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Full Length Research Paper

Ultrastructure of the pollen grains in the family Bignoniaceae Juss. in Nigeria

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Pollen morphology of eight species belonging to the Bignoniaceae in Nigeria was described using Scanning Electron Microscope (SEM). The species studied were *Crescentia cujete* Linn., *Jacaranda mimosifolia* D.Don., *Kigelia africana* (Lam) Benth., *Markhamia tomentosa* (Benth) K. Schum., *Newbouldia laevis* (P. Beauv.) Seemann ex Bureau., *Spathodea campanulata* P. Beauv., *Stereospermum acuminatissimum* Cham. and *Tecoma stans.* Reticulate exine ornamentation were observed in all pollen grains of the species studied. The shapes of most of the pollen grains were circular except from those of *J. mimosifolia* and *T.stans* which were elliptic. The pollen grains of all species examined were tricolpate (except from those of *C. cujete* which were non-aperturate) isopolar with long colpi and a reticulate tectum. The pollen grains of the species studied were radially symmetrical, isopolar or apolar. The general shapes were subspheroidal to prolate. The AMB is rounded to triangular. The apertures are usually three in number, always equatorially placed. This study points to a reclassification of the Bignoniaceae.

Key words: Scanning electron microscopy, bignoniaceae, pollen grains, ultrastructure, bignoniaceae juss.

INTRODUCTION

Bignoniaceae Juss. is made up of about 100 genera and 800 species (Watson and Dallwitze, 1992). The family is mainly distributed in the tropical regions (greatest diversity in Brazil) and forms part of the vegetation (Ugbabe *et al*, 2009), while a few species are found in the temperate and sub-tropical regions of the world. Hutchinson & Dalziel (1954) recorded five genera and seven species in Nigeria. These are *Kigelia africana* (Lam.) Benth.; *Markhamia lutea* (Benth.) K. Schunm.; *Markamia tomentosa* (Benth.) Schum.; *Newbouldia laevis* Seem.; *Spathodea campanulata* P. Beauv.*; Stereospermum acuminatissimum* K. Schum. and *Stereospermum kunthianum* Cham. Beside these, there are also introduced species in Nigeria such as *Crescentia cujete* Linn.; *Tabebuia rosea* (Berthol.) D.C.; *Tecoma stans* (Linn.) H.B&K and *Tecoma capensis* (Ugbabe & Ayodele, 2008).

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The family was divided by Schumann (1895) into five tribes: Bignonieae, Tecomeae, Eccremocarpeae, Crescentieae and Tourrettieae. Much later Gentry (1980) recognized eight tribes, adding Oroxyleae, Coleeae, Schlegelicae. In Nigeria, considering native and exotic species it is possible to find representatives of: Tecomeae, Cresentieae, Eccremocarpeae and Coleeae.
From a palynological perspective, the family is known as eurypalynous, however each genus shows great uniformity. Generic diagnoses based on current taxonomic criteria are difficult, making the stable pollen pattern a useful additional character for identification. The palynology of the Bignoniaceae was studied at an early stage (e.g., Mohl 1835, Schumann 1895, Urban 1916) and by many subsequent authors: Erdtman (l952), Gomes (1955. 1957). Natarajan (1957). Palacios (1966), Mitra (1968), Heusser (1971), Huang (1972), Ikuse (l956), Ferguson & Santisuk (l973), Salgado-Labouriau (l973), Suryakanta (l973), Buurnian (l977), Markgraf & d'Antoni (1978), Andrade & Miranda (1979), Gentry & Tonib (1979), Carreira & Barth (1987), Silvestre & hlelhern (1989) and Bove & Barth (1992). The remaining

family members that are poorly known and studied are the African and Asian groups, which account for approximately 29 genera and 115 species (Lohmann and Ulloa, 2007).

Pollen is composed of sporopollenin which is very durable and does not easily decay, from pollen identification, the geographical origin of the plant from which the pollen came can be determined because of the sporopollenin that does not decay. Pollen architecture has great significance in the taxonomy of Angiosperms and interpreting inter-relationships among them (Ugbabe et al., 2009). The first successive attempt at using pollen characters in the classification of plants was made by Lindley (1837). Since then, Erdthman (1952), Patel and Datta (1958), Sowunmi (1973) and several others have worked on the morphology of the pollen grains from different regions and emphasized its phylogenetic significance and this is important when there is temporal and geographical variation in the distribution of the identified plants. Earlier works on pollen morphology covered little or no African species.

Genera of the Bignoniaceae are notoriously difficult to recognize (Gentry, 1973). This has largely been due to taxonomic over-splitting at the genus level which produced very similar genera. In fact there are fewer species per genus in Bignoniaceae than in any other large or medium sized plant family (Gentry, 1973) Willi's Dictionary (Airy Shaw, 1966) listed 54 monotypic genera in Bignoniaceae. Therefore, critical redefinition of genera is clearly the major taxonomic problem in the family. The careful use of pollen morphology has been a valuable tool in generic delimitation and in understanding relationships in the family. For example, the taxonomic potential of pollen morphology in Bignoniaceae has long been appreciated Urban's (1916), Pichon (1945), Gomes (1955) and other subsequent workers followed Urban's lead in proposing numerous new genera based on palynological differences. Unfortunately, the lack of any overall understanding of the family has led to repeated taxonomic misinterpretations.

Previous studies of the pollen of this family had relied mostly on light microscopy and some of the misinterpretations of the pollen morphology were due to the difficulties of the method. Although several papers on Bignoniaceae pollen have been based largely on SEM work and have provided taxonomically important new information (Ferguson & Santisuk, 1973, Buurman, 1977, Roubik and Moreno, 1991).

This paper describes the pollen morphology of native and exotic species that occur in Nigeria.

The aim of this study is to obtain characters of the pollen grains of the family Bignoniaceae in Nigeria using SEM which may contribute to the understanding and identification of the family. The pollen morphology of the family has value as a paleo-ecological indicator. The characterization and recognition of the species will assist palynologists in identifying pollen of fossil and recent deposition in the Bignoniaceae.

MATERIALS AND METHODS

Specimen collection

Anthers of the eight species studied were collected from the field. The various locations and the distribution of Bignoniaceae based on studies from 4 herbaria: Forestry Research Institute of Nigeria (FRIN); University of Ibadan herbarium (UIH); Ahmadu Bello University herbarium (ABUH) and National Institute for Pharmaceutical Research and Development herbarium (NIPRDH) are shown in Figures 1, while the collection sites are shown in figure 2.

Scanning Electron Microscopy (SEM)

Procedure

Pieces of the anthers were taken and crushed directly on labeled stubs with double sided adhesive tape. Each sample was coated with 100% Platinum portion in a sputter coating unit, scanned and photographed using the JEOL JSM – 6360A Analytical Scanning Electron Microscope with Energy Dispersive Spectrometer at the Scanning Electron Microscopy unit of the Department of Physics, University of Pune, Pune – India.

Mean, range and standared error were calculated for the quantitative variables based on eleven measurements: { minimum (mean ± standard error) maximum}. Terminologies used are based on Punt et al. 2007.

RESULTS

Plates 1-3 show the pollen morphology of some of the Nigerian Bignoniaceae while table 1 show pollen grains characters of the species studied. Distribution of the Species of the Bignoniaceae is mostly in the Western, Eastern and North-Central Nigeria (Fig. 1 & 2).

The pollen grains of the species studied were radially symmetrical, isopolar or apolar and also tricolporate. The AMB/Shape is either circular or elliptic. The circular ones included those of *K. africana* (Plate 1G-I), *M. tomentosa* (Plate 2A-C), *N. laevis* (Plate. 2D-F) *Spathodea campanulata* (Plate. 3A-C) and *Stereospermum acuminatissimum* (2G-I), while those of *J. mimosifolia* (Plate 1D-F) and *T. stans* (Plate 3D-F) were elliptic. The sizes of the grains showed variation from species to species. There was diversity in the exine ornamentation of the pollen grains which were reticulated in various degrees. The pollen grains of *M. tomentosa* (Plate 2A)

Figure 1. Map showing the Distribution of Bignoniaceae in Nigeria.

Figure 2. Map showing Collection sites of species of the Bignoniaceae in Nigeria.

and S. acuminatissimum (Plate 2G) were folded, probably being dehydrated.

The shape varies from prolate to subprolate. The prolate grains include those of *J. mimosifolia*, *K. africana, M. tomentosa N. laevis* and *T. stans* (having P/E = 10.2 – 18.6). While *C. cujete*, *S. campanulata, S. acuminatissimum* showed subprolate grains. Pollen of the introduced species (*J. mimosifolia* and *T. stans*) apart from those of *C. cujete* had long colpi measuring up to 0.86 times the length of the polar axis (P) which were elliptic. While colpi of indigenous species (*K. africana, M. tomentosa, N. laevis, S. campanulata* and *S. acuminatissimum*) were relatively shorter. The species studied had 3 apertures except *C. cujete*. The exine orna-

Table 1. Pollen grain characters of species of the Bignoniaceae in Nigeria.

S.No.	Genus / Species	AMB/shape	Pollen type	Polar axis (P)	Equatorial axis (E)	P/E%	Pollen classes
1.	*Crescentia cujete*	Elliptic	Non-aperturate	65.4 (32.0 ± 4.1) 85.0	42.6 (44 ±2.8)46.4	140 (11.3 ±4.8)209	Prolate-sheroidal
2.	*Jacarada mimosifolia*	Elliptic	Tricolporate	55.2 (34 ±2.5)74.6	28.6 (24.5 ±3.4)39.3	110 (13.8 ±20)185	Prolate
3.	*Kigelia africana*	Circular	Tricolporate	34.4 (38 ± 2.6) 42.4	24.8 (28.4 ± 2.5)32.0	123 (135.2 ±17)171	Subprolate
4.	*Markhamia tomentosa*	Circular	Tricolporate	36.4 (34 ±3.6) 49.2	26.8 (25.6 ±3.6)31.2	106 (132.8 ±22)186	Subprolate
5.	*Newbouldia laevis*	Circular	Tricolporate	38.4 (41.6 ±2.2)46.4	24.8 (28.4 ± 3) 30.4	124 (146.5± 12)162	Subprolate
6.	*Spathodea campanulata*	Circular	Tricolporate	30.8 (35.2 ±5.2)48.4	29.2 (23.6 ±3.8)35.4	90 (149.2 ±15)149	Subprolate
7.	*Stereospermum accuminatissimum*	Circular	Tricolporate	28.2 (30.8 ±3.6)47.2	19.2 (27.2 ±4.4)32.8	100 (113.2 ±19)150	Subprolate
8.	*Tecoma stans*	Elliptic	Tricolporate	34.0 (33 ±3.5)60.8	25.6 (23.6 ±4.4)30.4	111 (140.7 ±22)183	Prolate

Key: Minimum (mean ± standard error) maximum
All measurements in microns. AMB = Ambient

mentation of the all the pollen were reticulated. Perforation density is variable ranging from sparsely perforated as in *C. cujete* to densely perforated as in *K. africana, S. campanulata* and *S. acuminatissimum* and quite densely perforated as in *T. stans* to very densely perforated as in *N. laevis, J. mimosifolia* and *M. tomentosa.* Often giving the outline a slightly rugulate appearance. In general, the pollen grains of the species studied were densely perforated. In *C. cujete, J. mimosifolia, T. stans* and *N. laevis* the perforations are evenly distributed while in *K. africana, M. tomemtosa, S. campanulata* and *S. acuminatissimum* the perforations are more densely perforated between the colpi.

DISCUSSION AND CONCLUSION

The pollen grains can be grouped according to their shape, classes and in their relation between Polar axis (P) and Equatorial axis (E) into three classes based on Erdthman (1952): Prolate-spheroidal with P/E% of 100-114, which occurred in *C. cujete*, subprolate with P/E% of 114-133 in *K. africana, M. tomentosa, N. laevis, Spathodea campanulata* and *Stereospermum acuminatissimum*; and the prolate type with P/E% of 133-200 in *J. mimosifolia* and *T. stans*. The pollen grains of the species studied were radially symmetrical, isopolar or apolar. The general shape is spheroidal - prolate.

The polar axis ranged from 28 to 85 micrometers while the equatorial axis ranged from 19 to 46 micrometers. The AMB/shape is circular to elliptic. The apertures are

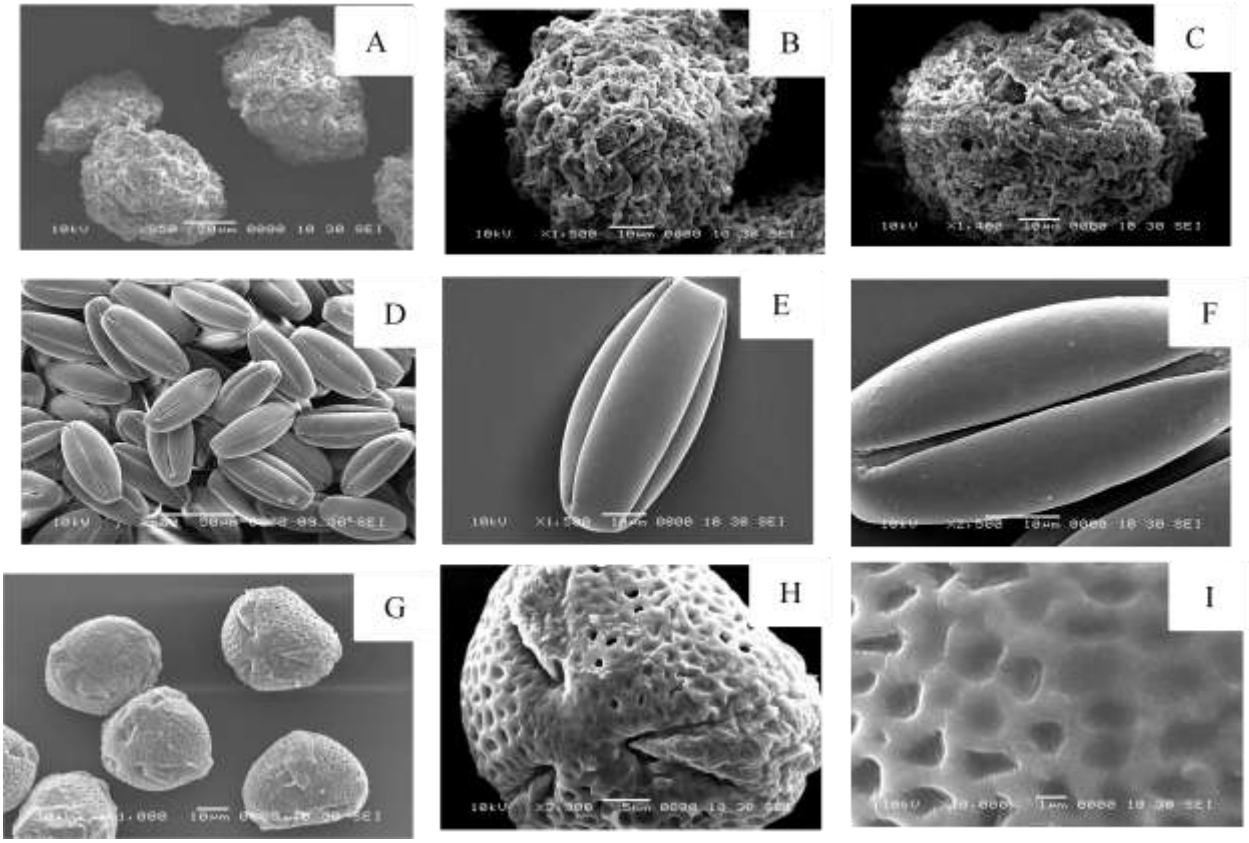

PLATE 1. Scanning Electron Micrograph of Pollen Grains of Nigerian Bignoniaceae
(A-C)*Crescentia cujete (*A) general appearance showing elliptic, non-aperturate pollen grains, B-C Detail of ornamentation showing reticulated ornamentation; (D-F) *Jacaranda mimosifolia (*D) general appearance showing elliptic tricolporate pollen (E-F) detail of colpi and ornamentation; (G-I) *Kigelia africana* (G) general appearance showing circular tricolporate pollen (H-I) detail of ornamentation

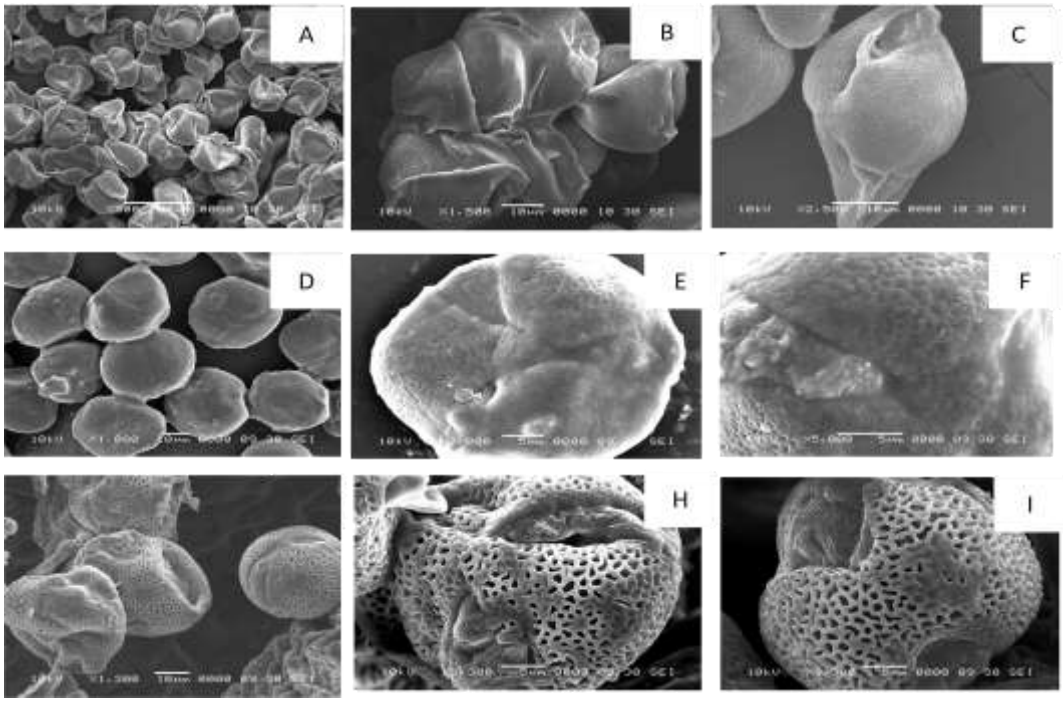

PLATE 2. Scanning Electron Micrograph of Pollen Grains of Nigerian Bignoniaceae
(A-C)) *Markhamia tomentosa* (A) general appearance showing circular tricolporate pollen (B-C) detail of ornamentation (D-F)*Newbouldia laevis* (D) general appearance showing circular tricolporate pollen (E-F) detail of ornamentation; (G-I). *Stereospermum acuminatissimum* (G) general appearance showing circular tricolporate pollen (H-I) detail of ornamentation

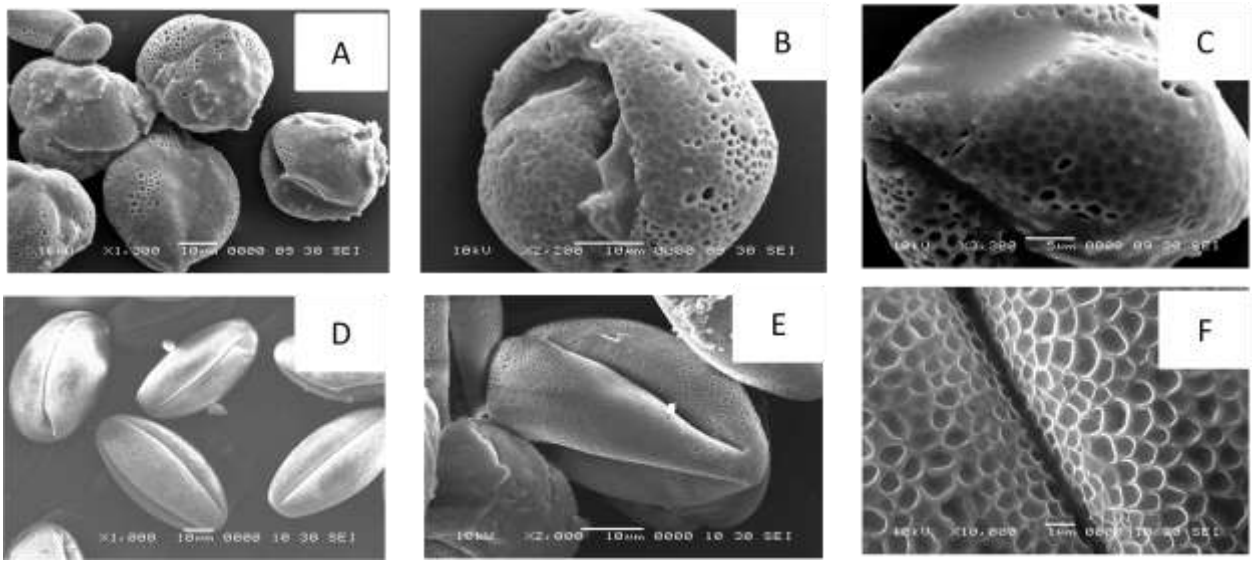

Plate 3. . Scanning Electron Micrograph of Pollen Grains of Nigerian Bignoniaceae.
(A-C) *Spathodea campanulata* (A) general appearance showing circular tricolporate pollen (B-C) detail of reticulated ornamentation; (D-F) *Tecoma stans* (D) general appearance showing elliptic tricolporate pollen (E-F) detail of colpi and ornamentation

usually three in number. The pollen grains were all tricolporate except from those of *C. cujete* which were non-aperturate and mostly circular except from those of *J. mimosifolia* and *T. stans* which were elliptic. Ornamentation of the all the pollen were reticulated. Pollen grains of the indigenous members of the Bignoniaceae (*K. africana, M. tomentosa, N. laevis, Spathodea campanulata* and *Stereospermum*

acuminatissimum) were circular while those of the introduced species (*C. cujete*, *J. mimosifolia* and *T. stans*) were elliptic.

According to previously published studies [Grayum, 1986, Dransfield *et al*, 2008, Hesse, 2000], our results suggest that the pollen ornamentation may actually depend on the species/taxon. The high diversity of ornamentation type in pollen grains of angiosperms has often been suggested to be linked to diversity in pollination systems (Sannier et al, 2009). It is commonly stated that smooth pollen grains are associated with wind or water pollination while sculptured pollen grains are associated with biotic pollination (Sannier et al, 2009). The exine wall of the pollen grains of flowering plants displays patterns of ornamentation (the external aspect of pollen grains, also called sculpturing) that are highly diversified. Among the different types of relationship implying pollen ornamentation that have been suggested, the existence of a link between exine sculpturing and pollinator type has often been proposed and was even evidenced in certain situations. It is often considered that smooth pollen grains are associated with abiotic pollination (wind or water) while echinulate or reticulate pollen grains are associated with biotic pollination, particularly entomophily [Lumaga *et al* 2006, Tanaka *et al* 2004]. Pollination in the Bignoniaceae is either entomophilous (via insects), ornithophilous (via birds) or chiropterophilous (via bats).

In conclusion, four of the seven tribes are represented in Nigeria based on this study, and especially on the SEM of pollen and from studies of the leaf epidermis (Ugbabe *et al*, in press) and the phylogenetic studies (Ugbabe *et al,* in press), it would be suggested that the genera *Tecama, Tabebuia* (shown to have elliptic pollen in Ugbabe *et al*, 2007) and related genera be put in a separate tribe because they have elliptic pollen grains while the others in the tribe (*Tecomeae*) have circular pollen grains, there were also striations on their leaf epidermis from the SEM work carried (Ugbabe *et al*, in press) and they occupied lower positions in the phylogenetic trees when the *rbcL* and the *ndhF* gene were used as primers (Ugbabe *et al,* in press).

It is suggested that the genus *Tabebuia* and *Tecoma* and related genera be the true *Tecomeae* and the others (*Markhamia*, *Newbouldia,*
Spathodea and *Stereospermum etc.*) be placed in a separate tribe. A reclassification of the family is hereby suggested and the tribe Spathodeae is hereby being proposed.

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