

## Pollen Morphology of some Species of Genus *Ficus* L. (Moraceae) from Egypt

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IN THE PRESENT work, the pollen morphological characters were extensively studied in 17 taxa from genus *Ficus* (Moraceae), based on examination with light and scanning electron microscopy. The studied taxa represent three subgenera (*Ficus*, *Sycomorus* and *Urostigma*), collected from three Egyptian Botanical Gardens (Orman, Ain-Shams and Zoo-garden). The obtained results of fundamental and evolutionary significance were: pollen size (less than 20 µm), pollen shape (circular or elliptic), pollen class (vary from di- to triporate), and the pollen surface sculpture (psilate or finely scabrate). The dendrogram produced from the cluster analysis of the characters of pollen grains have been discussed, and shed light on the relationships, similarities and dissimilarities between the studied taxa. Pollen data only does not support macromorphological evidence for the identification of *Ficus* species.

**Keywords:** Moraceae, *Ficus*, pollen, morphology, taxonomy, Egypt.

The first and very simple subdivisions of *Ficus* was proposed by Thunberg in 1786 and adopted by Vahl in 1805 (Berg 2003a). *Ficus* is the old Latin name of fig (Stearn, 1996), and constitutes one of the largest genera of angiosperms (Frodin, 2004), consisting of about 1000 species from pan-tropical and subtropical origins (Wagner *et al.*, 1999) and form a distinctive monophyletic clade within the family (Judd *et al.*, 1999).

*Ficus* is represented in wild Egyptian flora by three taxa: *F. salicifolia* Vahl, *F. pseudosycomorus* Decne and *F. carica* L. var. *rupestris* Hausskn. All of which are either of rare or of very rare occurrence (Täeckholm, 1974). Boulos (1999) changed the name of these taxa; *F. salicifolia* Vahl into *F. cordata* Thunb. subsp. *salicifolia* (Vahl); *F. pseudosycomorus* Decne into *F. palmata* Forssk., and *F. carica* L. var. *rupestris* Hausskn. into *F. carica* L. However, the number is compensated by numerous cultivated taxa that were introduced to Egypt, particularly during the late 19<sup>th</sup> century and the 1<sup>st</sup> half of the 20<sup>th</sup> century. *Ficus carica* L. is cultivated in Egypt since historic ages.

One of the most widely adopted infrageneric classification of *Ficus* is that of Corner (1965). In that classification, *Ficus* is divided into four subgenera (*Ficus*, *Pharmacosycea*, *Sycomorus* and *Urostigma*), with the functionally dioecious species united under the subgenus *Ficus*, but in the most recent classification by Berg and Corner (2005), *Ficus* is divided into six subgenera and a number of sections.

Burn and Mayle (2008) studied pollen morphology of some genera of Moraceae, Urticaceae and Cecropiaceae which were represented in Amazonian rainforest communities. They demonstrated that all grains are isopolar small monads with tectate exine; pores are always circular and some grains exhibit a slightly protrusion or thickening of the exine around the pores (annulus sensu Punt *et al.*, 2007). Sculpturing of the sexine is usually psilate or scabrate. The shape class (P/E) varies between oblate and subprolate while some genera exhibiting spheroidal characteristics.

El-Ghazali (1989) studied the pollen morphology of *Ficus bengalensis*, *F. inteophylla*, *F. religiosa* and *F. sycomorus*. All are characterized by ellipsoidal, diporate apertures; pores simple open with annulus situated at the opposite ends of the grain, slightly sunken. Exine tectate imperforate of uniform thickness and endexine slightly thicker than ectexine. Collumellae indistinct and sculpturing psilate. Dimensions less than 25µm in long axis or in short axis.

*Ficus* have species which are occasionally monoporate or triporate (Jago and Boyd, 2003). Khan *et al.* (2011) reported all of *Ficus* species had monad and diporate pollens and they showed very little variation in their exine thickness. *Ficus* pollens tend to have thinner and smoother exine and they tend to become more asymmetric than other genera of Moraceae family.

Little attention has been paid to the pollen morphology of *Ficus*. Therefore, the present study was conducted to show how far the pollen morphological variations could be used to distinct between the studied taxa of *Ficus*.

### Material and Methods

In total, 17 taxa including 15 species; one variety and one subspecies from *Ficus* were examined (Table 1). The samples were collected from three recognized subgenera *Ficus*, *Urostigma* and *Sycomorus* (Corner, 1965) obtained from three Egyptian gardens: Orman Botanical Garden (OBG), Dokky, Giza, Egypt; Ain Shams Botanical Garden (AB), Ain Shams University, Egypt and Zoo-Garden (ZG), Dokky, Giza, Egypt. Identification was verified according to W<sup>3</sup> TROPICOS (2008), GRIN (2008) and IPNI (2008).

TABLE 1. Species collection.

No.	Species	Subgenus	Source
1	<i>Ficus afzelii</i> G. Don. in J. C. Loudon, Hort. Brit. ed. 1: 416. 1830 = <i>F. saussureana</i> Dc.	<i>Urostigma</i>	OBG
2	<i>F. benghalensis</i> L. in Sp. Pl. 2:1059. 1753 = <i>F. indica</i> L.	<i>Urostigma</i>	OBG
3	<i>F. benjamina</i> var. <i>comosa</i> (Roxb.) Kurz in Forest Fl. Burma 2: 446. 1877	<i>Urostigma</i>	AB
4	<i>F. cordata</i> Thunb., subsp. <i>salicifolia</i> (Vahl.) = <i>F. salicifolia</i> Vahl in Symb. Bot. 1: 82. 1790.	<i>Urostigma</i>	AB
5	<i>F. cunninghamii</i> Miq. In Ann. Mus. Bot. Lugd. Bat. iii, 286-Austral.	<i>Urostigma</i>	OBG
6	<i>F. deltoidea</i> Jack, In Malayan Misc. 2(7): 71. 1822. = <i>F. diversifolia</i> Blume.	<i>Ficus</i>	AB
7	<i>F. elastica</i> Roxb. ex Hornem in Hort. Bot. Hahn. Suppl. 7. 1819 = <i>F. decora</i> hort.	<i>Urostigma</i>	BGA
8	<i>F. infectoria</i> Roxb. In Ann. Bot. Gard. Calcutta, i. l. t. 75, 84(1887) = <i>F. virens</i> Aiton.	<i>Urostigma</i>	ZG
9	<i>F. laurifolia</i> Hort. ex Lam., In Encycl. Meth. (Bot.) 2: 495. 1786. = <i>F. inspida</i> wika = <i>F. glabrata</i> H. B. K. = <i>F. anthelemintica</i> Mart	<i>Urostigma</i>	ZG
10	<i>F. macrophylla</i> Desf. ex Pers. In Syn. Pl. 2: 609. 1807 = <i>F. magnolioides</i> Borzi	<i>Urostigma</i>	OBG
11	<i>F. mysorensis</i> B. Heyne ex Roth in J. J. Roemer & J. A. Schultes, Syst. Veg. 1: 508. 1817(A. W. Roth, Nov. Pl. sp. 390. 182 = <i>F. drupacea</i> var. <i>pubescens</i> (Roth) corer	<i>Urostigma</i>	OBG
12	<i>F. platypoda</i> (Miq.) A.Cunn ex Miq. In, Ann. Mus. Bot. Lugduno-Batavum 3: 287. 1867 = <i>Urostigma platipodum</i> .	<i>Urostigma</i>	OBG
13	<i>F. racemosa</i> Wall-Cat. 1799 = <i>F. glomorata</i> Roxb. In Pl. Coromandel 2: 13, t. 123.	<i>Sycomorus</i>	OBG
14	<i>F. religiosa</i> L. in Sp. Pl. 2: 1059. 1753	<i>Urostigma</i>	AB
15	<i>F. retusa</i> L. = <i>F. nitida</i> Thunb. In <i>Ficus</i> 10. 1786.	<i>Urostigma</i>	AB
16	<i>F. spragueana</i> Mildbr. & Burret in, Bot. Jahrb. Syst. 46: 253. 1911.	<i>Urostigma</i>	OBG
17	<i>F. sycomorus</i> L. In Sp. Pl. 1059. 1753.	<i>Sycomorus</i>	OBG

Fresh material was used for all the sampled species and pollen extraction method was prepared according to Moore *et al.* (1991). The dimensions of the polar axis (P), equatorial axis (E), pore diameter and the ratio of polar and equatorial axis (P/E) were calculated using the Light Microscopy (LM). The mean value ( $\bar{x}$ ) of 20 counts, the range of each parameter and the ratio of polar and equatorial axis (P/E) was calculated (Table 2). A standard x100 phase oil immersion objective was used with an X<sub>16</sub> photo eye piece.

To obtain the maximum amount of systematic data from SEM studies of pollen, both acetolyzed and non-acetolyzed materials were studied. Valuable systematic characters may be lost in acetolysis and true pollen shape may be greatly changed. Study of fresh (non acetolyzed pollen) can give greater insight into the functional significance of pollen characters which, in turn, can contribute to better systematic treatment (Harley and Ferguson, 1990).

Scanning Electron Microscopy (SEM) examination was done by two models of SEM at different official centers: (1) A JEM-1200 EX II EL Micrographs with a JEOL UHR Camera 90561 Japan 6X7 cm Adapter and JFC-1100 E Ion Sputter Coated in Central Lab at Faculty of Science, Ain Shams University, Cairo, Egypt; (2) A JOEL JSM-25 SII SEM and JFC-1100 E, Ion Sputtering Device at Faculty of Science, Alexandria University, Alexandria, Egypt. The terminology follows that of Punt *et al.* (2007).

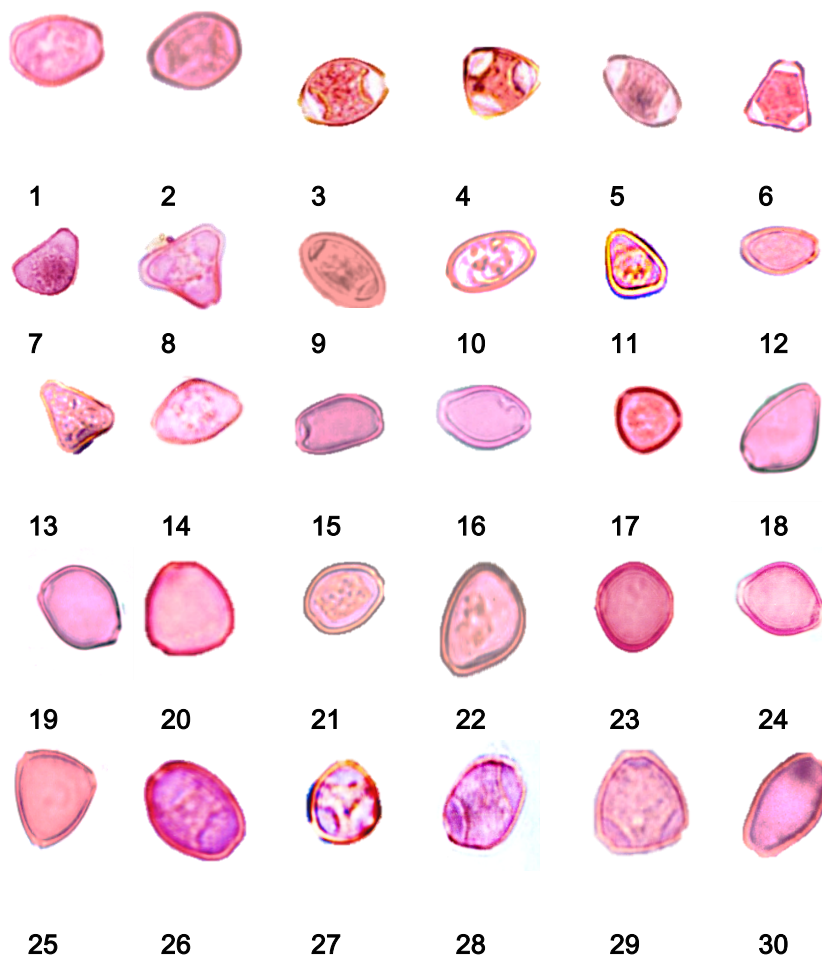
Pollen morphology was analyzed using the NTSYS-pc program version 2.02 (Exeter Software, NY, USA; Rohlf, 1998). The results were presented in dendrogram which have been discussed to shed light on the relationships, similarities and dissimilarities between the studied taxa. The similarity matrix was used in the cluster analysis by using the NTSYS-pc software version 2.02 (Exeter Software, NY, USA; Rohlf, 1998). This method is known as Unweighted Pair Group Method using Arithmetic Average (UPGMA) using Sequential Agglomerative, Hierarchical and Nested cluster (SAHN) (Sneath and Sokal, 1973).

### Results and Discussion

The variation in the shape class as showing in Table 2: Prolate; P/E=2-1.33 (8:4-8:6); in *Ficus afzelii*, *F. benamina* v. *comosa*, *F. cunninghamii*, *F. deltoidea*, *F. infectoria*, *F. laurifolia*, *F. religiosa* and *F. sycomorus*. Subprolate; P/E=1.33-1.14 (8:6-8:3), in *Ficus benghalensis*, *F. mysorensis*, *F. racemosa*, *F. cordata* and *F. spragueana*. Prolate spheroidal; P/E=1.14-1.00 (8:7-8:8), in *Ficus elastica*, *F. macrophylla*, *F. platypoda* and *F. retusa*.

The pollen grain shape is ellipsoidal in the following *Ficus* species: *afzelii*, *cunninghamii*, *platypoda* and *religiosa* (Table 2; Plate1; 1, 2, 9, 21, 22 & 26). While it is ellipsoidal and triangular; in *benghalensis*, *benamina* var. *comosa*, *deltoidea*, *elastica*, *racemosa*, *infectoria*, *laurifolia*, *macrophylla*, *mysorensis*, *retusa*, *cordata* and *sycomorus* (Table 2; Plate1; 3-8, 10-20, 23-35 & 27-30). From the obtained data, it was found that the use of the pollen shape character in evaluating the phylogenetic relationships between the studied taxa is of little value; this is in agreement with Scotland (1992a, b and 1993).





**Plate I.** 1-30: LM X1600. Pollen morphology: 1, 2. *Ficus afzelii*; 3, 4. *F. benghalensis*; 5, 6. *F. benamina* var. *comosa*; 7, 8. *F. cordata* subsp. *salicifolia*; 9. *F. cunninghamii*; 10, 11. *F. deltoidea*; 12, 13. *F. elastica*; 14. *F. infectoria*; 15, 16. *F. laurifolia*; 17. *F. macrophylla*; 18, 19, 20. *F. mysorensis*; 21, 22. *F. platypoda*; 23, 24, 25. *F. racemosa*; 26. *F. religiosa*; 27. *F. retusa*; 28, 29. *F. sperguana*, 30. *F. sycomorus*.

Regarding dimensions (Table 2) and according to the length of the longest grain axis; whatever this axis is polar or equatorial, all investigated pollen grains are small sized (less than 20  $\mu\text{m}$ .); this is in agreement with (Kremp, 1965; Bank *et al.*, 2000; Ueckermann and Rooyen, 2000). The pollen aperture which recorded in the current study was porate type. Concerning the number of apertures the obtained results showed that it was diporate in *F. afzelii*, *F. cunninghamii*, *F. platypoda* and *F. religiosa* (Table 2; Plate I; 3 Plate II, 1, 13 & 16) while it was diporate and triporate in the rest of the studied taxa (Table 2; Plate I; 3 & 4 - Plate II, 3, 4, 10 & 11). Jago and Boyd (2003) reported that *Ficus* have species which

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are occasionally monoporate or triporate, while Khan *et al.* (2011) studied some *Ficus* species had diporate.

Moore *et al.* (1991) recorded that the increased number of apertures provides a better opportunity for the pollen tube to emerge close to the stigma surface than does a single one. The obtained results as in Table 3 showed that the studied taxa grouped according to the pore width into three categories. Pore width narrow (1.2-1.4  $\mu\text{m}$ ) in *F. deltoidea*, *F. infectoria*, *F. laurifoila*, *F. retusa* and *F. platypoda*. Relatively narrow (1.5-2  $\mu\text{m}$ ) in *F. cunninghamii*, *F. elastica*, *F. racemosa*, *F. macrophylla*, *F. mysorensis*, *F. religiosa* and *F. sycomorus*. Relatively broad (2.1-3.2  $\mu\text{m}$ ), in *F. afzelii*, *F. benghalensis*, *F. benjamina* var. *comosa*, *F. cordata* and *F. sperguana*.

Membrane texture of apertures in all the studied taxa was glabrous and surface level unsunken in three taxa (*F. cunninghamii*, *F. deltoidea* and *F. racemosa* (Table 3; Plate II; 6, 7, 8 & 12) while it was slightly sunken; in the remaining 14 taxa.

Pore visibility is indistinct in *F. benjamina* var. *comosa*, *F. infectoria*, *F. platypoda*, *F. religiosa* and *F. sycomorus*, on the other hand it appeared distinct in the remaining 12 taxa. Pore shape more or less elliptic; in *F. benghalensis*, *F. benjamina* v. *comosa*, *F. cunninghamii*, *F. cordata*, *F. spragueana* and *F. sycomorus*; more or less circular; in the remaining studied 11 taxa (Table 3). Columellae (LM) indistinct and tectum tectate-imperforate.

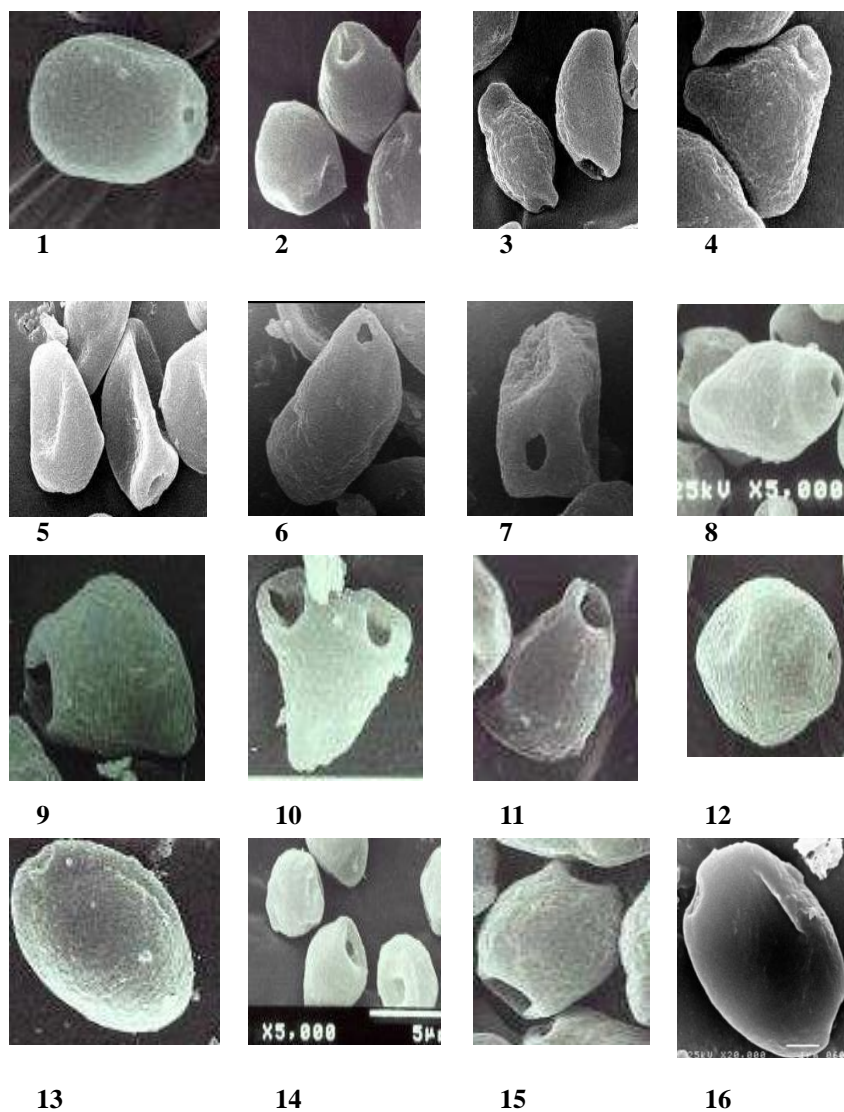
The characters of exine sculpturing as revealed by SEM is considered a fundamental character used in the identification and delimitation between the taxa under investigation. Sculpturing was psilate in *F. afzelii*, *F. cunninghamii*, *F. elastica*, *F. infectoria*, *F. macrophylla*, *F. mysorensis*, *F. platypoda*, *F. religiosa* and *F. sycomorus* (Table 3; Plate III; 1, 6 & 8). Yet, it was finely scabrate in the remaining studied 8 species (Table 3; Plate III; 2, 3, 4, 5 & 7).

The findings of the study revealed that pollen morphological characters such as the surface sculpturing type, pollen size, shape and outline are mostly useful in distinguishing the studied taxa, but they do not provide strong evidence for the infrageneric delimitation of the genus.

The data collected so far were not sufficient to offer hypotheses on the possible evolutionary trends in the pollen of *Ficus* as a whole. However, the following subsequent characters were considered to show the magnitude of these characters as being primitive versus advanced. Elliptical shape of pollen grains was considered primitive versus angular (advanced). Porate could be considered as more advanced, the diporate versus triporate (advanced), the greater number of apertures the better the opportunity of the pollen. The leveled aperture is considered primitive than the sunken aperture (advanced). The evolutionary trends of aperture shape from circular versus elliptic, which the elliptic was considered the advanced form. Concerning the pollen sculpturing, the psilate pattern (sculpture less) is primitive, where the ornamented patterns are considered the advanced state (Waker and Doyle 1975). The obtained data

referred to a degree of advancement in the pollen criteria; the members of the *F. afezelii*, *F. religiosa*, *F. platypoda* and *F. cunninghamii* have lowest grades and occupied a primitive position.





**Plate II. 1-16 SEM X5000: 1. *Ficus afzelii*; 2. *F. benghalensis*; 3, 4. *F. benjamina* var. *comosa*; 5. *F. cordata* subsp. *salicifolia* ; 6, 7. *F. cunninghamii*; 8. *F. deltoidea*; 9. *F. laurifolia*; 10, 11. *F. macrophylla*; 12. *F. racemosa*; 13. *F. religiosa*; 14. *F. retusa*; 15. *F. sperguana*, 16. *F. sycomorus*.**

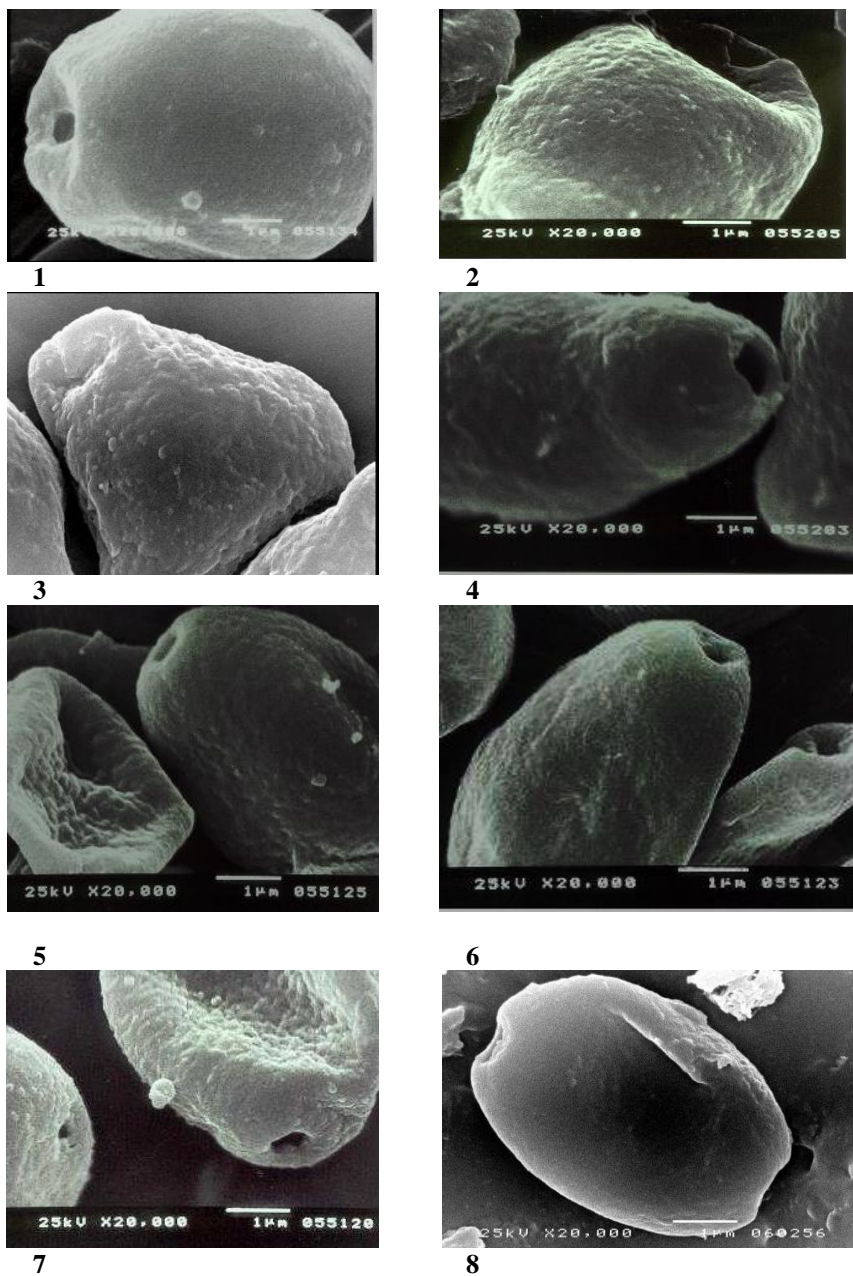


Plate III. Fig. 1-8 SEM x20000: Pollen morphology: 1. *Ficus afzelii*; 2. *F. benghalensis*; 3. *F. benjamina* var. *comosa*; 4. *F. deltoidea*; 5. *F. laurifolia*; 6. *F. mysorensis*; 7. *F. racemosa*; 8. *F. sycomorus*.

The pollen morphology does not appear to be particularly useful as a taxonomic technique in the identification of *Ficus* species.

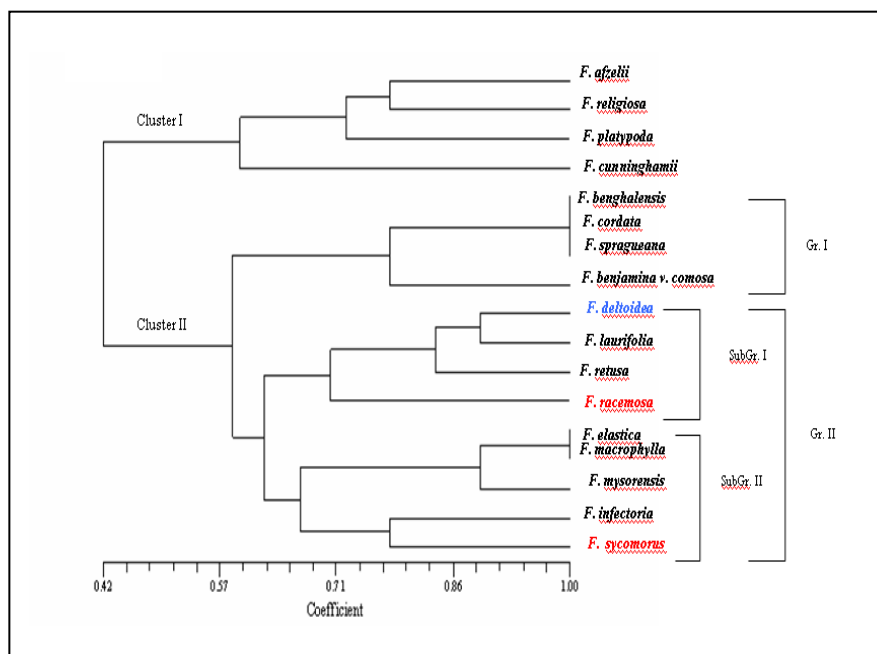
Numerous studies were made on genus *Ficus* to clarify the phylogenetic relationships and evolution in certain subgenera, sections and lower taxonomic ranks, utilizing data sets from entirely different criteria as macromorphology (Berg and Corner, 2005; Chen and Chen, 2005), micromorphology (Dixon, 2002; Neves *et al.*, 2002; Alejandra and José, 2004; Sonibare *et al.*, 2006), chemotaxonomy (Pistelli *et al.*, 2000; Sharaf *et al.*, 2000; Sandabe *et al.*, 2006; Andrzej *et al.*, 2007), reproductive biology (Douglas *et al.*, 2004; Weiblen, 2004), molecular criteria (Noth *et al.*, 1996; Weiblen, 2000; Anuntalabhochai *et al.* 2008) and chromosome studies (Azizian and Sonboli, 2001). However, the obtained results were different; none of these studies have reached a conclusive result in determining the inter-specific relationships between the taxa of *Ficus*. This may be attributed to the following facts:

1- The investigated taxa in a particular study were unlike that included in another study, utilizing different criteria (Heywood, 2001). So they could not be compared objectively, this fact is particularly true when dealing with exceptionally large and diverse genera as in the case of *Ficus*.

2- Only a few number of *Ficus* taxa were studied since new ones are still discovered annually (Berg, 2002; Van Noort *et al.*, 2007), thus continuously changing our view on the relationships within the taxa of the genus.

#### *Numerical analysis*

The dendrogram produced from the cluster analysis of 18 palynological character states (Fig. 1) shows that the taxa are divided into two main clusters at similarity level 0.42. The first cluster includes *F. cunninghamii* at similarity level 0.66, *F. platypoda*, *F. religiosa* and *F. afzelii* at similarity level 0.77. The second cluster is divided into two groups. The first group (Gr. I) includes *F. benjamina* v. *comosa* which splits off other species in the same group at similarity level 0.77 and *F. spragueana*, *F. cordata* subsp. *salicifolia* and *F. benghalensis* which are closely related to each other at similarity level 1.00. The second group (Gr. II) also is divided into two sub-groups; the first sub-group consists of *F. racemosa* (subgenus *Sycomorus*) at similarity level 0.66, *F. retusa* (subgenus *Urostigma*) at similarity level 0.88, *F. laurifolia* (subgenus *Urostigma*) and *F. deltoidea* (subgenus *Ficus*) at similarity level 0.88. The second sub-group included *F. sycomorus* (subgenus *Sycomorus*) and *F. infectoria* (subgenus *Urostigma*) at similarity level 0.77, *F. mysorensis* at similarity level 0.88 and *F. macrophylla* and *F. elastica* which are also closely related to each other at similarity level 1.00. The dendrogram illustrates the relationship between *F. deltoidea* (subgenus *Ficus*) with *F. laurifolia* and *F. retusa* (subgenus *Urostigma*). While the two studied taxa of subgenus *Sycomorus* (*F. racemosa* and *F. sycomorus*) were separated in 2 different subgroups and showed relations with the taxa of subgenus *Urostigma*. Also studied taxa of subgenus *Ficus* (*F. deltoidea*) possessed relation with members of subgenus *Urostigma*.



**Fig.1.** UPGMA- dendrogram based on 18 palynological characters illustrating similarity between the studied taxa.

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## مورفولوجيا حبوب القاح لبعض انواع جنس فيكس (الفصيلة التوتية) من مصر

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في هذه الدراسة تم دراسة مورفولوجيا حبوب القاح لسبعة عشر نوعاً من جنس فيكس (الفصيلة التوتية) على نطاق واسع باستخدام الميكروسكوب الضوئي والميكروسكوب الإلكتروني. وقد تم تجميع هذه الأنواع من ثلاثة حدائق مصرية (الأورمان ، عين شمس وحديقة الحيوان) و تدرج هذه الانواع تحت ثلاثة تحت جنس وهي فيكس *Ficus* وسيكومورس *Sycomorus* و يوروستيجمما *Urostigma* . إن النتائج المكتسبة ذات الأهمية الأساسية والتطورية على النحو التالي: حجم حبوب القاح صغير جدا (أقل من ٢٠ ميكرون) ، شكل حبة اللقاح بين (الدائري *Circular* أو الإهليجي *Elliptic*) وتتراوح عدد الفتحات في حبوب اللقاح ما بين اثنين *Diporate* وثلاث فتحات *Triporate* و أن صفة الزركشة الموجوده على السطح الخارجى ما بين السطح الأملس *Psilate* أوالسطح ذات البروزات الرفيعه *Finely scabrate* الرسم الشجرى *Dendrogram* الناتج من التحليل العدى للصفات المختارة لقت الضوء على العلاقات البينية بمدى التشابه والاختلاف وصلة القرابة بين الوحدات المدروسة. بيانات حبوب اللقاح فقط لاتدعم دلائل الشكل الظاهرى لتعريف جنس الفيكس.