

## Systematic Significance of Pollen Morphology of Citrus (Rutaceae) from Owerri

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### Abstract

Palynological features of *C. sinensis*, *C. limon*, *C. aurantifolia*, *C. paradisi*, *C. reticulata* and *C. maxima* collected from various parts of Owerri were examined and evaluated in order to determine the taxonomic value of the observed internal peculiarities. Features related to pollen shape showed circular to elliptic shapes in all the taxa while rectangular shape distinguished *C. limon*, *C. paradise* and *C. maxima*. Polar view showed that *C. paradisi* and *C. maxima* have closer affinity than the other taxa studied. Palynological characters as observed in this study were found useful especially in delimiting the investigated taxa and thus could be exploited in conjunction with other evidences in species identification and characterization.

**Keywords:** Citrus; Pollen morphology; Systematic; Rutaceae

### Introduction

The use of palynological studies in solving taxonomic problems is gradually gaining grounds with vigorous extensive investigation in the field of science. Diversified morphological details of pollen characters like pollen shape, symmetry, size, polar equatorial outlines, number of aperture wall or exine thickness, pore diameter and exine or wall ornamentation are significant attributes of potential taxonomic importance that are diagnostic at the species level and provides a useful data for the intergeneric classification of the larger genera [1]. The differences and similarities in pollen morphology of some investigated species showed significant evidences and could be exploited for biosystematic purposes. Mou and Zhang [2] investigated pollen morphology of eleven species of *Murraya* and *Micromelum* in the family Rutaceae. They indicated that species of *M. sect. Bergera* and *sect. Murraya* have very distinct pollen morphology. Species in *sect. Bergera* have egulate to foveolate exine whereas the tectum in *sect. Murraya* is cross-striate. This supports the redefinition of the genus *Murraya*, retaining only the species of *sect. Murraya*, while *M. sect. Bergera* is better treated as a separate genus. From the palynological point of view, it is suggested that *Bergera* and *Murraya* are not closely related, with the latter being closer to *Micromelum* and *Merrillia*, a result in accordance with phytochemical, chromosomal and molecular evidences, thus supporting the reinstatement of the generic name *Bergera* L.

The variations in shape, aperture, pollen unit, symmetry and differences in wall sculpture of pollen grains have been used by many authors in the delimitation of various taxa; Okwulehie and Okoli [3] in *Corchorus* species; Mbagwu and Edeoga [4] on *Vigna*. Edeoga et al. [1] on *Ludwigia* and *Aneilema*; Nyananyo, [5] on *Talineae*; Soladoye and Crane [6] on *Baphia* and Ferguson [7] on *Macrotylome* confirmed interspecific relationships based on affinities between related taxa.

Despite these contributions by these authors, there is little or no information documented on pollen morphology of *Citrus* species studies. This paper therefore reports the pollen morphology of the six species of *Citrus* as observed. It assesses the relevance of and discusses the extent to which pollen morphology might be utilized in the systematic consideration of the six *Citrus* species in relation to their perceived morphological similarities in some of the species.

### Materials and Methods

#### Specimen collection

The studies were made on matured living materials of the six *Citrus* species collected from Ministry of Agriculture and Natural Resources Nekede, Agricultural Development Programme (ADP) farms, Owerri.

The specimen were authenticated by a professional taxonomist, Prof. S.E. Okeke of Imo State University, Owerri. They were confirmed at Forest Herbarium Institute (FHI) Ibadan and voucher specimen were deposited at Imo State University Herbarium with Herbarium No. IMSUH 001-006. The study was conducted at the Plant Science and Biotechnology laboratory of Imo State University, Owerri.

#### Palynological studies

Mature flower head of each of the *Citrus* species studied was collected and teased out on a slide. Samplings were made on flesh flowers from plants in their natural conditions as these do not undergo any form of deterioration [1,8]. 5mls of distilled water were used and the materials filtered into a centrifuge tube. 5% KOH (potassium hydroxide) was added to each sample and heated for 20 minutes. The samples were centrifuged and the filtrate decanted. 5mls of acetolysed mixture comprising acetic anhydride mixed with concentrated sulphuric acid ( $H_2SO_4$ ) in the ratio of 9:1 were added to each of the centrifuge materials in the plastic test tubes containing the samples. These test tubes were heated through a water bath for 3-5 mins. After cooling, they were centrifuged for 5 minutes at 2000 rpm (revolution per minutes). The residues were treated with glacial acetic acid, centrifuged and decanted. Residues were washed 3 times with water,

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each washing followed by centrifugation and decanting for about 10-20 minutes. The acetolyzed residues containing pollen grains treated with 2-5 drops of glycerin alcohol in the ratio of 2:1 were added to the precipitate in order to preserve them and make them much clearer. The precipitate was stored in a labeled specimen bottles. Unstained acetolyzed pollen grains were embedded in glycerin jelly and sealed with wax after covering with zero cover slip. Photomicrographs of the pollen grains were taken using a Laitz Wetzler ortholux microscope fitted with vivitar-v-335 camera at 100x magnification.

## Result

The palynological features of the six species of Citrus studied are summarized in Table 1. The pollen morphology of the six species of Citrus showed the following results. The descriptions of the pollen morphology and species possessing them are summarized as follows:

Pollen grains in all the species of Citrus studied are isopolar and shed in monads. Pollen grains are radially symmetrical in *C. sinensis*, *C. limon*, *C. aurantifolia* and *C. reticulata* while *C. paradisi* and *C. maxima* are bilaterally symmetrical. Pollen grains are circular to elliptical in *C. sinensis*; *C. aurantifolia* and *C. reticulata*, circular to elliptic to rectangular in *C. limon*; *C. paradisi* and *C. maxima*. The polar view is circular in all the species while the equatorial view is elliptical in *C.*

*sinensis*, *C. limon*, *C. aurantifolia* and *C. reticulata* but rectangular with straightened ends in *C. paradisi* and *C. maxima*.

Pollen wall ornamentation and exine thickness showed striate pattern and thick exines respectively in all species investigated. Ectocalpus is long narrow at centre, sunken, distinct and regular in all the species of Citrus studied.

Pollen aperture is monocolpate to tetracolpate in *C. sinensis*; *C. limon*; *C. aurantifolia* and *C. reticulata* while *C. paradisi* and *C. maxima* have monocolpate pollen aperture (Figure 1A-1F).

## Discussion

The result of the pollen morphology of the six species of Citrus studied showed combination of shapes. The pollen shape in the Citrus ranged from circular to elliptical in all the taxa investigated. But rectangular shaped observed in *C. limon*, *C. paradisi* and *C. maxima* delineate them from the other three. Applying these variations in pollen shape to the Citrus species studied showed that *C. limon*, *C. paradisi* and *C. maxima* have closer affinity and can be distinguished from the rest of the taxa. The pollen colpate/aperture shows monocolpate to tetracolpate in *C. sinensis*; *C. limon*, *C. aurantifolia* and in *C. reticulata* but only monocolpate in *C. paradisi* and *C. maxima*. This distinguished

Species	<i>C. sinensis</i>	<i>C. limon</i>	<i>C. aurantifolia</i>	<i>C. reticulata</i>	<i>C. paradisi</i>	<i>C. maxima</i>
Pollen shape	Circular-elliptical	Circular Elliptical-rectangular	Circular to elliptical	Circular-Elliptical	Circular-Elliptical Rectangular	Circular-Elliptical rectangular
Polar view	Circular	Circular	Circular	Circular	Circular	Circular
Equatorial view	Elliptic	Elliptic	Elliptic	Elliptic	Rectangular	Rectangular
Pollen wall ornamentation	Striate	Striate	Striate	Striate	Striate	Striate
Exine thickness	Thick	Thick	Thick	Thick	Thick	Thick
Pollen aperture	Monocolpate to tetracolpate	Monocolpate to tetracolpate	Monocolpate to tetracolpate	Monocolpate to tetracolpate	Monocolpate	Monocolpate

Table 1: Types of pollen characters in the species of Citrus studied.

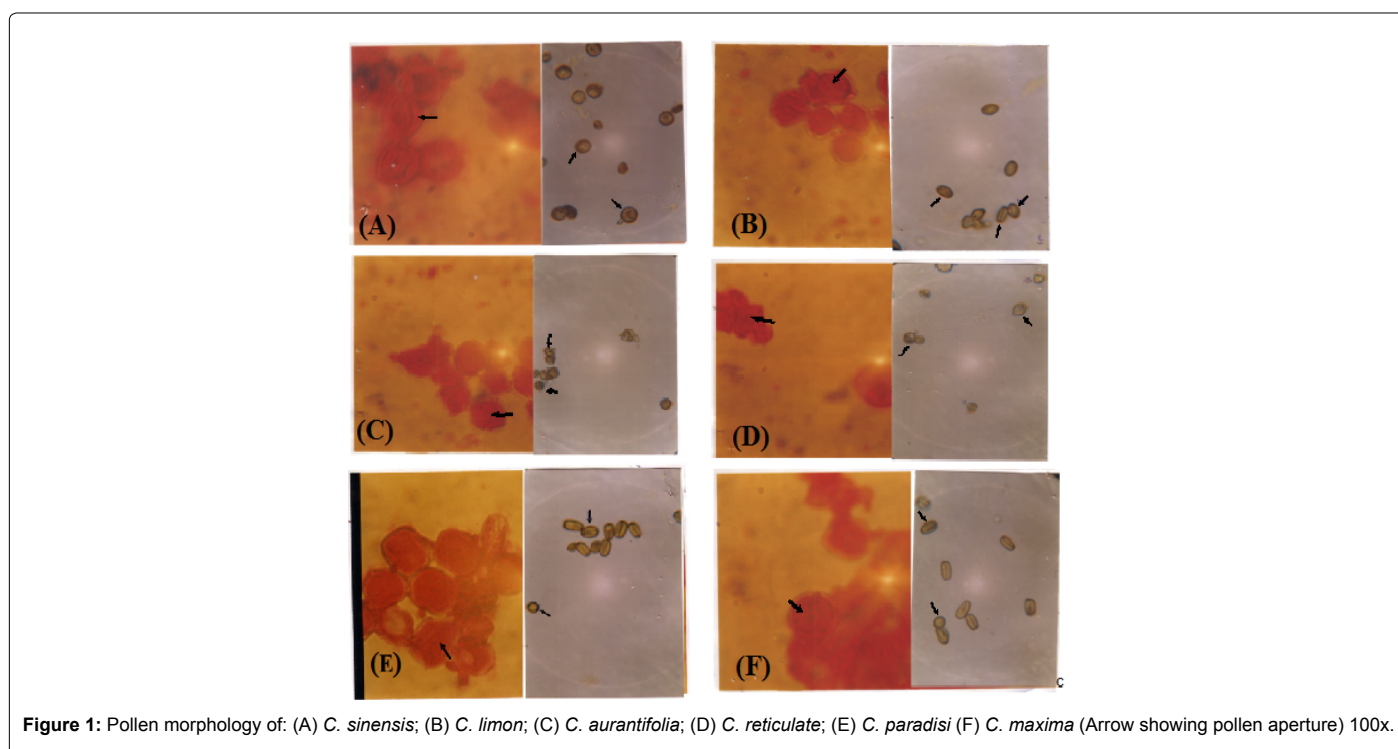


Figure 1: Pollen morphology of: (A) *C. sinensis*; (B) *C. limon*; (C) *C. aurantifolia*; (D) *C. reticulata*; (E) *C. paradisi* (F) *C. maxima* (Arrow showing pollen aperture) 100x.

*C. paradise* and *C. maxima* from *C. limon* thus, affirming their close relationship. These findings do not agree with those of Terradillos-Lamas [9] who reported tricolpate for *Citrus limon*. No tricolpate grains were found in the samples studied which is in congruence with the studies made by Breis, et al. [10]. This study also disagrees with the findings of Grant, et al. [11]. WHO reported that 26 of the 28 genera of the tribe Citreae have pollen which is 4/5 colpate with micro-perforate or reticulate ornamentation.

From this point of view, variations exist in pollen shape and aperture of the pollen grains investigated and these features could serve as a point of differentiation between the different species of Citrus and also to reinforce close relationships among the six species investigated and this justifies the placement of the six species under the same family and genus.

## Conclusion

The differences in pollen grains are not enough for re-classification of the species studied, but the similarities in structure among the investigated taxa showed interspecific relationships of the individual species [3].

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