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POLLEN MORPHOLOGICAL STUDIES IN THE FAMILY SAPINDACEAE FROM PARTS OF IBADAN IN OYO STATE AND MORO IN OSUN SATE IN SOUTHWESTERN NIGERIA

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ABSTRACT

Pollen grains of nine (9) species of Sapindaceae randomly collected from parts of Oyo and Osun States of Nigeria were prepared using the acetolysis method and studied with the aim of identifying characters of taxonomic relevance in the family. Results showed that the pollen grains were either triporate or tricolporate. Pore or aperture was found to be a homologous character and the presence or absence of Os and/or their ornamentations can be reliably used to delimit the species. The largest pollen (46.94 x 51.7 µm) was observed in *Cardiospermum halicacabum* L. while the smallest pollen was observed in *Blighia sapida* K.D Koenig (16.80 x 15.84 µm). The exine pattern may be reticulate (*Paullinia pinnata* L), striate (*Allophylus africanus* P. Beauv, *Blighia sapida*, *B. unijugata* Baker, *Deinbolia pinnata* Schumach & Thonn. and *Lecaniodiscus cupanioides* Planch ex Benth) or reticulofoveolate in *Cardiospermum grandiflorum* Swartz and *C. Halicacabum.*

Keywords; Systematics, Sapindaceae, pollen grain, morphology, Nigeria, aperture, exine,

INTRODUCTION

The family Sapindaceae is native to Asia, although some members are found in South America, Africa and Australia (APG II, 2003). Most genera are predominantly medium-sized to large emergent trees or erect shrubs; less often they are lianas or understory palm-like treelets, exceptionally sub-shrubs or scandent shrubs (Acevedo-Rodriguez *et al*., 2011). The arboreal and fruticose habits are widespread throughout the distributional range, while treelets, lianas and sub-shrubs are restricted to the tropics. Leaves are most often pinnately compound, sometimes bipinnate or palmately compound, or just palmate with petiole lacking stipules, but having swollen base (Singh, 2004). Flowers are arranged in groups, usually creamy white but sometimes pinkish white as in *Dodonaea* Mill. Inflorescence is usually in form of raceme or cyme. Most often pollination can be anemophylous or entomophylous (Singh, 2004). Fruits are green in colour turning orange or red as they become ripe; they are brown in *Dodonaea* species (Adeyemi *et al*., 2013).

Pollen grains of some families are homogenous (Borokinni *et al.*, 2012; Ullah *et al.*, 2019) and are, therefore, easy to delimit, while others are heterogeneous, dividing the family into genera (Shokefun *et al*., 2014).

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Pollen morphology has often been used to ascertain groups to which such uncertain elements could be referred (Schols, 2005); aberrant genera have been more loosely attached to particular families as "enigmatic taxa", genera of uncertain systematic and taxonomic position to avoid excessive splitting. Shokefun *et al.* (2014) employed some basic pollen characters in the identification and delimitation of *Microcos* L. species in Nigeria. Schols *et al.* (2005) recorded monosulcate grains in 61 *Dioscorea* L. species, a type which Furness and Rudall (1999) has established as the most common aperture type in monocots. Borokinni *et al.* (2012) supported previous findings of Caddick *et al.* (1998, 2002) on Taccaceae whose pollen grains were monosulcate and spheroidal, with the sexine finely reticulate and similar to that of some species of *Dioscorea*. The literature on pollen morphology of Sapindaceae is quite extensive. The most complete work at the family level was done by Muller and Leenhouts (1976), in which pollen types were evaluated with respect to their systematic significance. More recent progress has been made on Sapindaceae by several workers (van der Ham, 1990; Acevedo-Rodrı´guez, 1993; Ferrucci and Anzo´tegui, 1993; William *et al.*, 2013; Ebigwai *et al.*, 2017). Generally**,** Sapindaceae pollen is 3-aperturate, but often small percentages of 2- and 4-aperturate grains co-occur. Colporate pollen is the commonest, being known from many genera in all four sub-families. Several other types are more restricted. Syncolporate and parasyncolporate pollen, with and without apocolpial fields, respectively, are known only in sub-family Sapindioideae. Moreover, several genera (e.g. *Alectryon* Gaertn, *Arytera* Blume and *Cupania* L.) possess both colporate and (para) syncolporate pollen as well as intermediates (van der Ham, 1990; van Bergen *et al*., 1995**)**. Porate pollen grains are found in a few species of *Allophylus* L, *Lepisanthes* Blume, *Pometia* J.R. Forst and G. Forst, *Talisia* Aubl. and the tribe Paullinieae. Pollen of several Paullinieae is heteropolar with a demisyncolporate aperture system. *Cardiospermum* L. pollen has short demicolpi on the proximal side of the pollen, whereas pollen of several related genera have a demisyncolporate aperture system, and a few species of *Serjania* Mill. and *Urvillea* Kunth. have a syncolporate aperture system proximally with short demicolpi distally. Sapindaceae pollen grains nearly always have lalongate, elliptic to sub-circular endoapertures, though usually hidden by the ectoaperture margins. *Acer* pollen may have apertures without or with indistinct endoapertures. The exine is usually clearly stratified, showing a tectum, a columellate infratectal layer, and a nexine. In *Diplopeltis* Endl. and *Dodonaea* Mill., the infratectal layer is granular/columellate, which might relate to wind-pollination.

Comparison with the molecular phylogenetic trees of 64 genera by Harrington *et al.* (2005) suggests that the colporate spheroidal is indeed basal in the Sapindaceae, sub-families Xanthoceroideae, Hippocastanoideae and Dodonaeoideae being characterised or heavily dominated by this pollen type. Different researchers (Faegri and Iversen 1975; Erdtman, 1952, 1957, 1965, 1971; Melhem *et al.,* 1981; Roubik and Moreno, 1991; Sowumi, 1995; Acevedo-Rodriguez *et al.,* 2011; Shubharani, 2013; Shokefun *et al*., 2014; Akinnubi *et al.,* 2014; Dalia, 2018) have stressed the importance and engaged the use of pollen morphological characters in describing different families and genera. The evaluation of literature on this family has revealed dearth of information on the pollen grain morphology of the species of Sapindaceae represented in Nigeria.

The present study aimed at assessing the pollen characters of some representatives of the family Sapindaceae in Nigeria with a view to determining their importance in the delimitation and recognition of the taxa.

MATERIALS AND METHODS

Collection of specimens

Fresh floral specimens of *Allophylus africanus* P. Beauv., *A. spicatus* (Poir)Radlk, *Blighia unijugata* Baker, *B. sapida* K.D. Koenig, *Cardiospermum grandiflorum* Swartz, *C. halicacabum* L., *Deinbolia pinnata* Schumach. & Thonn., *Lecaniodiscus cupanioides* Planch ex Benth and *Paullinia pinnata* L were collected randomly from Ibadan, Oyo state, and Moro, Osun state, Nigeria. The collections were identified at the University of Ibadan Herbarium in the Department of Botany, University of Ibadan, Ibadan where voucher specimens were also deposited.

Pollen Preparation

Fresh pollen bearing anthers of the taxa collected were cut using a blade and put in 50 ml plastic centrifuge tubes clearly labeled. They were crushed using glass rods. Glacial Acetic acid was poured into each of the centrifuge tubes (dehydration process); the glass rods were removed and the tubes were stirred on a mixer and then centrifuged at 4, 000 rpm for 10 minutes; the supernatant was decanted into a special bottle "Acetolysis waste".

Freshly prepared Acetolysis mixture (9 parts Acetic Anhydride to 1 part concentrated Sulphuric Acid) was poured into tubes containing the pollen samples. The tubes were heated on a water bath from 70^{o}C up to boiling point for 5 minutes. They were centrifuged and decanted into the special bottles labeled "Acetolysis waste". Distilled water was added to the mixed, centrifuged and decanted residue in the tube. This was the first rinsing. Rinsing was done two more times, first with 70% alcohol and, secondly, with absolute alcohol (thus rinsing off the acetolysis mixture). Absolute alcohol was added to the residue in the tube, mixed and poured into a well labeled storage vial from which slides for microscopic analysis were prepared. The mixture in the vial was mixed using mixer and immediately a drop of the mixture was spread on the cover slip and allowed to air-dry. A drop of DPX (mountant) was placed at the centre of the slide. The air-dried cover slip was taken upside down and placed on the drop of the mountant at the centre of the slide. A little pressure with the finger was applied to cause a complete spread, after which it was ready for microscopic study. Terminologies used were based on Erdtman (1952). Mean, range and standard error were calculated for all the quantitative characters.

Photomicrography

Photomicrographs of the pollen grain samples were taken using Leica CME with Digital microscope equipped eyepiece attachment.

Statistical analysis

Statistical analysis was carried out using SPSS 16.0 software to obtain minimum, maximum, mean and standard error (Butt *et al*., 2018; Shah *et al*., 2018a, 2018b). The average values were determined for the following measureable variables: polar diameter, equatorial diameter, exine thickness, length and width of pore.

RESULTS AND DISCUSSION

The pollen grains of species of Sapindaceae studied were either triporate or tricolporate. The triporate grains were observed in *Allophylus africanus, A. spicatus, Cardiospermum grandiflorum, C. halicacabum, Deinbolia pinnata* and Paullinia *pinnata* while tricolporate grains were found in *Blighia sapida, B. unijugata* and *Lecaniodiscus cupanioides*. The largest pollen (46.94 x 51.7 µm) was observed in *C. halicacabum* while the smallest pollen was found in *Blighia sapida* (16.80 x 15.84 µm). The exine pattern was either reticulate (*P. pinnata*), striate (*Allophylus africanus, Blighia sapida, B. unijugata, DeDeinbolia pinnata* and *Lecaniodiscus cupanioides*) or reticulofoveolate in *Cardiospermum grandiflorum* and *C. halicacabum*. Exine thickness ranged from 1.18 µm in *Allophylus africanus* to 2.55 µm in *C. Halicacabum* (Table I).

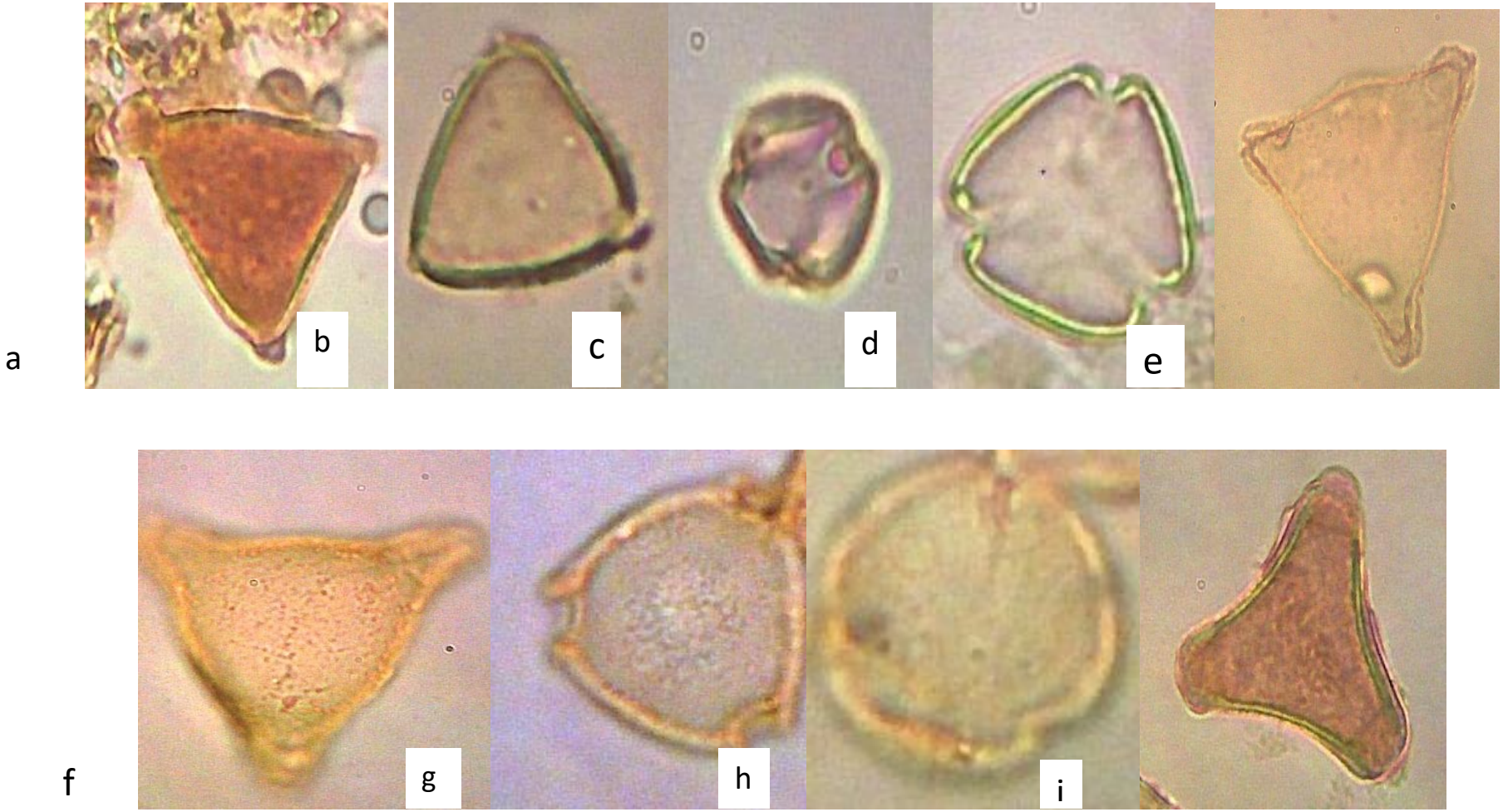

Plate I: Photomicrographs (x 400) of pollen grains of (a) *Allophylus africanus* (*b) Allophylus spicatus* (c) *Blighia sapida* (d) *Blighia unijugata* (e) *Cardiospermum grandiflorum* (f) *Cardiospermum halicacabum* (g) *Deinbolia pinnata* (h) *Lecaniodiscus cupanioides* (i) *Paullinia pinnata*

Table I: Quantitative pollen character
Minimum Maximum (µm)

Species/Characters	Polar View		Equatorial View		Exine	Exine Pattern	Os (pore)	Aperture
	PA	ED	PA	ED				
A. africanus	22.5-25	22.5-27.5	0	0	1.0- 1.25	Striate	0	Triporate
	24±0.52	25.5±0.85			1.18±0.05			
A. spicatus.	20.0-21.5	26.4-27.5	12.7-13.7	25.0-25.5	1.2-1.5	Reticulo-	2.0-3.0	Triporate
	21.5±0.42	26.9±0.24	13.18±0.18	25.34±0.24	1.37±0.06	Striate	2.5±0.18	
B. sapida	15.0-18.5	15.0-17.5	15.0-16.7	15.0-15.2	1.2- 1.5	Striate	2.5-2.9	Tricolporate
	16.8±0.60	15.84±0.46	15.68±0.31	15.06±0.04	1.35±0.06		2.56±0.04	
B. unijugata	18.7-20.0	17.5-20.0	13.5-15.0	17.5-18.5	1.23-1.5	Striate	3.5-4.0	Tricolporate
	19.34±0.22	18.6±0.43	14.1±0.29	17.94±0.20	1.29±0.05		3.74±0.08	
C. grandiflorum	40.0-45.0	35.5-52.5	32.5-36.5	45.0-50.0	2.2-2.7	Reticulate;	0	Triporate
	41.2±1.75	43.6±3.35	34.6±0.66	47.2±0.85	2.42±0.10	foveolate		
C. halicacabum	46.2-47.5	50.0-55.0	29.5-30.0	50.0-53.0	2.5-2.75	Reticulate;	0	Triporate
	46.94±0.26	51.7±0.99	29.6±0.19	51.6±0.53	2.55±0.06	foveolate		
D. pinnata	22.5-30.0	27.7-28.7	0	0	1.25-1.50	Striate	5.0-5.25	Triporate
	26.6±1.30	28.18±0.18			1.39±0.70		5.14±0.07	
L. cupanioides	20.0-21.5	20.2-25.0	20.0-22.5	19.5-20.0	1.25-1.5	Striate	2.7-3.7	Tricolporate
	20.6±0.29	22.54±0.78	21.2±0.51	19.74±0.11	1.34±0.05		3.22-0.18	
P. pinnata	33.7-37.5	37.5-40.0	0	0	1.21-1.25	Reticulate	4.5-5.0	Triporate
	35.44±0.77	38.2±0.46			1.22±0.01		4.66±0.09	

(Mean ± Standard error)

Definition of terms: PA: Polar axis (total length); ED: Equatorial diameter (total width); Os (pore): spherical opening in pollen; Exine: the outermost hard part of the pollen containing patterns or processes; PV: Polar view (view with all apertures displayed at same time); EV: Equatorial view (view with only one or two apertures displayed); NO: Not observed. All pollen grains were studied at a magnification of x400. Pollen micrographs were taken at x 100

Generally, Nigerian taxa of Sapindaceae studied can be assigned into two pollen types based on pollen grain size and aperture. The type A (climbers and shrubs) have large to medium- sized, reticulate ornamentations and triporate pollen grains. Pollen grains ranged in most species from small (21.5 µm) to medium- sized (46.94 µm) in *A. spicatus* and *C. grandiflorum,* respectively (Table I).

In the pollen grains of type B (comprising the tree species), small-sized, striate ornamentation and 3-colporate pollen grains were observed in agreement with the findings of Acevedo- Rodriguez *et al*. (2011). The smallest pollen size was observed in *B. sapida* and the largest was recorded in *C. halicacabum*. Porate pollen is the commonest pollen observed. It is relatively the basic type found in many other angiosperm families (Acevedo-Rodriguez *et al*., 1993). Pollen grains of the *Allophylus* species were similar in their shape and aperture (Plate I) but can be distinguished by their exine ornamentations. *A. africanus* had striate exine pattern while *A. spicatus* had the combinations of reticulate and striate exine patterns, i.e. reticulostriate (Table I).

Blighia sapida and *B. unijugata* were tricolporate; pollen grains in *B. unijugata* were comparatively larger in size than in *B. sapida*. *Paullinia pinnata* and *Deinbolia pinnata* were slightly similar in both pollen shape and aperture (triporate). *Paullinia pinnata* had more elongated arms with convex amb and reticulate ornamentation than *L. cupanioides* with tricolporate grains (Plate I).

There are three main classes of exine patterns in group A, namely reticulo: foveolate, striate and reticulo-striate. *Paullinia pinnata* had a unique pollen structure compared with the other taxa studied with reticulate, hemisyncolporate (a pomorphic character). *A. spicatus* had reticulo-striate ornamentation while *L. cupanioides, B. sapida, B. unijugata, Deinbolia pinnata* and *Allophylus africanus* had striate ornamentations (Plate I).

Moore *et al.* (1991) noted that pollen grain shape was less useful in taxonomic delimitation but stressed on the use of aggregate morphological characters. Ayodele (2005) and Shokefun *et al. (* 2014) noted that pollen grains could be useful at all levels of taxonomic hierarchy and could help in suggesting relationship at the specific level or to determine variation within and among species or even below the species level.

CONCLUSION

In classical taxonomy, gross morphological characters have constantly been weighed and examined carefully when decisions with respect to delimitations and classifications are concerned. Variations in interpreting and evaluating morphological characters most times result in disagreements regarding classification. Consequently, taxonomists have to, as a rule, look for characters aside morphological such as anatomical (Solereder 1908; Metcalfe and Chalk, 1950; Calquist, 1962; Akinnubi *et al.*, 2013, 2014), embryological (Maheshwari, 1950), cytological (Darlington, 1963; Manton, 1950;Oyewole *et al.,* 1990) and palynological (Erdtman, 1952; Wodehouse, 1959; Sowunmi, 1995; Ayodele, 2005; Akinnubi *et al.,* 2014; Shokefun *et al.*, 2014; Dalia, 2018). Characters of pollen grains have proven to be a useful tool in taxonomic and biosystematic studies and the importance of exine ornamentation in systematic studies and phylogenetic relationships involving species, subspecies, varieties and cultivars has been elucidated (Shokefun *et al.,* 2014). The palynological investigation of the nine species of the family Sapindaceae showed that pore or aperture is a homologous character but presence or absence of Os and/or their ornamentations can be used to further delimit species of the same genus as seen in *Allophylus* (porate) and *Blighia* (colporate).

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