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Pollen Variations among some Cultivated *Citrus* Species and its Related Genera in Egypt

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Abstract

The present investigation aims to study the pollen morphology and ultrastructure of pollen grain characteristics for nine *Citrus* species and three related genera cultivated in Egypt. The pollen grains were photographed by using both Light Microscope (LM) and Scanning Electron Microscope (SEM). Twelve qualitative and quantitative pollen morphological characters were used to differentiate among the studied taxa. Statistical analysis of palynological data indicated that the pollen size, shape, colpi length, apertures number and type, ora size, amb shape, mesocolpium diameter, and the exine ornamentation were the most distinguished characters in the circumscription of the studied taxa and were of taxonomic value. On the contrary, the other studied pollen characters including the ratio between Polar length/Equatorial diameter (P/E), ora shape and exine thickness were not found to be of taxonomic value in the differentiation among the closely related taxa of *Citrus, Fortunella margarita,* Limequat hybrid and *Poncirus trifoliate* in the present study.

Keywords: *Citrus; Fortunella margarita*; Limequat hybrid; Pollen morphology; *Poncirus trifoliate*; *Rutaceae*

Abbreviations: LM: Light Microscope; SEM: Scanning Electron Microscope; LSD: Least Significant Difference; SAS: Statistical Analysis System

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Introduction

Cultivated *Citrus* are derived from various *Citrus* species found in the wild. Some are only selections of the original wild types, while others are hybrids between two or more ancestors. Many *Citrus* types were identified and named by individual taxonomists, resulting in a large number of identified species: 870 by a 1969 count [1].

Citrus plants belong to family *Rutaceae*, they are characterized by having different life forms as trees and shrubs. *Citrus* species may frequently contain aromatic compounds with pellucid glands on the stems, leaves and fruits. The leaves are usually opposed, compound and without stipules, sometimes with thorns [2]. According to Engler [3], *Rutaceae* is divided into seven subfamilies, he defined these subfamilies primarily by gynoecium characters especially the fruit type. *Citrus* species and its related genera are closely related and all belong to subtribe Citrinae, tribe *Citreae*, of the orange subfamily *Aurantioideae*. The *Citrus* fruit is of berry or hesperidium type. Species within the genus *Citrus* are highly economic and medicinal plants distributed all over the world [4]. Several taxonomists have classified various kinds of *Citrus* species into groups and given them valid names [5-9]. Nicolosi [10] mentioned that there are two currently outstanding systems of classification for *Citrus*. They are those of Swingle [11] and Swingle and Reece [4] of the USA, and Tanaka [12] of Japan. From the standpoint of the grower, most horticulturists and other plant scientists, Swingle’s system appears to be the most useable.

According to Swingle's system the Citrinae subtribe is subdivided into three groups, the 'primitive *Citrus*' distant relatives, the closer 'near *Citrus*' including *Citrus*-related genera like *Atalantia*, and the 'true *Citrus*', which included *Poncirus*, *Citrus*, *Fortunella*, *Eremocitrus*, *Microcitrus*, and *Clymenia*, all but the first now viewed to fall within *Citrus*. He subdivided *Citrus* into two subgenera: the first subgenus *Eucitrus* (later called simply subgenus *Citrus*) includes citrons, *pomelos*, mandarins, oranges, grapefruits, and lemons, while the hardy but slow-growing trees with relatively unpalatable fruit he placed in subgenus *Papeda*.

Distinguishing of *Citrus* species and related genera according to morphological and geographical distribution are very difficult because *Citrus* contains an enormous degree of genetic variation, with abundant natural hybridization [13]. The classification of the genus *Citrus* are complex and the precise number of natural species is unclear, as many of the named species are hybrids clonally propagated through seeds (by apomixes) and there is genetic evidence that even some wild, true-breeding species are of hybrid origin [4,14]. As a result of hybridizations between *Citrus* species, there is confusion around correct botanical names of commonly known *Citrus* [10,15]. In Egypt, there are no wild *Citrus* species [16]. All the present species are introduced and cultivated. Now, Egypt is considered as one of the most leading countries in cultivating and exporting orange, ranking as the sixth-largest producer and the second-largest exporter in the world [17].

The use of pollen morphological characters is important in plant taxonomy, as Davis and Heywood [18] indicated. The use of pollen morphology in solving taxonomic problems has been used for a long time ago [19-26]. This work is considered as a step in finding way in differentiating between nine *Citrus* species and its related genera.

This study aims to investigate and assess the relationships among nine *Citrus* species as well as three related genera cultivated in Egypt using pollen morphological characters.

Materials and Methods

The present investigation was carried out on mature trees of nine *Citrus* species and three related genera; *Citrus aurantifolia* (Christm.) Swing., *Citrus aurantium* (L.), *Citrus grandis* (L.) Osbeck, *Citrus latifolia* Tanaka, *Citrus limetta* Risso. *Citrus paradisi* Macf., *Citrus reshni* Hort. ex Tanaka, *Citrus reticulate* Blanco, *Citrus sinensis* (L.) Osbeck, *Furtunella margarita* (Lour.) Swing. and Limequat hybrid, which is a cross hybrid between the *Citrus aurantifolia* (Christm.) Swing. and *Furtunella japonica* (Thunb.) Swing., and *Poncirus trifoliate* (L.) Raf. grown in a private orchard. This orchard located 120 Km away from Alexandria on Alexandria-Cairo desert road. These species are identified by the aid of the faculty of Agriculture, Alexandria University, as well as vouchers of the studied taxa are allocated there.

The trees were grown in sandy soil and received the same cultural practices as usually done in each orchard. Four uniform trees were selected from each *Citrus* species and related genera, from which mature anthers were taken from the uppermost flowers of the branches to obtain the mature pollen grains used in this investigation.

Pollen grain samples of all studied taxa were acetolyzed according to Erdtman's technique [19]. The acetolyzed samples were used for both Light and Scanning Electron Microscopy. Slides were prepared from acetolyzed portion of pollen grains for Light Microscope examination by mounting in glycerin jelly, examined and measured using Zeiss Light Microscope with a pre-calibrated eye-piece micrometer. Measurements given are the means of forty acetolyzed well-developed pollen grains from each taxon.

Pollen grains of the acetolyzed portion were dehydrated in ethanol series placed onto coverslips, left for ethanol evaporation then attached to copper stubs by double-sided tape, coated with 30 nm gold using fine coat ion sputter JEOL JFC 1100E, examined and photographed at 30 KV using JEOL JSM-3500 Scanning Electron Microscope present in the Faculty of Science, Alexandria University. The terminology used here is those of Faegri [27] and Erdtman [19].

Statistical analysis

The mean values of the pollen characters, of all the taxa under investigation, were separated and calculated. They were then compared using the Least Significant Difference (LSD) test at 0.05 level of probability, according to Snedecor and Cochran [28]. The statistical analysis was performed using Statistical Analysis System (SAS) version 9.13 [29].

Results

Pollen grain morphology

Shape: The results obtained from the twelve studied taxa are summarized in **Table 1** and were illustrated in plates 1-12. The pollen grains of all taxa were monads, radially symmetric, isopolar and were different in size. The pollen shape varied from prolate spheroidal to sub-prolate except in *F. margarita* **(Figure 1)**, where it was oblate spheroidal. The mean polar axis length varied from a minimum of 26 µm in both *F. margarita* and Limequat to a maximum of 34.48 µm in *C. grandis*. Moreover, the mean equatorial diameter ranged from a minimum of 26 µm in both *F. margarita* and Limequat to a maximum of 33.44 µm in *C. grandis*. While the minimum ratio of the mean length of Polar Axis/ Mean Equatorial Diameter (P/E) was 1 in both *F. margarita* and Limequat, while the maximum mean ratio was 1.2 in *C. latifolia*.

Aperture: The types of apertures were either colpate or colporate ranged from three to five in number. The variations in the type and number of apertures were found to be within the same taxa and within the same anther as well. Five groups of aperture types were found; the first group included three taxa characterized by tri-tetra-colporate aperture types, *C. limetta* **(Figure 2a and 2b)**, *C. reshni* **(Figure 3a, 3b and 3d)** and Limequat **(Figure 4a, 4b and 4d)**. The second group included also three taxa characterized by tetra-penta-colporate aperture types in *C. aurantifolia* **(Figure 5a, 5b and 5d)**, *C. reticulata* **(Figure 6a, 6b and 6d)** and *P. trifoliata* **(Figure 7a-7c)**. The third group has only *F. margarita* which included the tetra-penta-colporate types, in addition to the tri-colporate ones **(Figure 1a, 1b, 1d and 1e)**. The taxa in the fourth group characterized by tri-tetra-colpate and tri-tetra-colporate aperture types in *C. grandis* **(Figure 8a-8c and 8e)** and *C. sinensis* **(Figure 9a and 9b)**. Finally, group five comprised *C. aurantium*, *C. latifolia* and *C. paradisi* with pollen grains that have tetra-penta-colpate and tetra-penta-colporate types of apertures **(Figures 10a-10d, 11a-11c, 11e and 12a, 12c)**, respectively.

The ecto-aperture colpi, in all the studied taxa were long, wide, with rounded or pointed ends, equally spaced around the equator. They were characterized by uneven margins and covered with granular membranes. The mean colpi length varied within the studied taxa from a minimum of 20 µm in both *F. margarita* and

Table 1. Pollen morphological characters of the studied *Citrus* species and it's related taxa.

Sr. No.	Characters → / Taxa ↓	Common name	P. L. (µm)	E. D. (µm)	P/E R.	Pol. Sh.	Ap.	C. L. (µm)	Meso. D. (µm)	Ora L. (µm)	Ora W. (µm)	Amb Sh.	Ex. Th. (µm)	Ex. Or.
1	*C. aurantifolia* (Christm.) Swing.	Mexican lime	31.3	27.5	1.14	3	2	27	9.7	2.08	7.71	2	2.4	4
2	*C. aurantium* L.	Sour orange	30.7	28.3	1.09	2	5	25.4	9.36	3.2	8.3	2	2.7	3
3	*C. grandis* (L.) Osbeck	Pummelo	34.5	33.4	1.03	2	4	29.1	11.22	3.06	7.9	1	2.4	1
4	*C. latifolia* Tanaka	Tahiti lime	31.6	26.4	1.2	3	5	26.4	9.36	2.97	7.69	2	2.4	4
5	*C. limetta* Risso	Sweet lime	33.7	31.8	1.07	2	1	28.4	12.72	3.2	7.3	1	2.4	4
6	*C. paradise* Macf.	Marsh grapefruit	33.1	30.6	1.09	2	5	27.9	10.54	3.3	6.9	2	2.4	1
7	*C. reshni* Hort. ex Tanaka	Cleopatra mandarin	29.8	27.5	1.09	2	1	24.5	9.78	4.2	8.1	1	2.4	2
8	*C. reticulata* Blanco	Clementine tangerine	30.5	28.1	1.09	2	2	24.8	10.3	2.9	6.8	2	2.4	2
9	*C. sinensis* (L.) Osbeck	Succari orange	32.2	29.6	1.1	2	4	27.6	10.4	3.6	7.2	1	2.4	1
10	*Fortunella margarita* (lour.) Swing.	Oval Kumquat	26	26.2	1	1	3	21	9.82	3.5	6.08	3	2.4	3
11	Limequat (Hybrid) [*C. aurifolia* (Christm.) Swing.X *F. japonica* (Thunb.) Swing.]	Limequat (hybrid)	26.6	26.1	1.02	2	1	21	9.82	3	6.4	1	2.4	3
12	*Poncirus trifoliata* (L.) Raf.	Trifoliate orange	32.3	31.2	1.04	2	2	26.4	10.72	3.2	6.4	2	2.4	3
	$LSD_{0.05}$		0.93	0.89	0.03			0.94		0.71	0.44	0.8		0.04

P. L.: Polar Length in µm; E.D.: Equatorial Diameter in µm; P/E: Ratio between Polar length/Equatorial diameter; Pol. Sh.: Pollen Shape (1. Oblate spheroidal, 2. Prolate spheroidal, 3. Sub-prolate); Ap.: Aperture number and type (1. Tricolporate and tetracolporate, 2. Tetracolporate and pentacolporate, 3. Tricolporate, tetracoporate and pentacolporate, 4. Tricolpate, Tetracolpate and Tricolporate, Tetracolporate, 5. Tetracolpate, Pentacolpate and Tetracolporate, Pentacolporate); C.L.: Colpi Length in µm; Meso. D.: Mesocolpi Diameter in µm; Ora L.: Ora Length in µm; Ora W.: Ora Widthin µm; Amb Sh.: Amb Shape (1. Rounded, triangular and squared, 2. Squared and rounded, 3. Rounded triangular, Squared and rounded); Ex. Th.: Exine Thickness in µm; Ex. Or.: Exine Ornamentation (1. Tectate-perforate, 2. Tectate-perforate to microreticulate, 3. Foveolate 4. Reticulate)

Limequat to a maximum of 29.08 µm in *C. grandis*. Moreover, the mean mesocolpium diameter varied from a minimum of 9.36 µm in *C. aurantium* and *C. latifolia* to 12.72 µm in *C. limetta*. The endo-apertures pori were lalongate in all the studied taxa, where the ora width ranged from 6.08 µm-6.4 µm in *F. margarita*, Limequat and *P. trifolia*; slightly wider from 6.8 to 7.3 µm in *C. reticulate*, *C. paradise*, *C. sinensis* and *C. limetta* and more than 7.3 µm in the rest of the taxa. The amb shapes are mostly rounded triangular or square and sometimes both shapes are found. The pollen amb was rounded triangular and square in *C. limetta* **(Figure 2a and 2b)**, *C. reshni* **(Figure 3b and 3d)**, Limequat **(Figure 4b and 4d)**, *C. grandis* **(Figure 8c and 8e)** and *C. sinensis* **(Figure 9a and 9b)**. While it was square and rounded in *C. aurantifolia* **(Figure 5b and 5d)**, *C. reticulata* **(Figure 6b and 6d)** and *P. trifoliata* **(Figure 7b and 7d)**, *C. aurantium* **(Figure 10b)**, *C. latifolia* **(Figure 11c and 11e)**, *C. paradisi* **(Figure 12a and 12c)**. In addition, the amb was rounded triangular, square and rounded *F. margarita* **(Figure 1b, 1d and 1e)**.

Exine: The exine is considerably thin; it was 2.4 µm thick in all the studied taxa, except in *C. aurantium* it was 2.7 µm. The exine ornamentation of the pollen grains of the studied taxa; as observed by the scanning electron microscope appeared in four different types. The first type was tectate perforate with smooth tectum, which is provided by more or less rounded pores in *C. grandis* (**Figure 8d**), *C. sinensis* (**Figure 9c**) and *C. paradisi* (**Figure 12b**). The second type was tectate perforate to microreticulate with latimurate reticulum, which is characterized by more or less straight and smooth muri and rounded to oval small-sized lumina in *C. reshni* (**Figure 3c**), and *C. reticulata* (**Figure 6c**). The third type was foveolate with latimurate reticulum, which is characterized by more or less straight and smooth muri and nearly rounded large-sized lumina in *F. margarita* (**Figure 1c**), Limequat (**Figure 4c**) and *P. trifoliata* (**Figure 7c**) and *C. aurantium* (**Figure 10c and 10d**). The fourth type was reticulate with angustimurate reticulum, which is characterized by straight and rough muri and the lumina were different in size and shape in *C. limetta* (**Figure 2c**), *C. aurantifolia* (**Figure 5c**) and C. *latifolia* (**Figure 11d**).

Pollen grains classification

Accordingly, the studied taxa can be classified into three different

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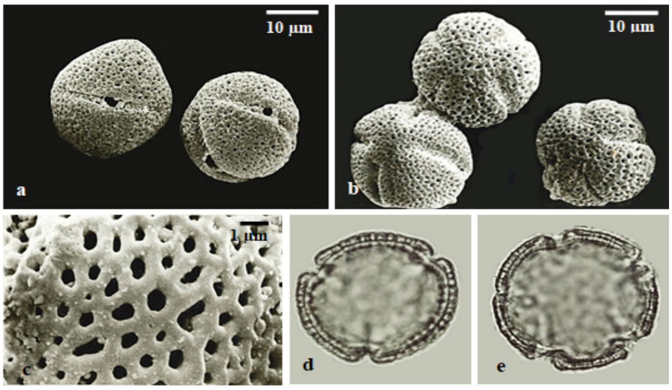

Figure 1 SEM (**a-c**) and LM (**d** and **e**) photomicrographs of *Fortunella margarita* (colpate, aperture number); **c:** Exine ornamentation; **d:** Polar view (aperture number).

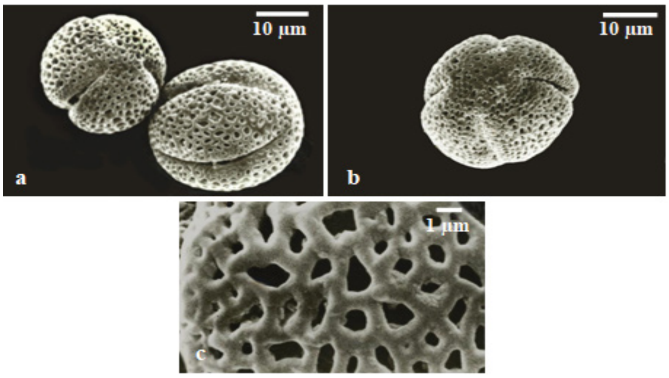

Figure 2 SEM (**a-c**) photomicrographs of *C. limetta* pollen grains; **a:** Equatorial and polar views (colporate, aperture number); **b:** Polar view (aperture number); **c:** Exine ornamentation.

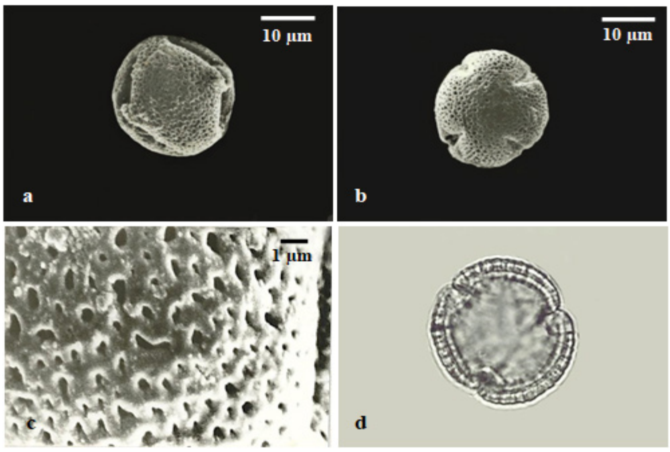

Figure 3 SEM (**a-c**) and LM (**d**) photomicrographs of *C. reshni* pollen grains; **a:** Equatorial view (colporate); **b:** Polar view (aperture number); **c:** Exine ornamentation; **d:** Polar view (aperture number).

groups according to their pollen characters: The first group included five taxa; *C. grandis*, *C. limetta*, *C. paradise*, *C. sinensis*

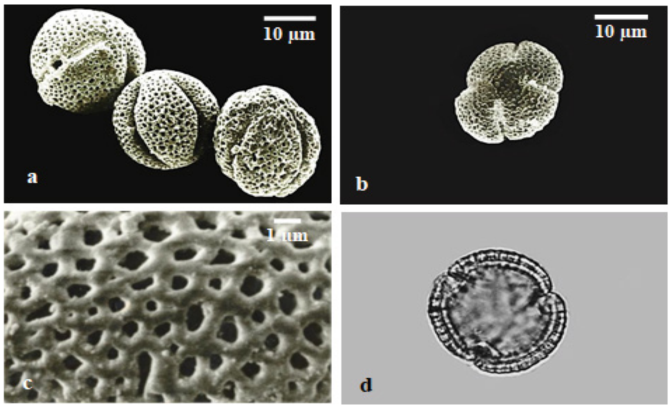

Figure 4 SEM (**a-c**) and LM (**d**) photomicrographs of Limequat hybrid pollen grains; **a:** Equatorial view (colpate, colporate); **b:** Polar view (aperture number); **c:** Exine ornamentation; **d:** Polar view (aperture number).

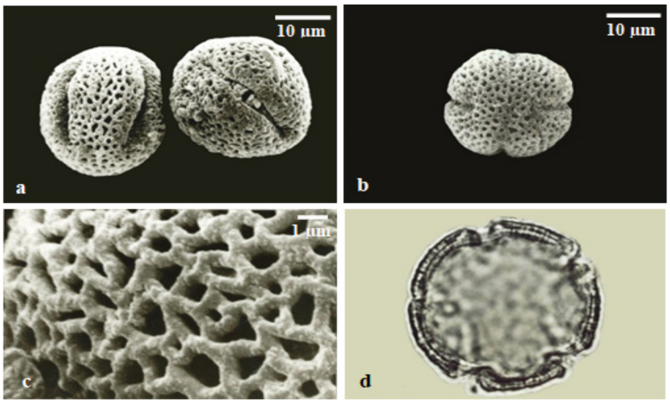

Figure 5 SEM (**a-c**) and LM (**d**) photomicrographs of *C. aurantifolia* pollen grains; a: Equatorial view (colpate and colporate); **b:** Polar view (aperture number); **c:** Exine ornamentation; **d:** Polar view (aperture number) .

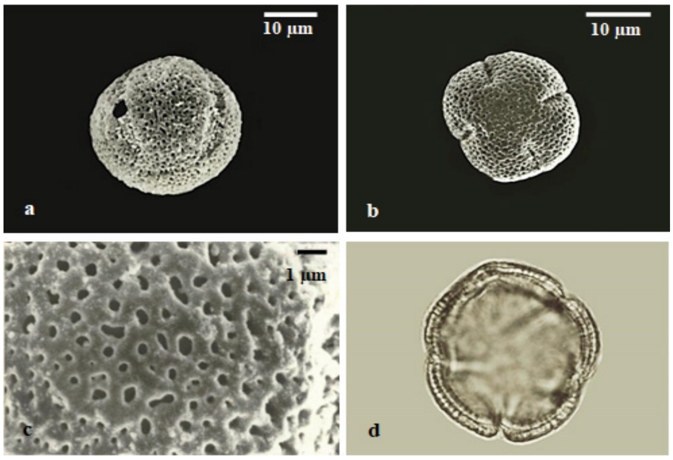

Figure 6 SEM (**a-c**) and LM (**d**) photomicrographs of *C. reticulata* pollen grains; **a:** Equatorial view (colporate); **b:** Polar view (aperture number); **c:** Exine ornamentation; **d:** Polar view (aperture number).

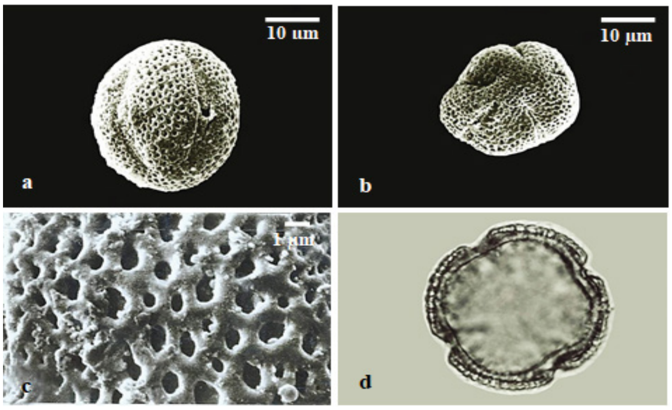

Figure 7 SEM (**a-c**) and LM (**d**) photomicrographs of *Poncirus trifoliata* pollen grains; **a:** Equatorial view (colporate); **b:** Polar view (aperture number); **c:** Exine ornamentation; **d:** Polar view (aperture number).

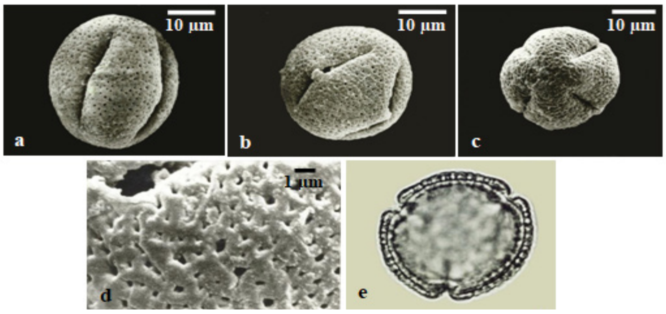

Figure 8 SEM (**a-d**) and LM (**e**) photomicrographs of *C. grandis* pollen grains; **a:** Equatorial view (colpate); **b:** Equatorial view (colporate); **c:** Polar view (aperture number); **d:** Exine ornamentation; **e:** Polar view (aperture number).

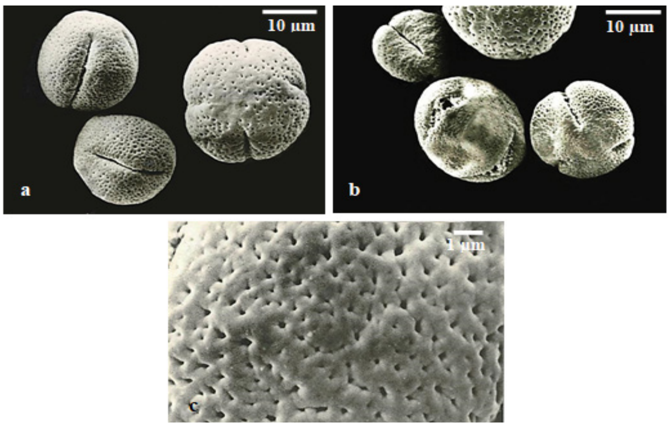

Figure 9 SEM (**a-c**) photomicrographs of *C. sinensis* pollen grains; **a:** Equatorial and polar views (colpate, aperture number); **b:** Equatorial and polar views (colporate, aperture number); **c:** Exine ornamentation.

and *P. trifoliate*. These five taxa were characterized by the biggest pollen size; where the polar axis length was more than 32.20 µm, with prolate spheroidal pollen shape, colpi length more than 26.44 µm and mesocolpium diameter exceed 10.30 µm.

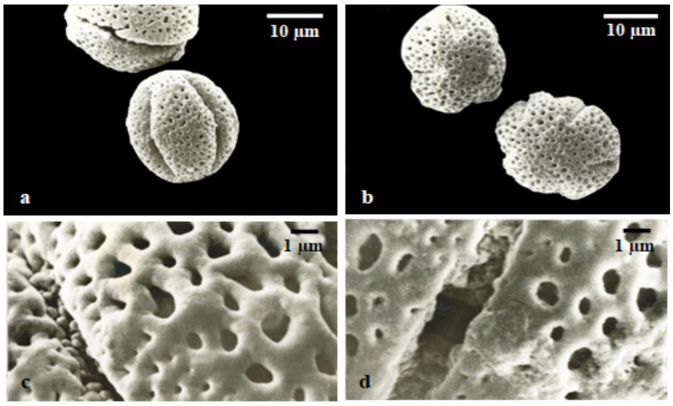

Figure 10 SEM (**a-d**) photomicrographs of *C. aurantium* pollen grains; **a:** Equatorial view (colpate and colporate); **b:** Polar view (aperture number); **c:** Exine Ornamentation (colpate); **d:** Exine ornamentation (colporate).

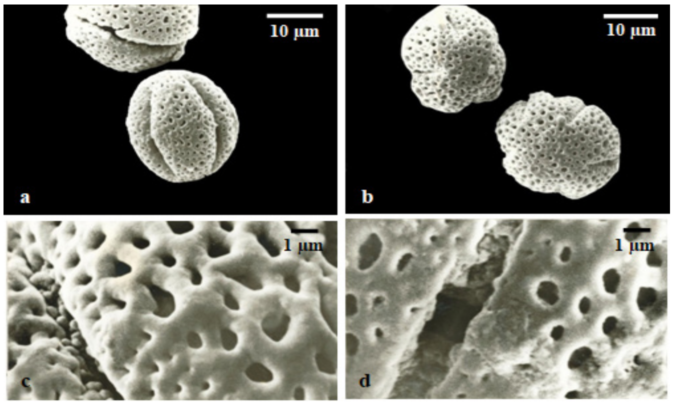
10 μm
a
10 μm
b
1 μm
c
1 μm
d

Figure 10 SEM (**a-d**) photomicrographs of *C. aurantium* pollen grains; **a:** Equatorial view (colpate and colporate); **b:** Polar view (aperture number); **c:** Exine Ornamentation (colpate); **d:** Exine ornamentation (colporate).

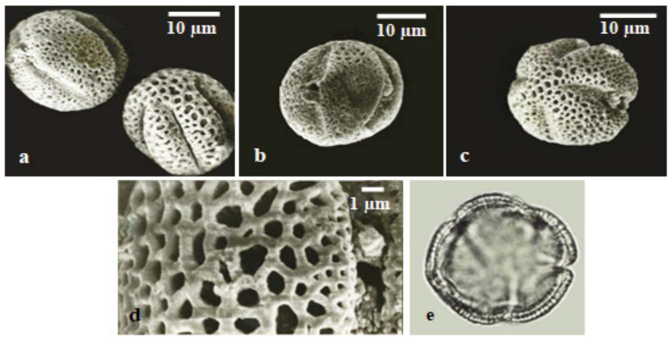

Figure 11 SEM (**a-d**) and LM (**e**) photomicrographs of *C. latifolia* pollen grains; **a:** Equatorial view (colpate); **b:** Equatorial view (colporate); **c:** Polar view (aperture number); **d:** Exine ornamentation; **e:** Polar view (aperture number).

The second group included five taxa as well; *C. aurantifolia*, *C. aurantium*, *C. latifolia*, *C. reshni* and *C. reticulate*. These taxa

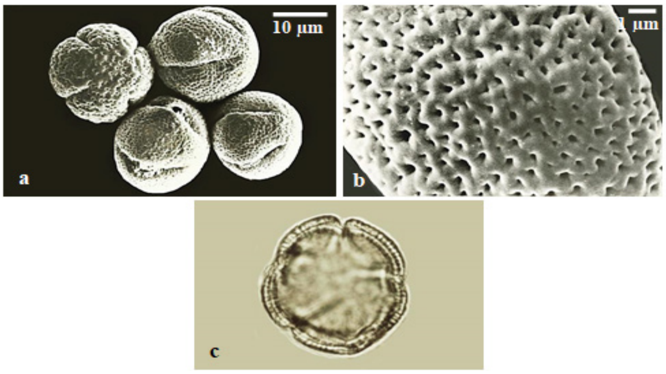

Figure 12 SEM (**a-b**) and LM (**c**) photomicrographs of *C. Paradise* pollen grains; **a:** Equatorial and polar views (colpate, colporate, aperture number); **b:** Exine ornamentation; **c:** Polar view (aperture number).

have medium polar axis length, ranged from 29.80 to 31.58 with prolate spheroidal and sub-prolate shapes, colpi length ranged from 24.48 µm to 26.44 µm and mesocolpium diameter ranged from 9.36 µm to 10.30 µm. While the third group included two taxa only; *F. margarita* and Limequat. Both species have the polar axis length ranged from 26 µm to 26.6 µm, oblate spheroidal or spheroidal in shape, with shorter colpi ranged from 20.96 µm to 20.02 µm and mesocolpium diameter of about 9.82 µm.

Statistical analysis

In the present study, palynological investigations indicated that variations in pollen morphological characters were of taxonomic significance. In particular, the twelve studied taxa were found to be significantly different from each other in six quantitative pollen characters out of eight; this includes polar length, equatorial diameter, colpi length, ora length, ora diameter, mesocolpi diameter. While the ratio between the Polar length and Equatorial diameter (P/E) and the exine thickness were insignificantly different from each other (**Table 1**).

Discussion

Pollen morphology has been used since a long time ago in solving taxonomic problems at different levels; families, genera and species [19,23,27,30] Genus *Citrus* and its related two genera: *Fortunella* and *Poncirus* are considered one of the important economic and medicinal fruits in the world, they are rich plants in vitamin C and volatile oils. The problem within these taxa is the frequent hybridizations between their species, which made their taxonomy very confusing.

The classification and species delimitation of the genus has long been a controversial issue by a number of authors as Swingle [11], who included only 16 species in *Citrus*, while Tanaka [9] described 162 species, but Scora [15] and Barett and Rodes [31] defined only three true species within the genus *Citrus*, which are Pummelo (*C. grandis L. Osbeck*), Citron (*C. medica L.*) and Mandarin (*C. reticulata Blanco.*). They indicated that all other *Citrus* species resulting from hybridization between these basic species. Later, Scora [32] added another true species *C. halimii* Stone.

Pollen morphology of Rutaceae has been examined by several workers; they found their taxonomic significance in different taxonomic levels, depending on the plant groups [19,25,33,34]. However, there are no reports on pollen morphology of *Citrus* species in Egypt. Inyama et al. [25] found that palynological characters were useful in delimiting six studied *Citrus* species and thus could be exploited in conjunction with other evidence in species identification and characterization, while they were insignificant in the reclassification of the investigated taxa. In the present study, palynological investigations indicated that variations in pollen morphological characters were of taxonomic significance.

In particular, the twelve studied taxa were significantly different from each other in six quantitative pollen characters: polar length, equatorial diameter, colpi length, ora length, ora diameter, mesocolpi diameter. These results were in agreement with those reported by Breis et al. [35] and Mohammad et al. [36]. The pollen shape varies from oblate-spheriodal, prolate-spheriodal to sub-prolate in all the studied taxa. This finding agrees with that found by Ye et al. [37] and Mohammad et al. [36]. The variations of pollen size were suggested by Kozaki and Hirai [38] and Mohammad et al. [36] where they reported that pollen grain of *C. grandis* and *Poncirus trifoliate* had larger pollen than *C. latifolia*, *C. limetta* and *F. margarita*, while those of *C. aurantium*, *C. sinensis* and *C. reshni* were intermediate in size. These suggestions were in agreement with the results of the present study where the studied taxa classified into three different groups according to their pollen size. The first group included *C. grandis*, *C. limetta*, *C. paradise*, *C. sinensis and P. trifoliate*, which have the largest pollen grains. The second group included *C. aurantifolia*, *C. aurantium*, *C. latifolia*, *C. reshni* and *C. reticulate* have medium size pollen grains. While the third one included two taxa *F. margarita* and Limequat with the smallest pollen grains. On the contrary, these groups did not coordinate with Al-Anbariet al. [34], which recognized four groups in the Iraqi pollen grains based on pollen size and exine ornamentation only.

Meanwhile, the most variable characters found in the present investigation were within the number and type of apertures, exine ornamentations, ora width as well as mesocolpium diameters. Ye et al. [37] and Mohammad et al. [36] used both ora width and mesocolpi diameters as valuable characters in the identification of *Citrus* species; these results agree with the results of this work.

Grant et al. [39] found considerable variation in pollen morphology of subfamily Aurantioideae, which divided the studied taxa into five pollen types. The differences include aperture number, ecto-colpus shape and size, exine ornamentation and wall structure. When designating pollen types for the subfamily Aurantioideae, the principal characters used were the aperture number and exine ornamentation. These characters were in concomitant with the obtained results and as a conclusion, the aperture type and ora size were the most distinguished characters in the circumscription of the studied taxa. According to the type and number of aperture five types were observed in the studied taxa. Type (1) Tri-tetra-colporate was found in *C. limetta*, *C. reshni* and Limequat. Type (2) Tetra-penta-colporatewas found in *C. aurantifolia*, *C. reticulata* and *P. trifoliata*. Type (3) Penta-

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colporate was found in *F. margarita*. Type (4) included both "tri-tetracolpate and tri-tetra-colporate" were found in *C. grandis* and *C. sinensis*. Finally, type (5) included both "tetra-penta-colpate and tetra-penta-colporate" were found in *C. aurantium*, *C. latifolia* and *C. paradisi*. These multi types of pollen apertures were found in the studied species from the same anther which may be due to chromosomal abnormalities as mentioned by Stace et al. [40].

In this study, the exine thickness was an insignificant character, while the exine ornamentations showed great variations in the sculpturing types and have taxonomic value in the classification of the studied taxa. According to the exine ornamentations four different types were observed. Type (1) Tectate perforate in *C. grandis*, *C. paradisi* and *C. sinensis*. Type (2) Tectate perforate to micro reticulate in *C. reshni*, *C. reticulata*. While Type (3) Foveolate with latimurate reticulum in *C. aurantium*, *F. margarita*, Limequat and *P. trifoliata*. Type (4) Reticulate with angustimurate reticulum, in *C. aurantifolia*, *C. latifolia* and *C. limetta*. These findings agree with those found by Ye et al. [37] and Mohammad et al. [36]. While disagree with the results of Kozaki and Hirai [38], who stated that the exine patterns was sub-reticulate in the species of *Citrus*, *Poncirus* and *Fortunella*.

Conclusion

In the present investigation, the pollen size, pollen shape, colpi length, the apertures number and type, ora size, amb shape, mesocolpium diameter, and exine ornamentation were the most distinguished characters in the circumscription of the studied taxa. All the studied pollen grain characters except ora shape and exine thickness could be considered of taxonomic value in the differentiation among the closely related taxa of *Citrus*, *Fortunella*, Limequat hybrid and *Poncirus* in the present study.

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