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Pollen Characterization of Woody Species of the Cross River National Park, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author JKE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AEE managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Pollen description for fifty two woody species obtained from the Cross River National Park was made. Standard methods of pollen collection (conducted in 12 months), storage, preparation (Erdmann's method) and quality assurance protocols were employed. The study revealed 51% of species with tricolporate pollen, 13% with tricolpate pollen, 11% with triporate, 7% with monocolpate pollen, about 6% with inaperturate, 2% species each with zonocolpate, 4-colporate pollen and pantoporate pollen respectively. Reticulate, perforate and granulate ornamentations accounted for almost two-thirds of exine wall patterns with baculate, striate, tectate, granulate scabrate and echinate made up the remainder. The shapes ranged from subprolate, prolate to prolate spheroidal, suboblate, oblate to oblate spheroidal and to peroblate to circular. All grain arrangements were monad. While forty-six of the species could easily be separated on the basis of pollen class, exine sculpturing and pollen shape, seven others could only be delimited based on slight differences on

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polar to equatorial ratio (P/E). All members with psilate sculpture were observed as species with abiotic agents (water and wind) of dispersal. These findings would undoubtedly enhance taxonomic practice in the Park.

Keywords: Pollen description; woody species; pollen key; Nigeria and P/E ratio.

1. INTRODUCTION

Pollen grains, the fertilizing element present in flowering plants, is a valuable food resource for insects [1,2]. It has found wide applications in allergenic studies, characterization and classification of honey samples, crime detection (forensic), environmental reconstruction/paleo ecological studies, climate change modelling, biostratigraphy and geochronology, organic palynofacies, geothermal alterations, limnological and archaeological studies [3]. The science of pollen analyses has also been applied to taxonomy and evolutionary sequences [4].

The possession of sporopollenin, that resistant organic marker present in pollen grain was the fundamental criterion for its wide applicability [5]. Similarly, the pollen specific signature of each species has played an important role in the investigation of natural clustering analysis and has helped in the assessment of the taxonomic relationship between species especially with the invention of the light microscope [6]. Pollen markers have proven to be a useful tool in resolving taxonomic conflicts existing among plant taxa [7]. Pollen grains according to [8,9] have several morphological characters (such as exine ornamentation, pollen shape, polar to equatorial ratio, pollen class) that are of diagnostic importance and are more often than not species specific. It has been used most notably in delimiting species in the Family and Genus ranks.

Species specific pollen characters were employed by [10,11,12] in delimiting some members of Asclepiadaceae, Brassicaceae and Poaceae respectively, while [13,14,15,16,17,18] applied the eurypalynous character present in Acanthaceae, Rubiaceae, Verbanaceae, Asteraceae, Euphorbiaceae, Gentianaceae, and Saxifragaceae in resolving genus conflicts. Based on pollen tetrad and monad, the family Podophyllaceae was established from the circumscribed Berberidaceae. [19] used the porate pollen of Poaceae, the spinulose exine of Malvaceae and Asteraceae [20] and verrucate

exine of Plumbaginaceae [21] in characterizing members of these families.

The reticulate exine sculpture of the tribe bombaceae was used in establishing members as a separate family Bombacaceae from the spinulose exine ornamentation of the Malvaceae where both were formerly nested [20].

Palynological evidence helped established relationships among members of Faeniaceae from Ranunculaceae, Fumariaceae from Papavariaceae and Nelumbonaceae from Nymphaeaceae [22].

Pollen characters were used almost exclusively in delimiting genera in Acanthaceae and Primulaceae. The possession of 3-zonocolpate in the genus *Phytolacca* and pantocolpate in *Rivinia* was the sole candidate for establishing dissimilarity between these genera of Phytolaccaceae [23,24,25] had long advocated for the split of the genus *Polygonum* into *Koenigia*, *Persicaria*, *Polygonum*, *Pleuropterygium*, *Bistoria*, *Tiniaria* and *Fagopyrum* based on pollen homologies. This view has received taxonomic supports in recently published result [26,27].

[28] showed how thickening of exine wall around pores is a species distinctive feature for which taxonomic circumscriptions were made on the genus *Betula* (Knob like in shape), *Corylus* (club-shaped), *Caprinus* (unexpanded) and *Alnus* (presence of arcus between adjacent pores). The genera *Salix* and *Populus* under the family Salicaceae can be distinguished only by the possession of 3- long narrow furrowed pollen and spherical pollen without distinct apertures respectively [29]. Pollen characters have also been employed in delimiting species even to intra-specific levels. [30] separated 16 species of *Cyperus* based on pollen information. The possession of 1-colpate apertural morphotype in *C. iria*, *C. difformis*, *C. squarosus*, *C. triceps*, *C. flabelliformis*, *C. panicus* Var *roxburghianus*, 2-colpus in *C. exaltatus* and *C. pumilus*, 4 - Aperturate (with 3- Colpi and 1 pore) in *C. rotundus*, *C. laevigatus*, *C. alalatus*, *C. bulbosus*, pantoporate in *C. compactus*, *C. kyllingia* and

C. globosus and 1-porate in *C. digitatus* was used solely in delimiting the *Cyperus* taxon.

Four species of *Anemone* was distinguished based on the possession of 3-zonocolpate pollen in *A. obtusiloba*, Pantoporate in *A. alchemillaefolia*, Pantocolpate in *A. rivularia* and spiraperturate in *A. fulgens* [31]. [32] employed exine sculpture in delimiting species of *Bauhinia*. The occurrences of Psilate sculpture in *B. acuminata*, strait in *B. krugii*, spirulate in *B. malabarica*, reticulate tuberculate in *B. purpurea*, reticulate in *B. racemosa* and verrucate in *B. retusa*.

The pollen sizes of 70-84 μm and 105 -126 μm were sufficient to characterize *Malva rotundifolia* from *Malva silvestris* [23].

The overlaps of some pollen characters in dicot and monots have helped infer evolutionary trends. For instance, the presence of monocolpate element in monocots helped established closer affinities to the Magnolian stock, so do the possession of monocolpate element in monocot and the magnolia dicots helped infer close relationships with preangiospermous archeogoniaties than do the ranalian dicots with absence of monocolpate elements and new apertural morphoforms [33].

Recent studies have supported [34] assertion that Helobiae are not taxonomically affiliated to other monocots but are specialized Polycarpiceae with ranalian affinities. Information from pollen spectra was used to establish the evolution of most monocots from either liliacea or araceae.

It was evident from the reviewed literatures that understanding the pollen spectrum of a species was the underlying the basis for its application in plant taxonomic studies and the array of various uses of pollen studies. More over, since literature was scanty on pollen structures of woody species in Nigeria, it is imperative that pollen album of trees and shrubs be developed. It was based on the fore goings that fifty -three woody plant species were obtained for analysis on their pollen characters.

2. MATERIALS AND METHODS

2.1 Study Area

The Cross River National Park measuring about 4000 Km^2 has two divisions- Oban and

Okwangwo. The two divisions of the park were established to protect the vast primary moist tropical forests and the southern coastal mangrove forests (Fig. 1). As one of the centers of endemism [35] in the world made possible by its peculiar geographic and topographic features, species evolution is rife. The park forms a continuous liner corridor with the Korup National Park in the neighboring Cameroun.

2.2 Sample Collection

Pollen samples were obtained manually from closed anthers and in some challenging circumstances (Tall trees), a trap was constructed and used for pollen collection. Ten replicates were collected for each species. All samples were collected in the Cross River National Park.

Species were identified using field reference materials of [36,37,38], and herbarium materials in the Department of Botany, University of Calabar, Nigeria.

2.3 Storage

Collected samples were stored in zip lock bags, preserved in glacial acetic acid and transported to the laboratory for analysis.

2.4 Pollen Analysis

The widely accepted method of pollen analysis is that one reported by [39] as adopted by [24] and was used in this study. The obtained anthers were crushed with a glass rod, and the debris removed with a needle to release the pollen grains. Glacial acetic acid (GAA) was used to transfer the crushed anthers into plastic test tubes and centrifuged for about 15 minutes at 5,000 revolutions per minute (RPM). The centrifuged samples were decanted. The residues were washed three times with distilled water, each time, centrifuged and decanted. Samples were acetolysed according to Erdtman (1960). The acetolysed mixture (9 part acetic anhydride and 1 part sulphuric acid) was added to the samples, and water bathed at 84°C for 10 minutes. The heated samples were centrifuged and washed with distilled water three times, each decanted to remove the acetolysed mixture. The residues were transferred into sterile vials. Glycerine jelly was added to the prepared samples at a ratio of 50 part sample: 50 part glycerine. Ten replicates of each pollen sample were analyzed.

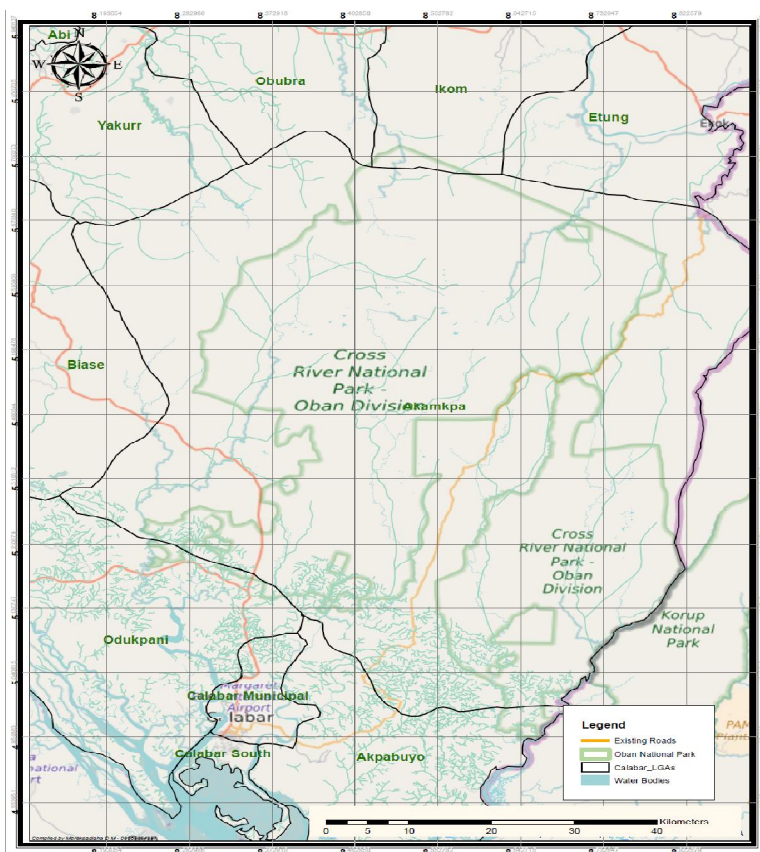


Fig. 1. Map of the study site in Cross River State, Nigeria
(Source: Bird Life International, 2010)

2.5 Mounting and Photomicrography

The prepared samples were pipette into a clean glass slides, covered with slid and sealed using a transparent nail hardener. The prepared pollen samples were properly examined under light microscope (AmScope microscope with X100 magnification). Photograph of the prepared pollen samples was taken with the aid of AmScope MA1000 camera with an in-built micrometer for measurement. Permanent slides of the prepared pollen samples were deposited in the Department of Botany, University of Calabar-Calabar, Nigeria.

3. RESULTS AND DISCUSSION

Pollen morphological studies of fifty-three woody species belonging to twenty-seven taxonomic families were carried out. The study revealed 51% of species with tricolporate pollen, 13% with tricolpate pollen, 11% with triporate, 7% with monocolpate pollen, about 6% with inaperturate, 2% species each with zonocolpate, 4-colporate

pollen and pantoporate pollen respectively. Reticulate and perforate and granulate ornamentation accounted for about two-thirds of the exine wall patterns with baculate, strait, tectate and scabrate made up the remainder. The shapes ranged from subprolate, prolate to prolate spheroidal, suboblate, oblate to oblate spheroidal and to peroblate to circular. Pollen grain arrangements were either monad, tetrad or polyad. Table 1 shows the summary of results obtained while Appendix 1 is a plate of the plant species and the recovered pollen structure.

Pollen architecture according to (40) has been a subject of discussion over the years and as such, is of great significance in the taxonomy of angiosperms and revealing inter-relationship among plant taxa. The result obtained from most of the species shows very high pollen morphological variations in aperture type, pollen shape, surface pattern, pollen size and grains arrangement. These differences serve taxonomic purposes.

Table 1. Pollen characters for studied species

S/N	Scientific Name	Common name	Family	H	Apertural type	Exine pattern (s)	Pollen size	Polar size			Equatorial diameter			P/E	Pollen Shape	PU
								Min	Max	Mean	Max	Min	Mean			
1	<i>Acacia seyal</i>	White whistling wood	Mimosoideae	T	Triporate	Reticulate	12	20	24.1	22	19.8	23	20.2	1.09	Prolate spheroidal	P
2	<i>Adansonia digitata</i>	Monkey-bread tree	Malvaceae	T	Triporate	Reticulate	53	42	52.1	48.3	47.1	61.5	59	0.82	Suboblate	M
3	<i>Azelia africana</i>	Azelia	Mimosoideae	T	Tricolpate	Reticulate	19	20.1	23	22	19.3	22.4	20	1.1	Prolate spheroidal	M
4	<i>Albizia lebbek</i>	Lebbek tree	Mimosoideae	T	Triporate	Psilate	18	81.2	90.3	85	82.7	99.2	87	0.98	oblate spheroidal	P
5	<i>Albizia procera</i>	-	Mimosoideae	T	Triporate	Reticulate	16	22	24	23	15.5	20	18	1.28	Subprolate	P
6	<i>Albizia saman</i>	Saman, Rain tree	Mimosoideae	T	Triporate	Psilate	22	19.4	23.1	21.2	32	37.3	34.6	0.61	Oblate	P
7	<i>Alstonia boonei</i>	stool wood	Apocynaceae	T	Tricolporate	Reticulate	29	21	27.1	24.4	28.3	34.5	30.2	0.81	Suboblate	M
8	<i>Alstonia congensi</i>	Dragon Tree	Apocynaceae	T	Tricolporate	Perforate	27	20.5	25.6	22.5	24.7	28	26.1	0.86	Suboblate	M
9	<i>Alstonia scholaris</i>	Blackboard tree	Apocynaceae	T	Tricolporate	Psilate	31	20.5	24	22.3	24.3	28.1	24.8	0.89	Oblate spheroidal	M
10	<i>Bombax buonopoonzes</i>	Red-flowered Silk	Bombaceae	T	Triporate	Baculate	207	40	48.4	44.4	37.8	43.4	39	1.14	Prolate spheroidal	M
11	<i>Bridelia micrantha</i>	Coastal Golden-leaf	Phyllanthaceae	T	Tricolporate	Reticulate	35	20	22	21	18.5	21	20	1.05	Prolate spheroidal	M
12	<i>Buahinia purpurea</i>	Butterfly-tree	Caesalpinioideae	T	Tricolporate	Reticulate	26	23.4	29	27.2	20.1.3	27.3	25	1.09	Prolate spheroidal	M
13	<i>Burkea africana</i>	Wild syringe	Mimosoideae	T	Inaperturate	Psilate	20	20.3	23.6	22	30.4	37.5	35	0.63	Oblate	M
14	<i>Callophyllum inophyllum</i>	Beach callophyllum	Calophyllaceae	T	Tricolporate	Granulate	68	31.2	39	40.6	27	37.1	38	1.05	Prolate spheroidal	M
15	<i>Canarium schweinfurthii</i>	African elemi or Canarium	Burseraceae	T	2-colporate	Finely Reticulate	49	19.4	26	27	17.3	23	25	1.08	Prolate spheroidal	M
16	<i>Ceiba pentandra</i>	Ceiba	Malvaceae	T	Tricolporate	Granulate	74	13	18.3	15.3	29.2	33.4	31.1	0.49	Peroblate	M
17	<i>Cleistopholis patens</i>	Head strap	Annonaceae	T	2-colpate	Tectate	22	40.6	45.3	43.1	26.1	31	27.9	1.54	Prolate	M
18	<i>Cola nitida</i>	kola nut	Sterculiaceae	T	Tricolporate	Reticulate	41	33.4	44.2	38.1	26	32.1	28	1.36	Prolate	M
19	<i>Cola rostrata</i>	-	Sterculiaceae	T	Tricolporate	Reticulate	34	32	36.7	34.5	34.2	38	36.7	0.94	Oblate spheroidal	M
20	<i>Cuala sp</i>	-	Burseraceae	T	Tricolporate	Reticulate	24	34.5	42.2	39	35	39.1	36.7	1.06	Prolate spheroidal	M

21	<i>Dalbergia sp.</i>	-	Papilionoideae	T	Tricolpate	Psilate	14	41.2	45	43	27.1	32.3	28	1.54	Prolate spheroidal	M
22	<i>Dialium guineensis</i>	velvet tamarind	Caesalpiinoideae	T	Tricolporate	Reticulate	28	20.5	25.6	22.5	24.7	28	26	0.87	Suboblate	M
23	<i>Diospyros mespiliformis</i>	African Ebony	Ebenaceae	T	Tricolporate	Psilate	33	48.3	52.5	49.5	31.7	36	33.7	1.47	Prolate	M
24	<i>Dracaena arborea</i>	Dragon Tree	Asparagaceae	T	Monocolpate	Psilate	67	87.7	90.2	88	63.3	67	64	1.61	Prolate	M
25	<i>Dracaena mannii</i>	Small-leaved Dragon-tree	Asparagaceae	S	Monocolpate	Scabrate	88	77	80.4	79	48.3	51	49	1.38	Prolate	M
26	<i>Drypetes chevalieri</i>	-	Euphorbiaceae	T	Tricolporate	Reticulate	23	23	26	25	26	33	30	0.83	Suboblate	M
27	<i>Erythrina senegalensis</i>	Coral tree	Papilionoideae	T	Triporate	Reticulate	11	35.1	43.2	38	42.7	50.4	44	0.86	Suboblate	M
28	<i>Erythrophylum suaveolens</i>	Sasswood tree	Caesalpiinoideae	T	Tricolpate	Reticulate	15	11	15	13.5	13	15	15	0.9	Oblate-spheroidal	M
29	<i>Eugenia nigerina</i>	Manding-bambara	Myrtaceae	S	Tricolpate	Granulate	88	18.3	23.5	19	15.4	18.7	12	1.58	Prolate	M
30	<i>Eugenia uniflora</i>	Native apple	Myrtaceae	T	Tricolpate	Granulate	92	18.6	23.3	19	11	18.3	14.3	1.37	Prolate	M
31	<i>Gossypium hirsutum</i>	Upland cotton	Malvaceae	T	pantoporate	Echinate	59	97	104	100	43.2	56	50	2	Prolate	M
32	<i>Heinsia crinata</i>	Bush Apple	Rubiaceae	S	Tricolporate	Scabrate	13	14.8	19	15.1	14.5	18.1	16.3	0.93	Oblate spheroidal	M
33	<i>Irvingia gabonensis</i>	Wild mango	Irvingiaceae	T	Tricolporate	Finely reticulate	76	21.3	24	19	11.6	17.4	14	1.36	Prolate	M
34	<i>Khaya ivorensis</i>	African mahogany	Meliaceae	T	Tricolpate	Reticulate	48	14	18.3	15	14.7	19	16.3	0.92	Oblate spheroidal	M
35	<i>Kigelia africana</i>	Sausage tree	Bignoniaceae	T	Tricolporate	Coarsely reticulate	35	23	26.7	25.3	29	33.1	30	0.84	Suboblate	M
36	<i>Lagestroemia speciosa</i>	Giant crape-myrtle	Lythraceae	T	Tricolporate	Psilate	22	32.5	37.1	33	30.6	33.4	31	1.06	Prolate spheroidal	M
37	<i>Mansonia altissima</i>	African black walnut	Malvaceae	T	Tricolporate	Reticulate	54	15.6	18	16.5	21.7	25.3	23	0.72	Oblate	M
38	<i>Morinda lucida</i>	Brimstone tree	Rubiaceae	T	4-colporate	Coarsely Reticulate	23	37.2	39.5	36	43.1	48.3	45	0.8	Suboblate	M
39	<i>Moringa oleifera</i>	Moringa	Moringaceae	T	Tricolporate	Psilate	18	31.5	37.2	33	30.6	34.3	32	1.03	Prolate spheroidal	M
40	<i>Myristica fragrans</i>	nutmeg	Myristicaceae	T	Monocolpate	Baculate	16	31.7	35.2	33	17.3	20.1	18	1.83	Prolate	M
41	<i>Nauclea latifolia</i>	African peach	Rubiaceae	T	Tricolporate	Reticulate	19	17	24	18	15	18	15	1.2	Subprolate	M
42	<i>Parkia biglobosa</i>	African locust bean	Mimosoideae	T	Inaperturate	Reticulate	67	24	32	28	17	25	22	1.27	Subprolate	P

43	<i>Prosopis africana</i>	Iron tree	Mimosoideae	T	Tricolporate	Psilate	39	31.3	33.1	29.5	21	24.2	22	1.34	Subprolate	M
44	<i>Pycnanthus angolensis</i>	African nutmeg	Myristicaceae	T	Monocolpate	Echinate	45	31.7	36	33.2	20	25.1	22.3	1.48	Prolate	M
45	<i>Rauvolfia vomitoria</i>	Poison devil's-pepper	Apocynaceae	T	Tricolporate	Reticulate	41	19.7	24.5	20	20	29.3	23	0.87	Suboblate	M
46	<i>Ruavolfia sp.</i>	-	Apocynaceae	T	Tricolporate	Psilate	34	12.2	16	14.7	14.1	16.4	15.9	0.92	Oblate spheroidal	
47	<i>Spathodea campanulata</i>	African tulip tree	Bignoniaceae	T	Tricolporate	Reticulate	35	39.3	54.1	42	28.5	39.7	34	1.24	Subprolate	M
48	<i>Spondias mombin</i>	Bitter kola	Anacardiaceae	T	Tricolporate		28	27.9	30.1	28	22.3	27	25	1.12	Prolate spheroidal	M
49	<i>Tabernaemontana pachysiphon</i>	Tagar	Apocynaceae	S	Tricolporate	Verrucate	15	30.7	33.3	31	38.9	43.1	40	0.78	Suboblate	M
50	<i>Tieghemella heckelli</i>	Cherry Mahogany	Sapotaceae	T	Inaperturate	Psilate	62	31	37.1	34.1	39.4	44	42.3	0.81	Suboblate	M
51	<i>Vitex acuminata</i>	Scrub Vitex	Lamiaceae	T	Tricolpate	Reticulate	18	32	35	33	30	37	33.3	0.89	Suboblate	M
52	<i>Zanthoxylum zanthoxyloides</i>	Senegal Prickly ash	Rutaceae	T	Tricolporate	Reticulate	17	14.2	18.6	16.2	20	23.1	21.3	0.76	Suboblate	M

* H=Habit; P/E=Polar/Equatorial ratio; L=Large (50-100µm); VL=Very Large (100-200 µm); S=Small; (10-25 µm) M=monad; P=Polyad; T=Tree; S=Shrub; PU=Pollen grain Arrangement; M=Medium; Min=Minimum and Max=Maximum

3.1 Apertural Type

Apertural type has been found to be a useful taxonomic character, especially at the tribal level [41]. As reported by Singh [40] pollen grains of earliest angiosperm were without an opening (acolpate type). The result of this study revealed nine apertural types.

Specifically, all members of the family Apocynaceae investigated in this study, revealed tricolporate apertural type while Fabaceae, a eurypalynous family, have been reported to be stenopalynous at the generic level [42]. This assertion which supports the result of this study is seen in the triporate aperture recorded for both *Albizia lebbbeck* and *A. saman*, with other members having different apertural types.

Taxonomic usefulness of aperture type was demonstrated by [43] when they distinguished members of the genus *Phytolacca* from *Rivinia* based on differences and similarity in the aperture type. [44,45] reported similar aperture type (tricolporate) for *Alstonia scholaris*, *Buahinia purpurea* and *Moringa oleifera*. Tricolpate pollen according to [46] is the main and basic type found in most eudicots while other aperture types such as 5-colpate, 6-colpate, porate, colporate and pororate are regarded as derived among eudicots. In the report of [47], taxa having tricolporate pollen grains reveals advanced evolutionary status while taxa with other types of aperture indicates primitive status. Inaperturate aperture recorded for *Burkea africana* and *Parkia biglobosa* agrees also with the report of [48]. [49,50], in their various works have utilized apertural attributes of pollen grains to establish probable evidences of relationships among some species of flowering plants and in some members of Clusiaceae family from Nigeria, thereby suggesting that pollen morphology can be useful as complimentary evidence with morphological characters to solve taxonomic challenges among taxa.

3.2 Pollen Shape

Pollen shape is another important diagnostic character of taxonomic value [24]. The pollen shape as given in this study is based on the values of P/E ratio as documented by [51]. From the result there exist morphological variations and similarities at the family level. For instance members of Fabaceae family in this study recorded oblate, prolate-spheroidal, suboblate

and oblate-spheroidal shapes. This result, thus agrees with the report of [52] which states that Fabaceae is eurypalynous. The stenopalynous character of this family was also evident in this although members of Mimosoideae sub family showed same pollen shape. For example, *Albizia procera*, *Parkia biglobosa*, *Prosopis africana*, all have subprolate shape.

Pollen shape recorded for *Acacia seyal*, *Albizia lebbbeck* and *A. procera*, however, was in line with the report of [53]. Also, the oblate-spheroidal shape observed in *Erythrophylum suaveolens* and the prolate spheroidal shape observed in *Azelia africana* in this study was in tandem with [54]. [55,56] suggested that the nature or shape of pollen grains could be an evolutionary modification often inherited to determine the mode of pollination and hence capable of perpetuating a particular group of plant in a given environment. Similarly, [49] were of the view that, where the pollen grain is longer than wide, it could be a structural adaptation for effective dispersal by wind while the nature of some of the pollen grains were attributed to structural adaptation. This assertion was supported by a re examination of the clustering patterns in the field of species analyzed to possessing Prolate shape.

3.3 Surface Pattern/Exine Sculpture

[57] related exine ornamentations among plant taxa to mode of pollinators. [58] drew strong correlation between species pollinated by entomophyllous agents and scabrate pattern of exine ornamentation. In this study, *Heinsia crinata* and *Dracaena mannii* was shown to possessing scabrate sculptures and insect is known as their agent of pollination and dispersal. This statement thus was in agreement with the report of [59,60], who reported that pollen grain characters do not differ much within most families and as such can be of great value in establishing affinity. The exine sculpture type for *T. pachysiphon*, *A. africana*, *M. lucida*, *K. africana*, *C. pentandra*, *C. Inophyllum*, *C. schweinfurthii*, was in line with the findings of [61]. [62] was able separate 24 of 33 species in the *Bomarea* sub genera on the basis of the reticulate exine possessed by its members which is different from other members of the Alstromeriaceae family possessing the spinose exine pattern. [45] also reported same exine sculpture for *Buahinia purpurea* as recorded in this study.

Eleven exine types were found among the 53 species. Verrucate type was found in Apocynaceae member only. Reticulate exine on the other hand was observed in Apocynaceae, Mimosoideae, Bursaraceae, Papilionoideae, and Bignoniaceae. Additionally, this exine type was found exclusively among members of Anacardiaceae, Caesalpinoideae, Euphorbiaceae, Lamiaceae, Meliaceae and Phyllantaceae. Rutaceae and Sterculiaceae were characterized by the reticulate exine ornamentation. This finding agreed with [63,64].

Perforate exine pattern was observed in Apocynaceae and Mimosoideae while Psilate exine patterns was observed in Apocynaceae, Papilionoideae, Ebenaceae, Asparagaceae, Lythraceae, Moringaceae and Sapotaceae. One member each of Malvaceae and Calophyllaceae and two members of Myrtaceae were ornamented with the Granulate exine. This was in agreement with [65]. Tectate exine was found in one member of Annonaceae while finely granulate exine type was found in one member each of Burseraceae and Irvingiaceae. [66,67] reported same as one of the various exine patterns in Sapindales and Malpighiales. The Scabrate exine pattern observed in Asparagaceae and Rubiaceae was in tandem with that shown in [68]. Echininate pattern was represented by one member in the Family Myristaceae as against one member each in the Rubiaceae and Bignoniaceae Family that were sculptured by the Coarsely Reticulate exine pattern. In addition to the latter type, [69] also reported Echininate ornamentation in these families. One member each of Bombacaceae and Myristaceae had the Baculate exine pattern. It was observed that all members ornamented by the Psilate exine type lend themselves to abiotic mode of dispersal. This agrees with [70] who drew relationship between the smoothness of the psilate ornamentation and wind and water dispersal agents.

In all, about 39.6% of the species studied were of the reticulate exine type as against 9.4% and 7.5% that were of the Perforate and Granulate exine type respectively.

3.4 Pollen Size

Pollen grain of the studied taxa varied greatly among the different species as well as among different pollen of the same species. Pollen grains according to [71] are grouped based on

their sizes into very small pollen (diameter <10 μm), small pollen (diameter 10-25 μm), medium pollen (diameter 25-50 μm), large pollen (diameter 50-100 μm), very large pollen (diameter 100-200 μm) and giant pollen (diameter >200 μm). This classification reveals that most pollen grains of the studied taxa were small, medium and large. As observed, the pollen size of *Alstonia scholaris*, *A. boonei*, *A. congensis*, *Tabernaemontana pachysiphon*, *Acacia seyal*, *Eugenia uniflora*, *Rauvolfia vomitoria*, *Ceiba pentandra*, *Cleistopholis patens*, *Bombax Ceiba*, *Dialium guineense* and *Adansonia digitata* corresponded with the reports of [72,73,74] showed pollen grains of members of Mimosoideae and *Malvaceae* to be among the largest among the angiosperms. This was shown to be correct as majority of the large pollen were mostly of these families. However some members of Myrtaceae and Sapotaceae also had large pollen. [75] demonstrated the taxonomic usefulness of pollen size at the tribal level and [76] suggested that the large to very large compound grains found in Mimosoideae serves as attraction for agents of pollination (insects, birds, bats). Evolutionary, [77] regarded pollen size as a tertiary character with little phylogenetic significance. However, several authors including [78] believe pollen size to be useful taxonomic tool at the tribal level.

3.5 Grain Arrangement

[79] reported that Monads are considered the simplest in the evolutionary line while Polyad are the most advanced. According to [80] the evolution of pollen grains from monads-to-tetrads-to-Polyad appears to have coincided in part with the development of bird and bat pollinated flowers. This report therefore suggests that species with monad arrangement evolved first, followed later by species with Polyad grains. Polyad grains reported by [81] in some members of Mimosoideae corresponded with that recorded for *Albizia lebbbeck*, *A. procera* and *A. saman* in this study. Although monads arrangement is considered the basic pollen unit for most angiosperms, Polyad pollen grains increases the reproductive capacity of species [82]. Going by this assertion, species having the Polyad grain arrangement such as *Parkia biglobosa*, *Acacia erythrocalyx*, *A. lebbbeck*, *A. procera*, *Acacia seyal* and *A. saman* would be considered more advanced and with Monad arrangement as primitives. This assertion may not be entirely correct as interplay of several factors is required before such categorical assertion could be made.

However forty six of the fifty three species studied are of the monads types.

4. CONCLUSION

This study showed specific pollen characters for most of the species studied. Most members had the tricolporate apertural type, reticulate exine pattern and the small and medium pollen sizes. Large pollen sizes were observed mostly in members of Mimosoideae. The pollen shape of most species were either sub oblate, prolate spheroidal or prolate just as the grain arrangement for all species studied were Monads except two members of Mimosoideae which were observed in Polyad form. The study also related the possession of some exine patterns to agents of dispersal. These findings would undoubtedly have fundamental bearings on the various applications of pollen studies particularly taxonomic practice.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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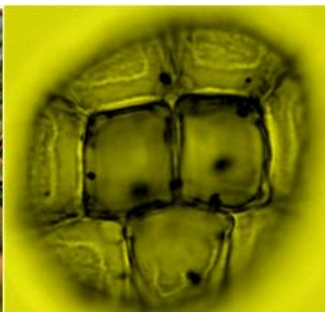
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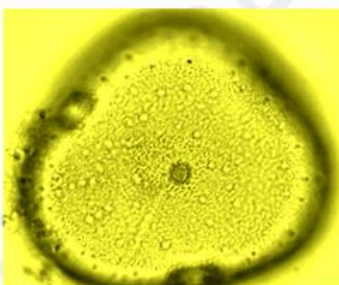
**Appendix 1. Pollen of fifty-three woody species of Cross River National Park, Nigeria
(The figures include photographs of plants and their respective pollen)**



Pollen characteristics

Pollen class: Triporate
Polar shape: Prolate-spheroidal
Exine sculpture: Reticulate
P/E ratio: 1.09 (0.00109mm)
Pollen size: 12µm
Grain Arrangement: Polyad

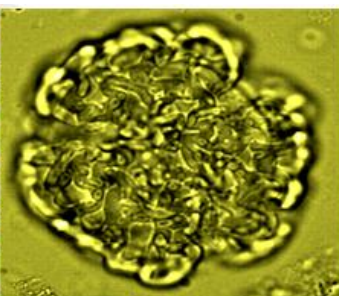
Plate 1. *Acacia seyal* Delile & its Pollen grain morphology



Pollen characteristics

Pollen class: Triporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E Ratio: 0.82(0.00082mm)
Pollen size: 53µm
Grain Arrangement: Monad

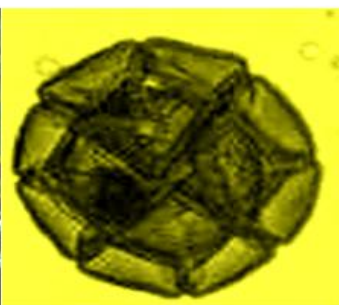
Plate 2. *Adansonia digitata* L & its Pollen grain morphology



Pollen characteristics

Pollen class: Tricolpate
Pollen shape: Prolate-spheroidal
Exine sculpture: Reticulate
P/E Ratio: 1.10(0.0011mm)
Pollen size: 19µm
Grain Arrangement: Monad

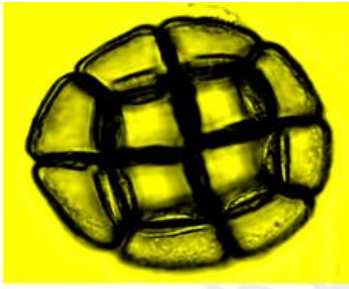
Plate 3. *Afzelia africana* Sm & its Pollen grain morphology



Pollen characteristics

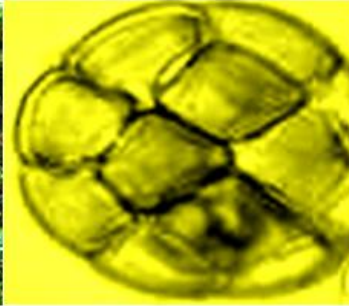
Pollen class: Triporate
Pollen shape: Oblate-Spheroidal
Exine sculpture: Psilate
P/E Ratio: 0.98 (0.00098mm)
Pollen size: 18µm
Grain Arrangement: Polyad

Plate 4. *Albizia lebeck* (L.) Benth & its Pollen grain morphology



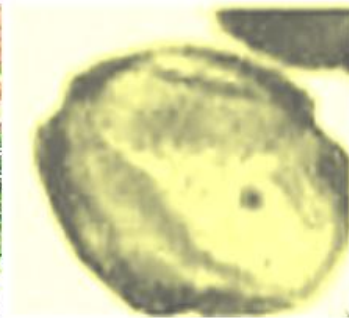
Pollen characteristics
Pollen class: Triporate
Pollen shape: Subprolate
Exine sculpture: Reticulate
P/E Ratio: 1.28
(0.00128mm)
Pollen size: 16µm
Grain Arrangement: Polyad

Plate 5. *Albizia procera* (Roxb.) Benth & its Pollen grain morphology



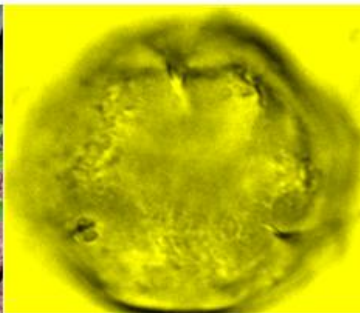
Pollen characteristics
Pollen class: Triporate
Pollen shape: Oblate
Exine sculpture: Psilate
P/E Ratio: 0.61
(0.00061mm)
Pollen size: 22µm
Grain Arrangement: Poyad

Plate 6. *Albizia saman* (Jacq.) Merr & its Pollen grain morphology



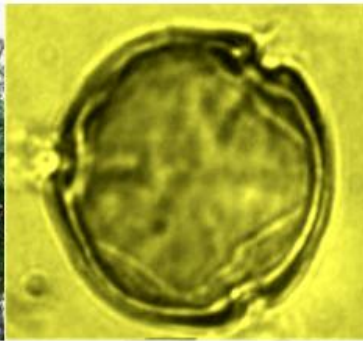
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E ratio: 0.81
(0.00081mm)
Pollen size: 29µm
Grain Arrangement: Monad

Plate 7. *Alstonia boonei* De Wild & its Pollen grain morphology



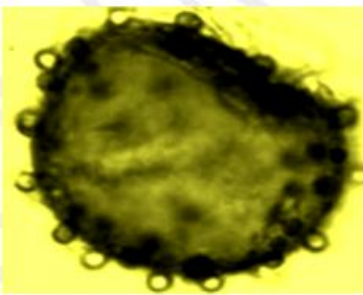
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Perforatepsilate
P/E ratio: 0.86
(0.00086mm)
Pollen size : 27 µm
Grain Arrangement: Monad

Plate 8. *Alstonia congensis* Engl & its Pollen grain morphology



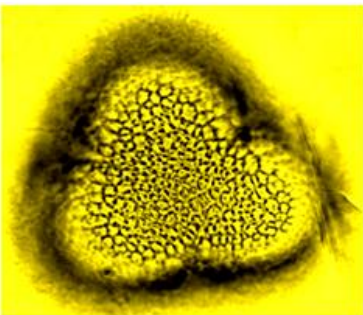
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Oblate-spheroidal
Exine sculpture: Psilate
P/E Ratio: 0.89 (0.00089mm)
Pollen size: 31µm
Grain Arrangement: Monad

Plate 9. *Alstonia scholaris* (L.) R.Br. & its Pollen grain morphology



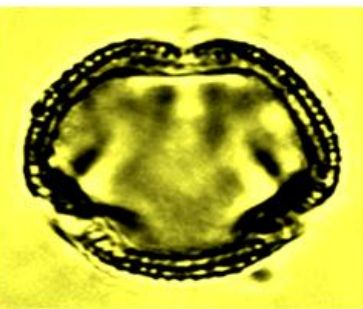
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate-spheroidal
Exine sculpturing: Reticulate
P/E Ratio: 1.09 (0.00109mm)
Pollen size: 26µm
Grain Arrangement: Monad

Plate 10. *Bauhinia purpurea* L & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate-spheroidal
Exine sculpture: Baculate
P/E Ratio: 1.14 (0.00114mm)
Pollen size: 207µm
Grain Arrangement: Monad

Plate 11. *Bombax buonopozes* P. Beauv & its Pollen grain morphology



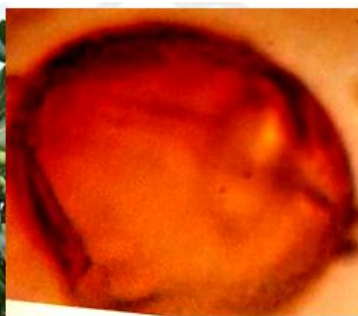
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate
Exine sculpture: Reticulate
P/E Ratio: 1.05 (0.00105mm)
Pollen size: 35µm
Grain Arrangement: Monad

Plate 12. *Bridelia micrantha* (Hochst.) Baill & its Pollen morphology



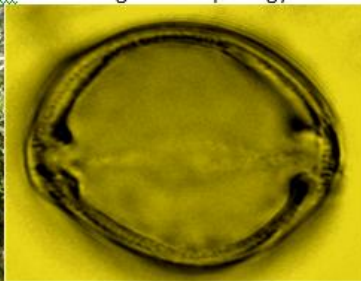
Pollen characteristics
Pollen class: Inaperturate
Pollen shape: Oblate
Exine sculpture: Psilate
P/E Ratio: 0.63 (0.00063mm)
Pollen size: 20µm
Grain Arrangement: Monad

Plate 13. *Burkea africana* Hook & its Pollen grain morphology



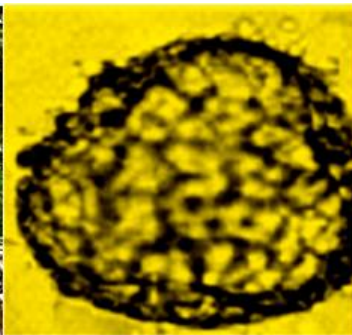
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate-spheroidal
Exine sculpture: Granulate
P/E Ratio: 1.05 (0.000105mm)
Pollen size: 68µm
Grain Arrangement: Monad

Plate 14. *Callophyllum inophyllum* L. & its Pollen grain morphology



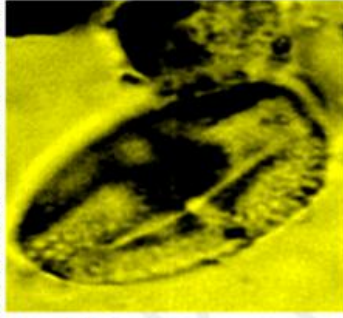
Pollen characteristics
Pollen class: 2-colporate
Pollen shape: Prolate-spheroidal
Exine sculpture: Finely Reticulate
P/E Ratio: 1.08 (0.00108mm)
Pollen size: 49µm
Grain Arrangement: Monad

Plate 15. *Canarium schweinfurthii* Engl & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Peroblate
Exine sculpture: Granulate
P/E Ratio: 0.49 (0.00049mm)
Pollen size: 74µm
Grain Arrangement: Monad

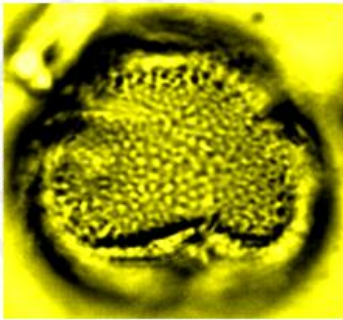
Plate 16. *Ceiba pentandra* L. & its Pollen grain morphology



Pollen characteristics

Pollen class: 2-colpate
Pollen shape: Prolate
Exine sculpture: Tectate
P/E ratio: 1.54
(0.00154mm)
Pollen size: 22µm
Grain Arrangement:
Monad

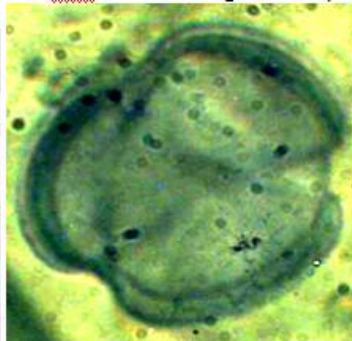
Plate 17. *Cleistopholis patens* (Benth.) Engl. & Diels & its Pollen grain morphology



Pollen characteristics

Pollen class: Tricolporate
Pollen shape: Prolate
Exine sculpture: Reticulate
P/E Ratio: 1.36
(0.00136mm)
Pollen size: 41µm
Grain Arrangement:
Monad

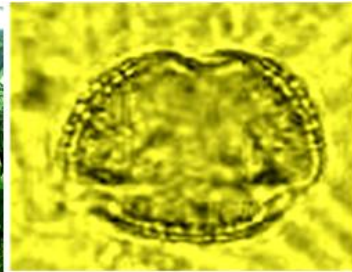
Plate 18. *Cola nitida* (Vent.) Schott & Endl & its Pollen grain morphology



Pollen characteristics

Pollen class: Tricolporate
Pollen shape: Oblate-Spheroidal
Exine sculpture: Reticulate
P/E Ratio: 0.94
(0.00094mm)
Pollen size: 34µm
Grain Arrangement:
Monad

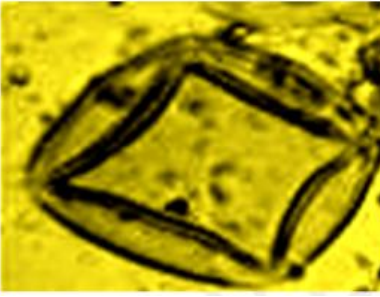
Plate 19. *Cola rostrata* K.Schum & its Pollen grain morphology



Pollen characteristics

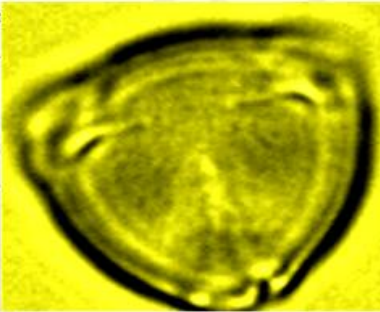
Pollen class: Tricolporate
Shape: Prolate-spheroidal
Exine sculpture: Reticulate
P/E ratio: 1.06
(0.00106mm)
Pollen size: 24µm
Grain Arrangement:
Monad

Plate 20. *Cuala* sp & its Pollen morphology



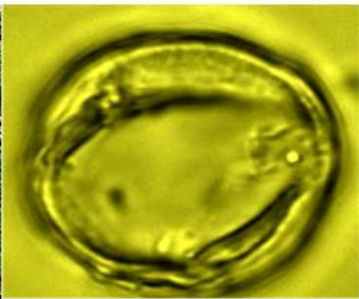
Pollen characteristics
Pollen class: Tricolporate,
Pollen shape: Prolate
Exine sculpture: Psilate
P/E Ratio: 1.54 (0.00154mm)
Pollen size: 14µm
Grain Arrangement: Monad

Plate 21. *Dalbergia* sp & its Pollen grain morphology



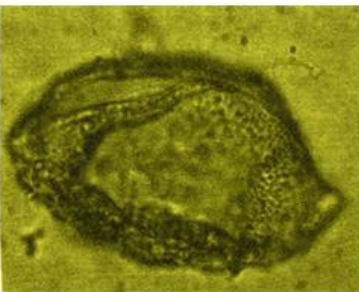
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E Ratio: 0.87
(0.00087mm)
Pollen size: 28µm
Grain Arrangement: Monad

Plate 22. *Dalium guineense* Willd & its Pollen grain morphology



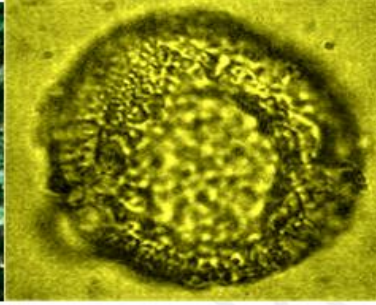
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate
Exine sculpture: Psilate
P/E Ratio: 1.47
(0.00147mm)
Pollen size: 33µm
Grain Arrangement: Monad

Plate 23. *Diospyros mespiliformis* Hochst. ex A. DC & its Pollen morphology



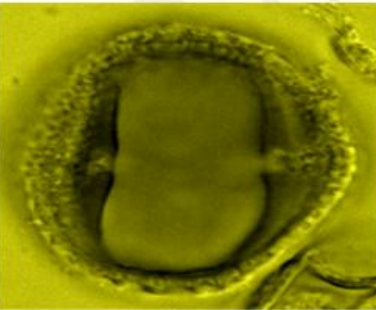
Pollen characteristics
Pollen class: Monocolpate
Pollen shape: Prolate
Exine sculpture: Psilate
P/E Ratio: 1.61 (0.00161mm)
Pollen size: 67µm
Grain Arrangement: Monad

Plate 24. *Dracaena arborea* (Willd.) Link & its Pollen grain morphology



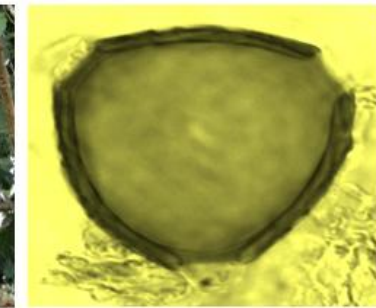
Pollen characteristics
Pollen class: Monocolpate
Pollen shape: Prolate
Exine sculpture: Reticulate
P/E Ratio: 1.38 (0.00138mm)
Pollen size: 88µm
Grain Arrangement: Monad

Plate 25. *Dracaena mannii* Baker & its Pollen grain morphology



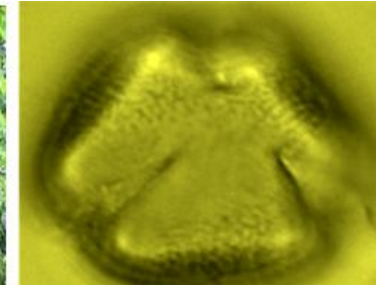
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E Ratio: 0.86 (0.00086mm)
Pollen size: 23 µm
Grain Arrangement: Monad

Plate 26. A=*Drypetes chevalieri* Beille ex Hutch. & Dalzie & its Pollen grain morphology



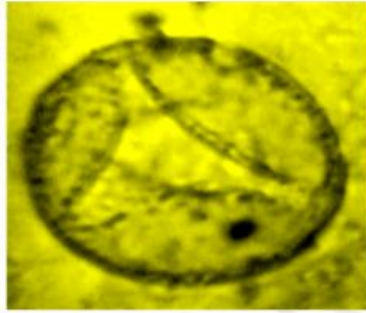
Pollen characteristics
Pollen class: Triporate
Shape: Suboblate
Exine sculpture: Reticulate
P/E ratio: 0.86 (0.00086mm)
Pollen size: 11µm
Grain Arrangement: Monad

Plate 27. *Erythrina senegalensis* DC., & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolpate
Shape: Oblate-spheroidal
Exine sculpture: Reticulate
P/E ratio: 0.90 (0.0009mm)
Pollen size: 15µm
Grain Arrangement: Monad

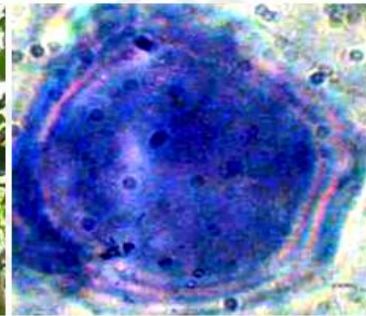
Plate 28. *Erythrophylum suaveolens* (Guill. & Perr.) Brenan, & its Pollen grain morphology



Pollen characteristics

Pollen class: Tricolpate
Pollen shape: Prolate
Exine sculpture: Granulate
P/E Ratio: 1.58 (0.00158mm)
Pollen size: 88µm
Grain Arrangement: Monad

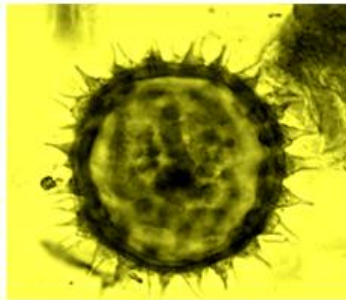
Plate 29. *Eugenia nigerina* & its Pollen grain morphology



Pollen characteristics

Pollen class: Tricolpate
Pollen shape: Prolate
Exine sculpture: Granulate
P/E Ratio: 1.37 (0.00137mm)
Pollen size: 92µm
Grain Arrangement: Monad

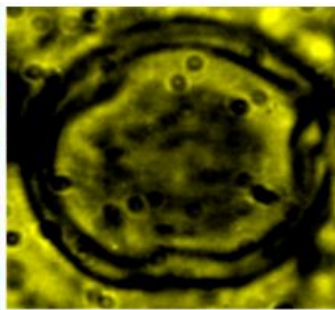
Plate 30. *Eugenia uniflora* L. & its Pollen grain morphology



Pollen characteristics

Pollen class: Pantoporate
Pollen shape: Prolate
Exine sculpture: Echinata
P/E Ratio: 2.0 (0.002mm)
Pollen size: 59µm
Grain Arrangement: Monad

Plate 31. *Gossypium hirsutum* L. & its Pollen grain morphology



Pollen characteristics

Pollen class: Tricolporate
Pollen shape: Oblate-spheroidal
Exine sculpture: Scabrate
P/E Ratio: 0.93 (0.00093mm)
Pollen size: 13µm
Grain Arrangement: Monad

Plate 32. *Heinsia crinata* (Afzel.) G.Taylor & its Pollen grain morphology



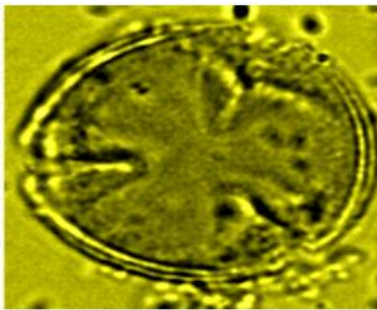
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate
Exine sculpture: Finely Reticulate
P/E Ratio: 1.36 (0.00136mm)
Pollen size: 76 μ m
Grain Arrangement: Monad

Plate 33. A=*Irvingia gabonensis* Baill. Ex Lanen & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolpate
Pollen shape: Oblate-spheroidal
Exine sculpture: Reticulate
P/E Ratio: 0.92 (0.00092mm)
Pollen size: 48 μ m
Grain Arrangement: Monad

Plate 34. *Khaya ivorensis* A. Chev & its Pollen grain morphology



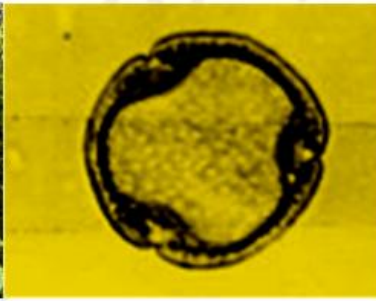
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Sub-oblate
Exine sculpture: Coarsely reticulate
P/E Ratio: 0.84 (0.00084mm)
Pollen size: 35 μ m
Grain Arrangement: Monad

Plate 35. *Kigelia africana* (Lam.) Benth & its Pollen grain morphology



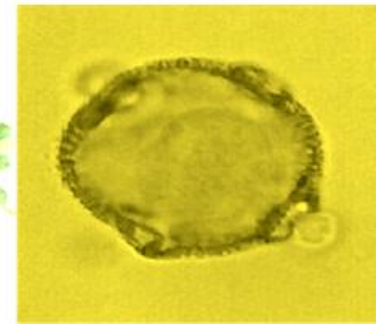
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate-spheroidal
Exine sculpture: Psilate
P/E Ratio: 1.06 (0.00106mm)
Pollen size: 22 μ m
Grain Arrangement: Monad

Plate 36. *Lagerstroemia speciosa* (L.) Pers & its Pollen grain morphology



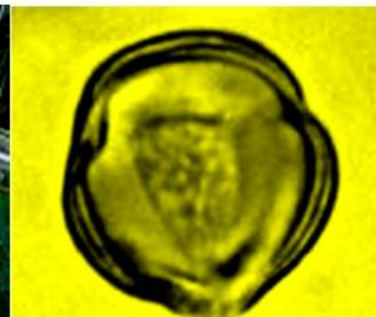
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Oblate
Exine sculpture: Reticulate
P/E Ratio: 0.72 (0.00072mm)
Pollen size: 54µm
Grain Arrangement: Monad

Plate 37. *Mansonia altissima* A. Chevalier & its Pollen morphology



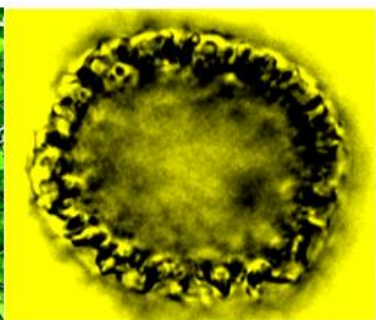
Pollen characteristics
Pollen class: 4-colporate
Pollen shape: Suboblate
Exine sculpture: Coarsely Reticulate
P/E Ratio: 0.80 (0.0008mm)
Pollen size: 23µm
Grain Arrangement: Monad

Plate 38. *Morinda lucida* Benth & its Pollen grain morphology



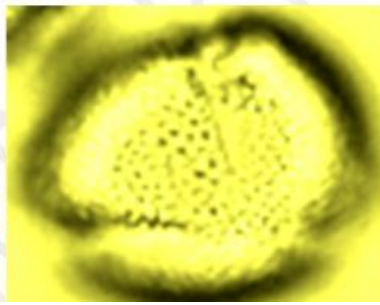
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate-spheroidal
Exine sculpture: Psilate
P/E Ratio: 1.03 (0.00103mm)
Pollen size: 18µm
Grain Arrangement: Monad

Plate 39. *Moringa oleifera* Lam & its Pollen grain morphology



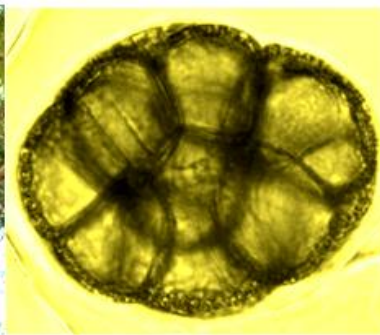
Pollen characteristics
Pollen class: Monocolporate
Pollen shape: Prolate
Exine sculpture: Baculate
P/E Ratio: 1.83 (0.00183mm)
Pollen size: 16µm
Grain Arrangement: Monad

Plate 40. *Myristica fragrans* Houtt. & its Pollen grain morphology



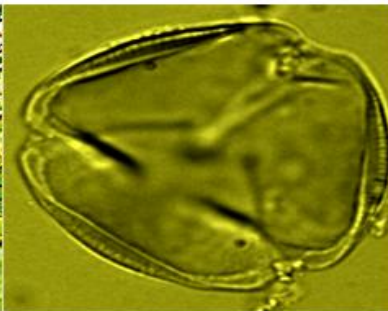
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Subprolate
Exine sculpture: Reticulate
P/E Ratio: 1.20 (0.0012mm)
Pollen size: 19µm
Grain Arrangement: Monad

Plate 41. *Nauclea latifolius* Sm. & its Pollen grain morphology



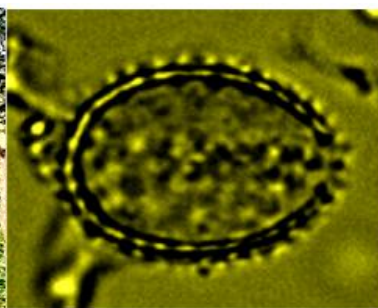
Pollen characteristics
Pollen class: Inaperturate
Pollen shape: Sub-prolate
Exine sculpture: Reticulate
P/E Ratio: 1.27 (0.00127mm)
Pollen size: 67µm
Grain Arrangement: Polyad

Plate 42. *Parkia biglobosa* (Jacq) G. Don & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Subprolate
Exine sculpture: Psilate
P/E Ratio: 1.34 (0.00134mm)
Pollen size: 39µm
Grain Arrangement: Monad

Plate 43. *Prosopis africana* (Guill. & Perr.) Taub & its Pollen grain morphology



Pollen characteristics
Pollen class: Monocolpate
Pollen shape: Prolate
Exine sculpture: Echinata
P/E Ratio: 1.84 (0.00184mm)
Pollen size: 45µm
Grain Arrangement: Monad

Plate 44. *Pycnanthus angolensis* (Welw.) Warb & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Oblate-spheroidal
Exine sculpture: Psilate
P/E Ratio: 1.92 (0.00192mm)
Pollen size: 34µm
Grain Arrangement: Monad

Plate 45. *Rauvolfia* sp & its Pollen grain morphology



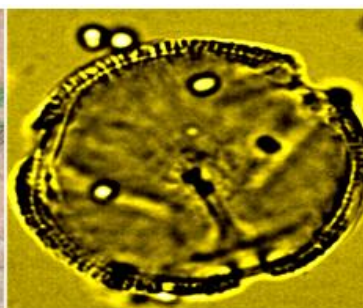
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E Ratio: 0.87 (0.00087mm)
Pollen size: 41µm
Grain Arrangement: Monad

Plate 46. *Rauvolfia vomitoria* Afzel & its Pollen grain morphology



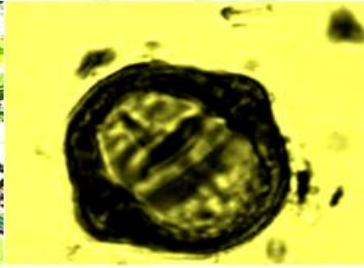
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Subprolate
Exine sculpture: Reticulate
P/E Ratio: 1.24 (0.00124mm)
Pollen size: 35µm
Grain Arrangement: Monad

Plate 47. *Spathodea campanulata* Beauv & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Prolate-spheroidal
Exine sculpture: Reticulate
P/E Ratio: 1.12 (0.00112mm)
Pollen size: 28µm
Grain Arrangement: Monad

Plate 48. *Spondias mombin* Jacq & its Pollen grain morphology



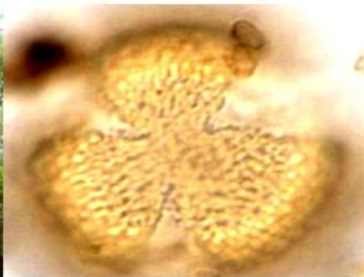
Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Verrucate
P/E Ratio: 0.78 (0.00078mm)
Pollen size: 15 µm
Grain Arrangement: Monad

Plate 49. A=Tabernaemontana pachysiphon Stapf. & its Pollen grain morphology



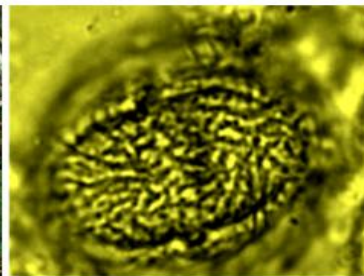
Pollen characteristics
Pollen class: Inaperturate
Pollen shape: Sub-oblata
Exine sculpture: Psilate
P/E Ratio: 0.81 (0.00081mm)
Pollen size: 62µm
Grain Arrangement: Monad

Plate 50. Tieghemella heckelli (A. Chev) Pierre ex Dubard& its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E Ratio: 0.89
(0.00089mm)
Pollen size: 18µm
Grain Arrangement:
Monad

Plate 51. Vitex acuminata R. Br & its Pollen grain morphology



Pollen characteristics
Pollen class: Tricolporate
Pollen shape: Suboblate
Exine sculpture: Reticulate
P/E Ratio: 0.76
(0.00076mm)
Pollen size: 17µm
Grain Arrangement:
Monad

Plate 52. Zanthoxylum zanthoxyloides (Lam.) Zepern. & Timler & its Pollen grain morphology

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