum rugosum* respectively. As for the remaining species, they are graded between them. The difference in the length of the colpus resulted in important taxonomic characteristics in isolating species, if its length ranged between isolating 13.8 -30.3 μm . The Apocolpium also contributed through the variance that ranged between 3.9-8.6 μm ., as well as the mesocolpium, which also varied between species, the shortest was 7.2 μm . in *Morettia philaeana* and the longest 18.3 μm . in *Myagrurn perfoliatum* as indicated in the measurement tables. The two species, *Eruca vesicaria* and *Strigosella africana* are distinguished by their thin pollen walls (1.3 μm .), while *Morettia philaeana* had a thick wall (2.7 μm .), and then the remaining species came between them. This study identified two types of Ornamentation, namely, Microreticulate in 7 species and Macroreticulale in 4 species.

Keywords. Micromorphology, Desert, Mesocolpium.

1. Introduction

The study of the different morphological characteristics of pollen grains is considered one of the micromorphological characters, which has a very high taxonomic value, and that their sizes, shapes, the presence of spinulate, furrows and pores, and the type of ornamentation have provided very important taxonomic information and evidence [1]. It is one of the important bases in the diagnosis and classification of plants belonging to different plant families [2]. Many researchers considered it to be one of the most accurate bases for the diagnosis and isolation of different plant species [3]. And some researchers [4] mentioned that the traits the morphology of pollen grains has contributed a lot to solving the complex problems and the interrelationships between the different taxa. Despite the modernity of the science of pollen grains, it has made rapid progress, and the results of studies have proven that the pollen grains of one species are



very similar, closer to symmetry, and their characteristics differ the farther they are for the species belonging to different genera and families [5].

The same researcher also referred to the close relationship between pollen science and plant taxonomy, so that this relationship took two directions, namely, a morphological direction, concerned with studying the shape, size and composition of the pollen grain, as well as the number of germination pores, their distribution, and the structure of the wall, the second direction is geological, the pollen grain walls and their extreme resistance to environmental factors during the time periods and their ability to fossilize, as they preserved their intact walls in the rock's characteristic of successive geological ages.

By studying the anatomical description of the pollen grain wall, it became clear that there are two main layers, the outer Exine and the inner Intine, and their components whose importance is reflected in giving the type of ornamentation [6]. It is known that the researcher Carl Jalrus was the first to use the two previous terms on the pollen wall in the year 1937 and called for attention to them taxonomically [2]. Although the characteristics of pollen are not included in Flora in general. The modern interest has become clear through some pollen Flora dealing with pollen only, which came as an extension of the emergence of the electron microscope, as some researchers tended to develop a special pollen Flora, such as the Pollen Flora of South Africa [7]. It was followed by the Pollen Flora of Japan [8].

In Northwest Europe, the same Flora was completed by [9]. And recently, the scanning electron microscope was adopted in the completion of the atlas of pollen grains of Tropical West African plants, which included 364 species [10]. On the Arab level, came the Pollen Flora of Qatar [11], in which he mentioned an accurate description of the pollen grains of plant species growing in the State of Qatar and was reinforced by microscopic electron images, explaining their shapes, dimensions, numbers of pores and colpus shapes.

As for the studies related to the Cruciferae family, they are very few and do not include all genera, as [1] indicated that the Cruciferae family is almost stable of pollen forms, which were characterized by containing three colpus for germination (Tricolpate), as the same researcher described later [12] Pollen of 85 species belonging to 40 genera of the Cruciferae family. One of the important and recent studies is the study of pollen grains for 77 species belonging to 36 genera of Cruciferae plants in Pakistan using optical and scanning electron microscopy, and they noticed that the pollen grains appeared radially symmetrical, isopolar, and with similar shapes, such as the spheroidal shape, prolate, subprolate, and rarely oblate [13].

2. Materials and Methods

The pollen grains were studied after they were obtained from the anthers of the flowers of the plant samples collected during the field trips, as they were fixed directly in the field, emphasizing the newly opened flowers with the mature flower buds by means of the fixative solution consisting of:

- Absolute ethanol alcohol (50 ml).
- Glacial acetic acid (5 ml).
- Formaldehyde concentration 40% (10 ml).
- Distilled water (35 ml).

It is symbolized by the name of (F.A.A.) solution, and prepared according to what was reported by [14], where the fixation process took about 20 hours, then the samples were washed with 70% alcohol, then the samples were kept at the same concentration in glass bottles until they were studied. To clarify the process of pollen study, it was done by placing the anthers in a small glass plate and adding to it drops of the preferred dye safranin, which was indicated by [15]. Also, a drop of glycerin was added to the dye when examining pollen grains, according to [16].

The plant samples of the species were selected from different collection places according to the spread of the species and its geographical distribution. At least 20 pollen grains were studied for each species. The study focused on the special measurements of each pollen grain through the

polar view (P), the equatorial view (E), and the largest and smallest values for the two dimensions were determined with their averages, and the value of P/E was calculated as well as the thickness of the grain wall, the precise scale of the ocular micrometer was used to adjust the precise measurements, then the pollen grains were photographed by a special camera for the optical microscope under the objective (oil) lens with a magnification of 100X and an eyepiece with a magnification of 10X.

3. Results and Discussion

It was found during the study of pollen grains that they were Monads, Homopolar, and Regular, and contain Furrows for germination. And according to [12] mentioned about the division of pollen grains on the basis of size in general, depending on the lengths of their axes, the pollen grains of the species under this study fell into two categories: The first category is small in size (10-25 μm), and the second category is medium in size (25-50 μm). The results of the pollen study concluded the following:

3.1. Shape

Depending on the value of the ratio between the polar and equatorial axes, pollen forms were determined as follows:

- Elongated pollen grains at the poles (Prolate):
This group included pollen of *Eruca vesicaria*, *Schimpera arabica* and *Zilla spinosa*.
- Semi-elongated pollen grains at the poles (Subprolate):
This group included pollen of the species *C. didymus*, *L. filifolium* and *M. perfoliatum*.
- Pollen grains are semi-spherical - semi-elongated at the poles (Subspheroidal - Subprolate):
This group included pollen of the species *Carrichtera annua*, *Morettia philaeana*, *Rapistrum rugosum*, *Strigosella africana* and *Strigosella grandiflora* var. *glabrescens*.

As for the polar view, pollen grains of all species were triangular rounded, in which the three angles alternate with the three furrows in the site (fig. 1, 2, 3 and, 4).

By studying the tropical view of pollen, three groups have been distinguished:

- A group of pollen grains characterized by a broadly elliptic shape (the shape with the right half similar to the left, the top half similar to the bottom, the length/width ratio being 3/2, the widest area in the center of the shape, and tapering towards the top and bottom ends of the shape) and the species included *E. vesicaria*, *S. arabica* and *Z. spinosa*.
- A group whose pollen grains were characterized by a very broadly elliptic or so-called Rotund shape (similar to the previous shape, except that the ratio of length/width is 6/5), as in the species *C. didymus*, *L. filifolium*, *M. Perfoliatum* and *R. rugosum*.
- The last group, which included the rest of the species (4 species), and whose pollen grains are in the tropical view, is as close as possible to the circular shape and is called subcircular, where the polar dimension increases from the equatorial dimension by a very small percentage, ranging between 10-13%.

Through the study of 15 species of this family in Turkey [17], among which the two species *R. rugosum* and *S. africana* under the current study, were very similar in the dimensions of pollen grains, and similarity in the nature of the shape, and this is evidence of the family's taxonomic stability.

3.2. Size

Depending on the measurement of the longest of the two axes (polar or equatorial) the species was located within the pollen groups that [12] divided into 5 groups, it was found that all the species under the current study fall within the third group (pollen size between 25-50 micrometers) Which is called medium-sized pollen, with the exception of the type *Strigosella africana*, which is distinguished by pollen grains that fall into the second group with small

pollen grains (10-25) micrometers, noting that some species within the medium-sized group have come close in size to the pollen group small (27.7, 26.9 and 25.2 μm) as in species *C. annua*, *L. filifolium* and *M. philaeana* respectively (Table 1).

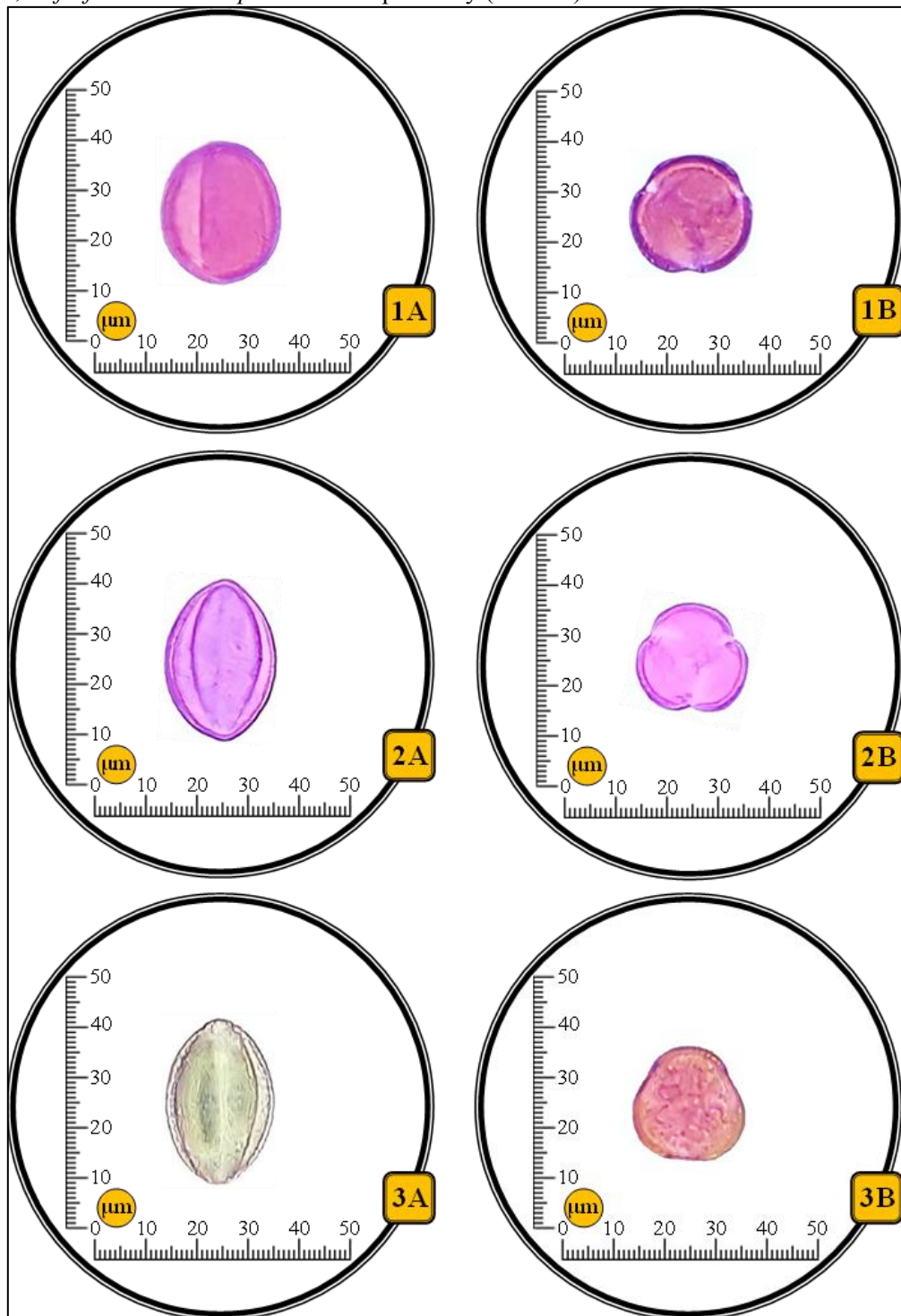


Figure 1. Species pollen (X 1000) 1- *Carrichtera annua* 2- *Coronopus didymus* 3- *Eruca vesicaria* ,Where: A= polar view, B= equatorial view.

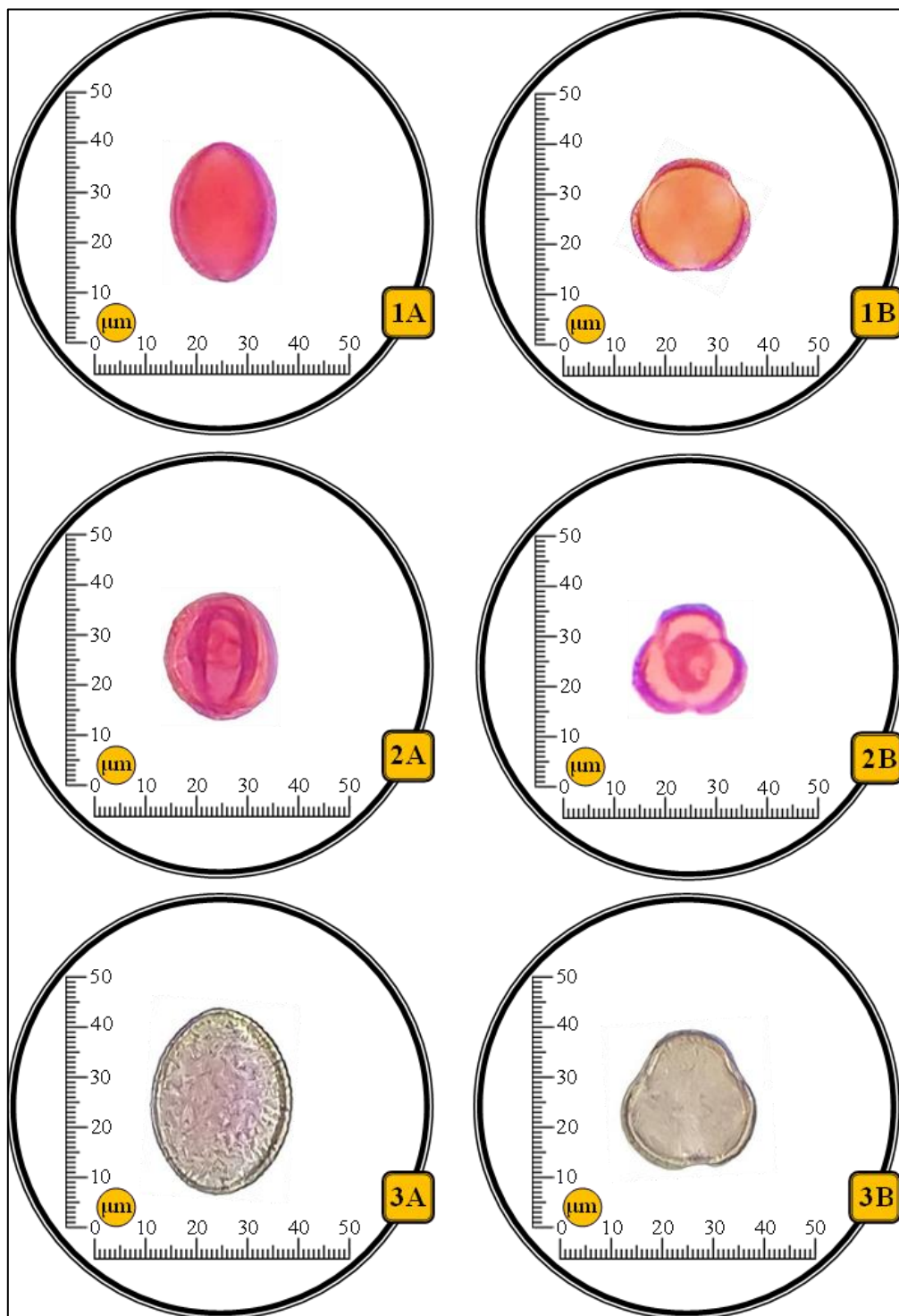


Figure 2. Species pollen (X 1000), 1- *Leptaleum filifolium* 2- *Morettia philaeana* 3- *Myagrurn perfoliatum* Where: A = polar view, B = equatorial view.

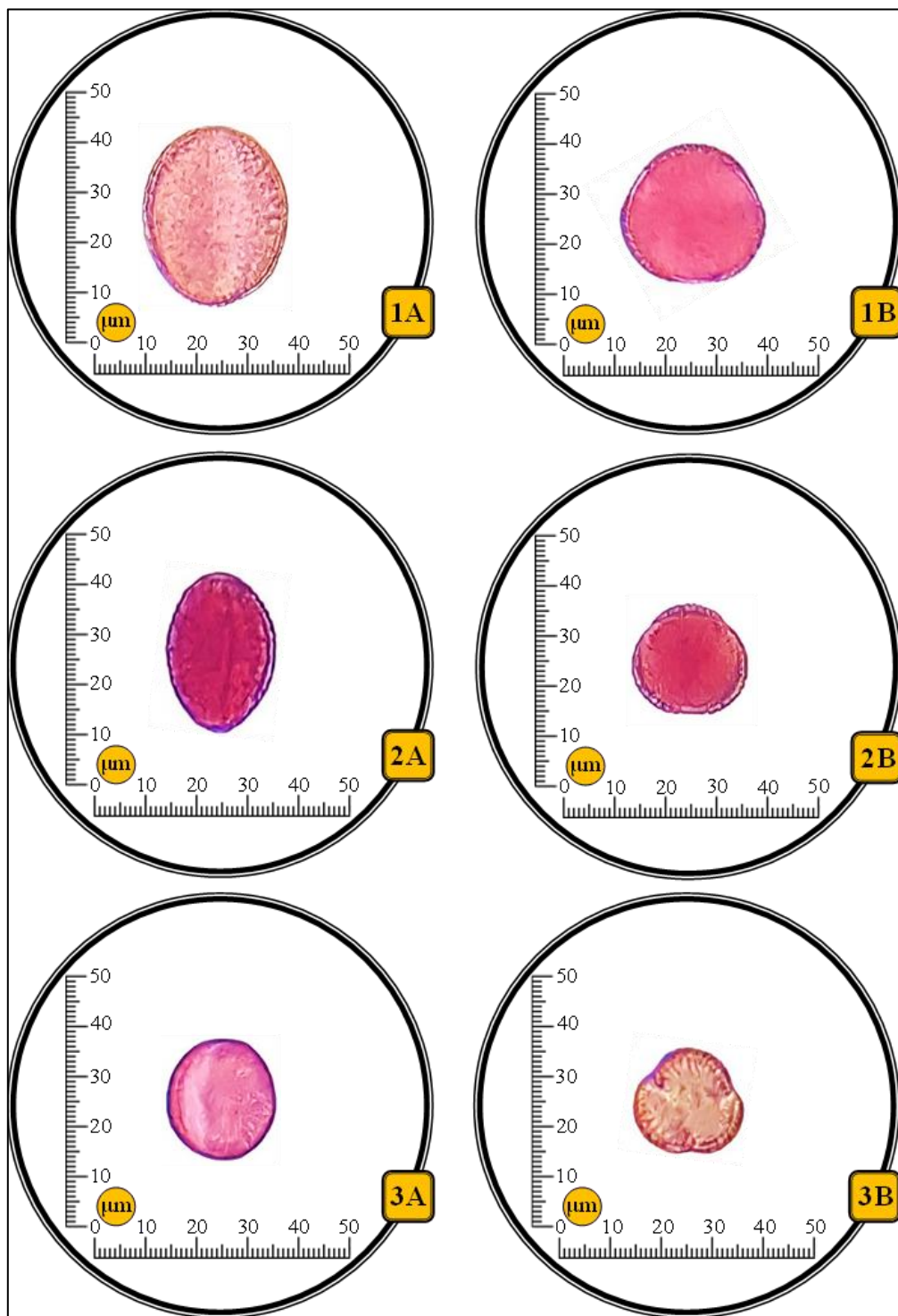


Figure 3. Species pollen (X 1000), 1- *Rapistrum rugosum* 2- *Schimpera arabica* 3- *Strigosella Africana*, Where: A = polar view, B = equatorial view.

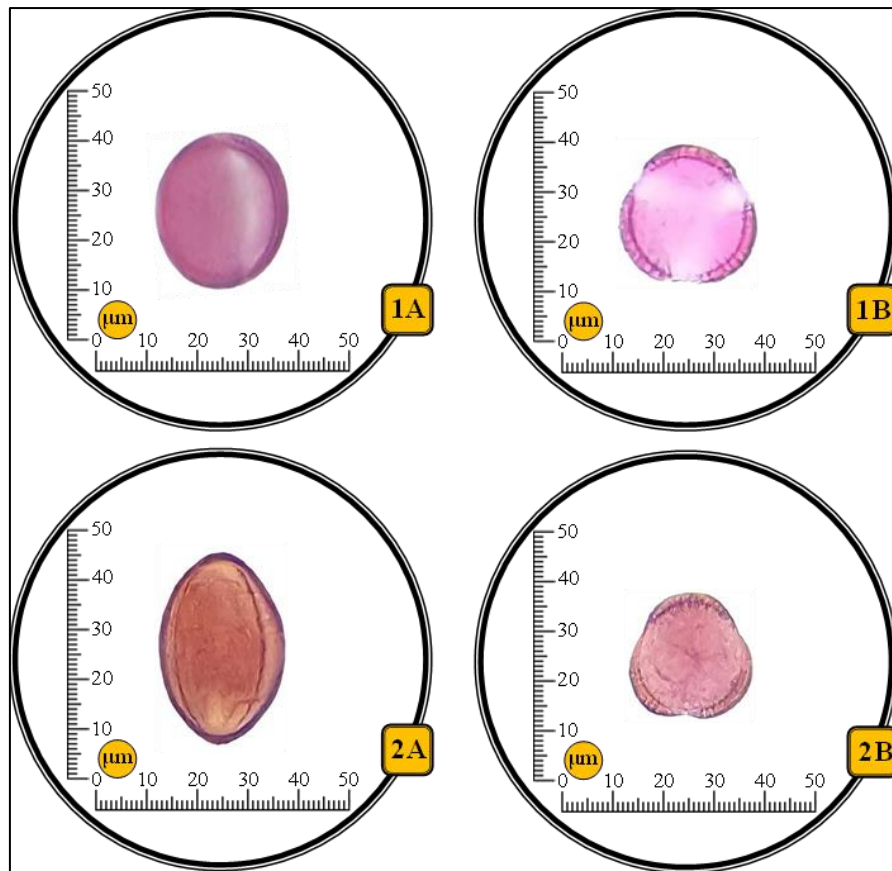


Figure 4. Species pollen (X 1000), 1- *Strigosella grandiflora* var. *glabrescens* 2- *Zilla spinosa*, Where: A = polar view, B = equatorial view.

3.3. Apertures

The current study of all species of pollen showed that it has germination pores represented by three furrows called Tricolpate, located between each of the two corners of the grain, and extending from the upper pole to the lower pole, and the furrows were characterized by being sharp or narrow ends, as for the middle region of the furrow, it is as wide as possible (the equator region). The lengths of the furrows varied between species, with the longest furrow (30.3 µm) in *Z. spinosa*, while the shortest furrow (13.8 µm) in *L. filifolium*, and the same is the case with the furrow width, which also varied, reaching a maximum Its width is (7.2 µm) in the species *S. arabica*, and its lowest width (2.9 µm) in the species *L. filifolium*.

Table 1. Dimensions of the polar and equatorial axes of pollen of species measured in micrometers.

Species	Polar axis length (Polar dimension) P	Equatorial axis length (Equatorial dimension) E	P / E
<i>Carrichtera annua</i>	(26.3-29.2) 27.7	(22.5-26.0) 24.4	1.13
<i>Coronopus didymus</i>	(29.0-31.8) 30.2	(23.0-25.5) 24.1	1.25
<i>Eruca vesicaria</i>	(25.3-28.1) 26.9	(20.2-22.8) 21.6	1.45
<i>Leptaleum filifolium</i>	(23.7-26.1) 25.2	(20.2-22.8) 21.6	1.25
<i>Morettia philaeana</i>	(30.1-33.3) 31.5	(21.3-24.7) 22.7	1.11
<i>Myagrurn perfoliatum</i>	(35.2-38.8) 37.1	(26.5-29.5) 28.8	1.28
<i>Rapistrum rugosum</i>	(33.6-37.5) 35.8	(28.0-32.2) 30.1	1.18
<i>Schimpera arabica</i>	(30.8-33.1) 32.2	(20.2-22.4) 21.5	1.49
<i>Strigosella africana</i>	(22.2-25.4) 23.8	(19.7-23.2) 21.6	1.10
<i>S. grandiflora</i> var. <i>glabrescens</i>	(29.8-32.7) 30.8	(25.4-28.3) 27.5	1.12
<i>Zilla spinosa</i>	(36.2-38.9) 37.3	(24.3-27.7) 26.2	1.42

* Measurements were taken from plant samples whose numbers ranged between 15-20 samples per species, the values inside the brackets represent the minimum and the highest, and the values outside represent the average.

3.4. Mesocolpium

The dimensions between each two furrows in the pollen grains of the species varied according to the sizes of the same grains. When the size of the pollen increases, the mesocolpium increases as well, and vice versa. The mesocolpium ranged from 7.2 μm in *M. philaeana* to 18.3 μm in *M. perfoliatum*, then the remaining species came between them.

3.5. Apocolpium

The values of the apocolpium for the species under current study ranged between 3.9 μm as an average diameter in *M. perfoliatum* to 9.8 μm as an average diameter in *S. arabica*, while the rest of the species were distributed between them, the apocolpium values varied among species, which is useful in successfully isolating it, sometimes the difference between the dimensions is almost double (4.4-8.6 μm) in the species *S. grandiflora* var. *glabrescens* and *C. annua* respectively. This study has proven that the apocolpium diameter is long when the Furrow is short, and vice versa when it is long, resulting in a very small apocolpium (Table 2).

3.6. wall Thickness

E. vesicaria and *S. africana* recorded the lowest value of pollen wall thickness among the species under current study, while *Z. spinosa*, *C. annua*, *S. grandiflora*, *L. filifolium* and *M. perfoliatum* recorded a gradual convergence in the wall thicknesses were 2.3, 2.4, 2.5, 2.6, and 2.7 μm , respectively. It seems that the five mentioned species are from dry desert plants whose pollen needs a thick wall to maintain their vitality from extreme environmental factors such as extreme heat, in contrast to the two species that have a thinner wall in pollen because they are spread in agricultural fields and wet places, and this what was confirmed by [18] when they studied pollen in 43 species belonging to 30 genera of Cruciferae plants in Turkey, among which was *Eruca vesicaria* (Table 3).

Table 2. Dimensions of the furrows, the distance between them and the polar spot measured in micrometers*.

Species	Colpus		Mesocolpium	Apocolpium
	length	width		
<i>Carrichtera annua</i>	20.4	4.8	11.6	8.6
<i>Coronopus didymus</i>	25.7	3.9	14.4	4.7
<i>Eruca vesicaria</i>	27.8	3.5	15.3	5.8
<i>Leptaleum filifolium</i>	13.8	2.9	15.6	6.2
<i>Morettia philaeana</i>	16.9	3.7	7.2	8.2
<i>Myagrurn perfoliatum</i>	28.7	4.6	18.3	3.9
<i>Rapistrum rugosum</i>	27.4	5.7	12.8	5.3
<i>Schimpera arabica</i>	18.1	7.2	10.3	9.8
<i>Strigosella africana</i>	17.2	4.2	11.6	6.2
<i>S. grandiflora</i> var. <i>glabrescens</i>	23.7	5.1	16.3	4.4
<i>Zilla spinosa</i>	30.3	4.4	17.2	6.3

* Measurements were taken from plant samples whose numbers ranged between 15-20 samples per species.

Table 3. The thickness of the pollen wall and some qualitative characteristics of the species measured in micrometers*.

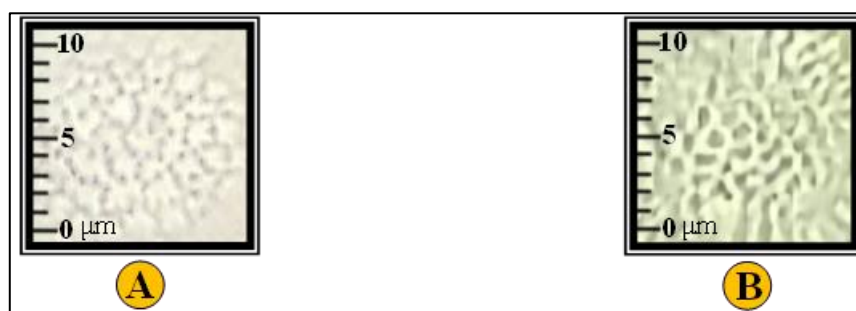
Species	an average wall thickness	pollen size	Pollen shape
<i>Carrichtera annua</i>	2.4	Medium - Small	Subspheroidal - Subprolate
<i>Coronopus didymus</i>	2.1	Medium	Subprolate
<i>Eruca vesicaria</i>	1.3	Medium	Prolate
<i>Leptaleum filifolium</i>	2.6	Medium - Small	Subprolate
<i>Morettia philaeana</i>	2.7	Medium - Small	Subspheroidal - Subprolate
<i>Myagrurn perfoliatum</i>	1.8	Medium	Subprolate
<i>Rapistrum rugosum</i>	1.5	Medium	Subspheroidal - Subprolate
<i>Schimpera arabica</i>	2.2	Medium	Prolate
<i>Strigosella africana</i>	1.3	Small	Subspheroidal - Subprolate
<i>S. grandiflora</i> var. <i>glabrescens</i>	2.5	Medium	Subspheroidal - Subprolate
<i>Zilla spinosa</i>	2.3	Medium	Prolate

* Measurements were taken from plant samples whose numbers ranged between 15-20 samples per species.

3.7. Ornamentation

The pollen grains of the species under study showed their stability through the great similarity in the nature of the Ornamentation, which was of the reticulate type, as the stitches or grid lines on the surface formed a group of polygonal cavities of different shapes and sizes called Heterobrochate, and depending on the size of the cavities were divided the pollen of the species into two groups:

- Pollen grains with a rough reticulated Ornamentation with large hollows called Macroreticulate, as in *M. perfoliatum*, *R. rugosum*, *S. africana* and *Z. spinosa*.
- Pollen grains with a very fine reticulated Ornamentation called Microreticulate, as in the rest of the species under current study (fig. 5).

**Figure 5.** Ornamentation of pollen grains. A: Macroreticulate, B: Microreticulate

[19] indicated that the Ornamentation of pollen grains, of most species of the Cruciferae family, was characterized by the smooth-walled Reticulate pattern, which appeared clear in 7 species under the current study, and this is another evidence of the taxonomic stability of the family. The Ornamentation became clearer and more important through the study carried out by [20] through the adoption of a scanning electron microscope to study 12 species of Cruciferae plants in Saudi Arabia, including *E. vesicaria*.

One of the signs of stability of the Cruciferae family in its different taxa is the lack of variations in relation to pollen grains and the stability of its various characteristics mentioned above (Figure 1), in contrast to some plant families that have wide variations that affected the taxonomic determinants sometimes, and the results of the current study showed a very large similarity in pollen characteristics compared to other studies in Iraq such as [21 and 22]. In addition to the study of [23] for pollen grains of plants of the same family in Jordan. And the study of [24] of the species of the genus *Arabis* in Turkey. And that this stability in traits has prompted many researchers to adopt pollen grains as a taxonomically, some researchers have successfully [25] been able to rely on the accurate micromorphological traits of pollen grains in isolating the two subspecies of *Alyssum strigosum* Banks & Sol. From the Cruciferae family in Afghanistan. As for [26], the optical microscope has adopted 6

varieties belonging to 3 species of the genus *Brassica* L. by studying the micromorphological pollen grains (shape, dimensions, furrows, Ornamentation and apocolpium). The researcher [27] was also interested in pollen through his study of three species belonging to different genera in Iraq, among which was the species *Rapistrum rugosum*, who's precise micromorphological characteristics were identical to the comprehensive and accurate description of pollen grains found in the current study.

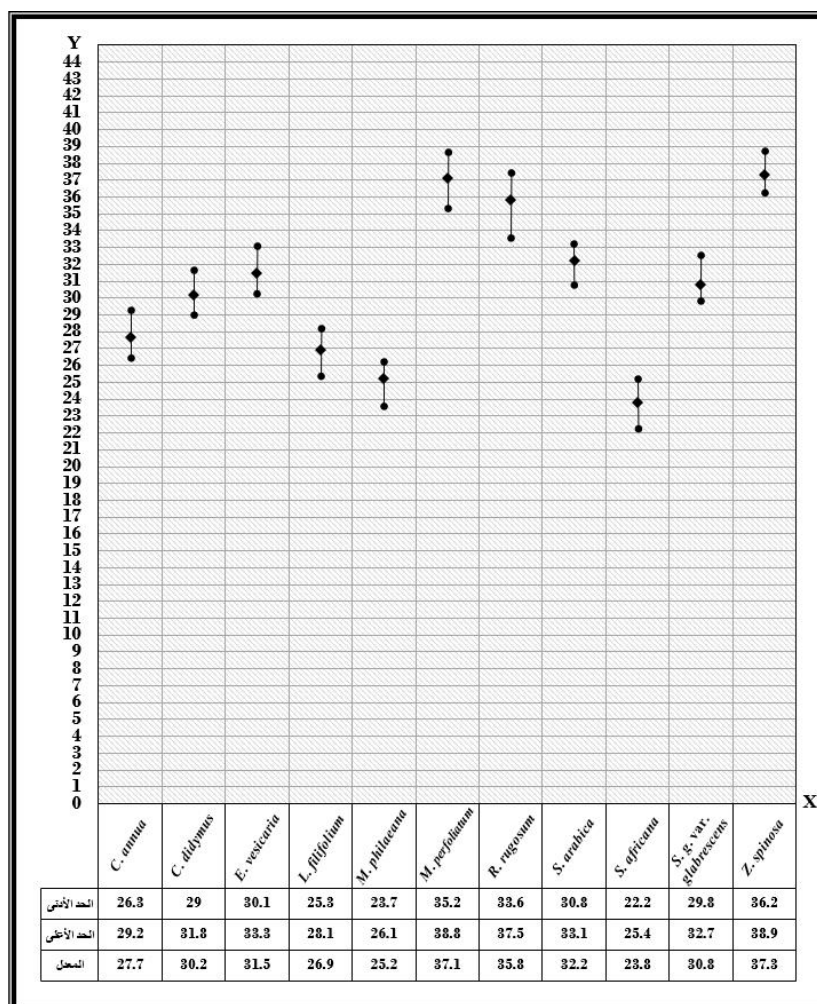


Figure 6. Variations in the polar axes of pollen of species measured in micrometers

Where: X= plant species, Y= pollen grain diameter

Conclusion

The precise characteristics of pollen have been characterized by stability in all taxonomic ranks to a very large extent, and they can be successfully adopted in diagnosis, and they can be sequenced according to taxonomic importance as follows: polar dimension, equatorial dimension, furrow length, apocolpium, pollen grain shape, decoration Surface and wall thickness.

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