Pollen Morphological Studies Of Ten Tree Species In Calabar As An Aid For Plant Identification


ABSTRACT: Pollen morphology of ten woody plant species was carried out using light microscope. The study adopted Erdtman method of pollen analysis. Pollen characters studied are pollen shape, aperture type, surface pattern, pollen size, and grain arrangement. The result revealed that 50% of the species accounted for Tricolporate class, 30% for triporate class, 10% each for tricolpate and pantoporate classes. 50% of the species accounted for reticulate exine pattern, 20% for psilate, while 10% each accounted for baculate, granulate and echinate pattern. Result of pollen shape indicates that 50% of the species accounted for prolate-spheroidal, 20% for suboblate and 10% each for spheroidal, oblate and perolbate shapes. 40% of the species accounted for medium size pollen, 30% small size pollen, 20% large pollen and 10% for very large pollen. All the species were observed to possessed monad grain arrangement. Variations observed in the morphology of the studied species revealed pollen characters that can be reliably used to separate and delineate taxa.

Keywords: Pollen, Palynology, sporopollenin, Morphology, Taxonomy and systematic.

1. Introduction

Pollen grain, a coarse to powdery substance produced by matured flowering plants is a distinctive natural marker that does not easily decay due to the possession of resistant organic-walled substance called sporopollenin. Pollen grain has often not been wide applied in several field of study such as taxonomy/systematics studies, epidemiological research, archaeology, biostratigraphy and geochronology, palaeo-environmental studies, food and pharmaceutical industries, biotechnology, melissopigolnylogy and many other applied disciplines [1]. Pollen can be used to determine pollination mechanisms, foraging resources, migration routes and source zones of insects and other pollinators. Thus, very specific information can be obtained from pollen study [2]. The studies of pollen grains have received much attention over the years especially with the development of light microscope. Important pollen characters of diagnostic values [3] include pollen shape, position and number, exine, aperture type, surface wall pattern, as well as grain arrangement. According to the reports of [4]; [5], exine sculpture, pollen size and aperture type have been considered very unique and specific characters in taxonomy and evolutionary studies. Pollen characters are therefore useful tools in the identification, characterization and delineation of taxa, especially at the generic and specific levels. The diagnostic attributes of most of these pollen characters, is due to the uniqueness which are often taxa specific [6]. Several authors however, have demonstrated the usefulness of pollen characters in taxonomy and evolutionary studies. For instance [7] on pollen morphology of the genus Hibiscus and [8] on pollen morphology of three species of Emilia. [9] also characterized and documented the taxonomic importance of pollen attributes of some woody plant species of the Cross River National Park, Nigeria.

Similar studies include those of [10], [11] and [12]. Aside from the role of pollen characters in solving taxonomic challenges, [4]; [13] opined that its specificity principle forms the basis for its wide application in allergy studies, crime detection and food standardization. For example, [14] reported the use of pollen as a tracer for hibernating butterflies. According to this report, when readily recognizable and possibly confounding taxa are excluded, pollen grains can therefore serve as direct evidence of life-history phenomena that are often difficult to verify in the field. It is based on the uniqueness and distinctiveness of pollen characters that this research aimed at studying the morphology of pollen of the ten selected plant taxa with the view of highlighting characters of diagnostics importance in taxonomy/systematics and to serve as a reference pollen guide for future research.

2.0 Materials and Method

2.1 Collection and storage of polleniferous materials

Pollen samples were obtained from the anthers of the sampled woody plant species labeled and stored in vials/sample bottles containing glacial acetic acid (GAA) for preservation and were taken to Mitof Consult laboratory in Calabar for analysis. Table 1 shows list of plant species from which pollen were obtained and coordinates of where they were obtained.

2.2 Pollen sample preparation

The widely accepted method of pollen analysis by [15] as adopted by [16] was used for 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 revolution per minute (RPM). The centrifuged samples were decanted. The residues were washed three times with distilled water, each time, centrifuged and decanted. Samples were acetylized according to [15]. The acetylated 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 acetylosis mixture. The residues were transferred into sterile vials. Glycerine jelly

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was added to the prepared samples giving a ratio of 50 part sample: 50 part glycerine.

2.3 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 were 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.

2.4 Quality assurance protocol
In other to avoid some technical errors, stringent measures were followed at each stage of the research to ensure accuracy of the result. Few of these measures include:
- Ensuring collected pollen grains from the field were preserved with glacial acetic acid, stored in a sterile vial/sample bottle and well labeled;
- Ensuring plant parts from which pollen were obtained were collected and well preserved for identification;
- The Obtained pollen samples were prepared according to standard palynological techniques.
Table 1: List of sampled plant species

<table>
<thead>
<tr>
<th>S/N</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Collection number</th>
<th>Flowering period</th>
<th>Collector</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Adansonia digitata</em> L.</td>
<td>Boabab tree</td>
<td>CA/PA/001</td>
<td>October to December</td>
<td>Egbe, Alexander E.</td>
<td>4.949183</td>
<td>8.345232</td>
</tr>
<tr>
<td>2</td>
<td><em>Bombax ceiba</em> L.</td>
<td>Cotton silk tree</td>
<td>CA/PA/002</td>
<td>February to April</td>
<td></td>
<td>4.946190</td>
<td>8.338366</td>
</tr>
<tr>
<td>3</td>
<td><em>Bridelia micrantha</em> (Hochst.) Baill</td>
<td>Coast Goldleaf</td>
<td>CA/PA/003</td>
<td>October to December</td>
<td></td>
<td>4.948926</td>
<td>8.339310</td>
</tr>
<tr>
<td>4</td>
<td><em>Bauhinia purpurea</em> L.</td>
<td>Purple Bauhinia</td>
<td>CA/PA/004</td>
<td>September to November</td>
<td></td>
<td>4.949183</td>
<td>8.342400</td>
</tr>
<tr>
<td>5</td>
<td><em>Canarium schweinfurthii</em> Engl.</td>
<td>Jackalberry</td>
<td>CA/PA/005</td>
<td>July - August</td>
<td></td>
<td>4.947216</td>
<td>8.337507</td>
</tr>
<tr>
<td>6</td>
<td><em>Diospyros mespiliformis</em> Hochst. ex A. DC.</td>
<td>African ebony</td>
<td>CA/PA/006</td>
<td>October to November</td>
<td></td>
<td>4.957221</td>
<td>8.331499</td>
</tr>
<tr>
<td>7</td>
<td><em>Erythrina senegalensis</em> DC.</td>
<td>English coral tree</td>
<td>CA/PA/007</td>
<td>August – October</td>
<td></td>
<td>4.952603</td>
<td>8.324461</td>
</tr>
<tr>
<td>8</td>
<td><em>Erythrophleum suaveolens</em> (Guill. &amp; Perr.) Brenan</td>
<td>Ordeal Tree</td>
<td>CA/PA/008</td>
<td>January – April</td>
<td></td>
<td>4.964917</td>
<td>8.336992</td>
</tr>
<tr>
<td>9</td>
<td><em>Gossypium hirsutum</em> L.</td>
<td>Upland cotton</td>
<td>CA/PA/009</td>
<td>May - July</td>
<td></td>
<td>4.967909</td>
<td>8.336649</td>
</tr>
<tr>
<td>10</td>
<td><em>Nauclea latifolia</em> Sm</td>
<td>African peach</td>
<td>CA/PA/010</td>
<td>December - April</td>
<td></td>
<td>4.956708</td>
<td>8.326178</td>
</tr>
</tbody>
</table>
3.0 Results
Pollen grains of ten (10) species of interest (SoI) were investigated. Pollen characters used in this study included pollen shape, size, aperture type, Polar/Equatorial (P/E) ratio, exine and surface pattern and grain arrangement. Results of these characters are presented in section 4.1.1 to 4.4.5 respectively. Table 1 presents details of pollen characters, why plate 1 to 3 shows pollen photomicrograph of the studied taxa.

3.1 Apertural type
Four (4) aperture types were recorded in the study (Table 1). This includes triporate grain (Adansonia digitata, Bombax ceiba and Erythrina senegalensis), tricolporate (Bauhinia purpurea, Bridelia micrantha, Canarium sweinfurthii, Diospyros mespiliformis and Nuuclea latifolia), tricolpate (Erythrophleum suaveolens) and pantoporate (Gossypium hirsutum).

3.2 Pollen shape
Five (5) pollen shapes were recorded for the study. These shapes were arrived at using ratio of the polar axis and equatorial diameter measurements (Table 1). The recorded shapes are prolate-spheroidal (Bauhinia purpurea, Bombax ceiba, Bridelia micrantha, Erythrina senegalensis and Nauclea latifolia), suboblate (Erythrophleum suaveolens), peroblate (Gossypium hirsutum), oblate (Diospyros mespiliformis) and spheroidal (Canarium schweinfurthii).

3.3 Surface pattern/exine ornamentation
Surface pattern recorded for this study as evident in Table 1 include reticulate (Adansonia digitata, Bridelia micrantha, Erythrina senegalensis, Erythrophleum suaveolens and Nauclea latifolia), echinate (Gossypium hirsutum), psilate (Diospyros mespiliformis and Bauhinia purpurea), granulate (Canarium schweinfurthii) and bacculate (Bridelia micrantha) respectively.

3.4 Pollen size
Pollen sizes recorded for this study as presented in Table 1 ranged from large pollen (Adansonia digitata and Gossypium hirsutum), medium size pollen (Bauhinia purpurea, Bridelia micrantha, Canarium sweinfurthii and Diospyros mespiliformis) to small size pollen (Erythrina senegalensis, Erythrophleum suaveolens and Nauclea latifolia).

3.4 Grains arrangement
All studied taxa possessed monad type of grain arrangement (Table 1).

3.5 Identification key
Identification key for the studied taxa was constructed for easy recognition of these taxa.

1. Pollen large, triporate, reticulate, suboblate and monad-------------Adansonia digitata
2. Pollen large, pantoporate, echinate, suboblate and monad-------------Gossypium hirsutum
3. Pollen very large, triporate, baculate, prolate-spheroidal and monad-------------Bombax ceiba
4. Pollen medium, tricolporate, psilate, prolate-spheroidal and monad-------------Bauhinia purpurea
5. Pollen medium, triporate, reticulate, prolate-spheroidal and monad---------Bridelia micrantha
6. Pollen medium, tricolporate, granulate, spheroidal and monad-------------Canarium sweinfurthii
7. Pollen medium, tricolporate, psilate, oblate and monad-------------------Diospyros mespiliformis
8. Pollen small, triporate, reticulate, prolate-spheroidal and monad---------Erythrina senegalensis
9. Pollen small, tricolporate, reticulate, prolate-spheroidal and monad-----------------------------------------------Erythrophleum suaveolens
10. Pollen small, tricolporate, reticulate, prolate-spheroidal and monad---------Nauclea latifolia
<table>
<thead>
<tr>
<th>S/N</th>
<th>Species</th>
<th>Family</th>
<th>H</th>
<th>Aperture type(s)</th>
<th>Surface pattern(s)</th>
<th>PS</th>
<th>Polar size</th>
<th>Equatorial diameter</th>
<th>P/E</th>
<th>Pollen shape</th>
<th>GA</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
<td></td>
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<tr>
<td>1</td>
<td><em>Adansonia digitata</em></td>
<td>Malvaceae</td>
<td>T</td>
<td>Triporate</td>
<td>Reticulate</td>
<td>L</td>
<td>42.0</td>
<td>52.1</td>
<td>48.3</td>
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<td>2</td>
<td><em>Bauhinia purpurea</em></td>
<td>Fabaceae</td>
<td>T</td>
<td>Tricolporate</td>
<td>Psilate</td>
<td>Med</td>
<td>23.4</td>
<td>29.0</td>
<td>27.2</td>
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</tr>
<tr>
<td>3</td>
<td><em>Bombax ceiba</em></td>
<td>Bombaceae</td>
<td>T</td>
<td>Triporate</td>
<td>Baculate</td>
<td>VL</td>
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<td>44.4</td>
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<td>4</td>
<td><em>Bombax micrantha</em></td>
<td>Phyllanthaceae</td>
<td>T</td>
<td>Triporate</td>
<td>Reticulate</td>
<td>Med</td>
<td>20.0</td>
<td>22.3</td>
<td>21.0</td>
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<td>5</td>
<td><em>Canarium schweinfurthii</em></td>
<td>Burseraceae</td>
<td>T</td>
<td>Tricolporate</td>
<td>Granulate</td>
<td>Med</td>
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<td>29.4</td>
<td>26.1</td>
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<td><em>Diospyros mespiliformis</em></td>
<td>Ebenaceae</td>
<td>T</td>
<td>Tricolporate</td>
<td>Psilate</td>
<td>Med</td>
<td>31.7</td>
<td>36.6</td>
<td>33.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* S/N = Serial number; CN = Common name; H = Habit; L = Large; VL = Very Large; S = Small; Med = medium; M = monad; T = Tree; GA = Grains Arrangement; Min = Minimum; Max = Maximum; PS = Pollen size and P/E = Polar/Equatorial ratio.

**Table 1 contd. Pollen characters of the studied taxa**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Species</th>
<th>Family</th>
<th>H</th>
<th>Aperture type(s)</th>
<th>Surface pattern(s)</th>
<th>PS</th>
<th>Polar size</th>
<th>Equatorial diameter</th>
<th>P/E</th>
<th>Pollen shape</th>
<th>GA</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Erythrina senegalensis</em></td>
<td>Fabaceae</td>
<td>T</td>
<td>Triporate</td>
<td>Reticulate</td>
<td>S</td>
<td>32.7</td>
<td>37.9</td>
<td>36.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Erythrophleum suaveolens</em></td>
<td>Fabaceae</td>
<td>T</td>
<td>Tricolporate</td>
<td>Reticulate</td>
<td>S</td>
<td>11.3</td>
<td>15.0</td>
<td>12.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Gossypium hirsutum</em></td>
<td>Malvaceae</td>
<td>T</td>
<td>Pantoporate</td>
<td>Echinate</td>
<td>L</td>
<td>91.0</td>
<td>104.0</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Nauclea latifolia</em></td>
<td>Rubiaceae</td>
<td>T</td>
<td>Tricolporate</td>
<td>Reticulate</td>
<td>S</td>
<td>17.1</td>
<td>24.3</td>
<td>18.1</td>
<td></td>
<td></td>
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</table>

* S/N = Serial number; CN = Common name; H = Habit; L = Large; VL = Very Large; S = Small; Med = medium; M = monad; T = Tree; GA = Grains Arrangement; Min = Minimum; Max = Maximum; PS = Pollen size and P/E = Polar/Equatorial ratio.
Plate 1: Photomicrograph of 1a-c=Adansonia digitata; 2a-c=Bauhinia purpurea; 3a-c=Bombax ceiba and 4a-c=Bridelia micrantha
Plate 2: Photomicrograph of 5a-c=Canarium schweinfurthii; 6a-c=Diospyros mespiliformis; 7a-c=Erythrina senegalensis and 8a-c= Erythrophleum suaveolens

Plate 3: Photomicrograph of 9a-c=Gossypium hirsutum and 10a-c=Nauclea latifolia
4.0 Discussion
Pollen morphological studies of fifty-three woody species belonging to twenty-seven taxonomic families were carried out. Pollen architecture according to [17] 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. Differences in pollen shape, aperture type, grains arrangement and exine surface pattern is useful in the delimitation of taxa. These characters which are useful in taxonomy/systematic studies are in section 4.1 to 4.5.

4.1 Apertural type
Apertural type has been found to be useful taxonomic character, especially at the tribal level [18]. As reported by [17] pollen grains of earliest angiosperm were without an opening (acolpate type) and subsequently having the monocolpate, multicoline and multiporate types developing at a later stage. The result of this study revealed four (4) apertural types. This includes tricolpate, triporate, tricolporate and pantporate types. Fabaceae, a euryvalynous family, have been reported to be stenopalynous at the generic level [19]. This assertion which supports the result of this study is seen in the aperture type recorded for *Erythrina senegalensis* (tricolpate), *Bauhinia purpurea* (tricolporate) and *Erythrophleum suaveolens* (tricolpate), with other families revealing only one type of aperture for its members. Taxonomic usefulness of aperture type was demonstrated by [20] when members of the genus *Phytolacca* were distinguished from *Rivinia* based on differences and similarity in the aperture type. [21] and [22] reported similar aperture type (tricolporate) for *Buahinia purpurea*. Tricolpate pollen according to [23] is the basic type of aperture found in most eudicots while other aperture types such as 5-colpate, 6-colpate, porate, colporate and porporate are regarded as derived among eudicots. In the report of [24] taxa having tricolporate pollen grains reveals advanced evolutionary status while taxa with other types of aperture revealed primitive status. In the different works of [13]; [25], the importance of 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, have been demonstrated thereby suggesting the use of pollen morphology in conjunction with morphological characters to solving taxonomic controversies existing in problematic taxa.

4.2 Pollen shape
Pollen shape is another important diagnostic character of taxonomic value [16]. The pollen shape as given in this study is based on the values of P/E ratio as documented by [26]. From the result there exist morphological variations and similarities at the family level. For instance members of Fabaceae family in this study recorded oblate, prolatespherooidal, suboblate and oblate-spherooidal shapes. This result, thus agrees with the report of [19] which states that fabaceae is euryvalynous but display stenopalynous character at generic level. Also, the oblat-spherooidal observed in *Erythrophleum suaveolens* for this present study, has been reported by [27]. According to the works of Lemmens [28]; [29] the shape of pollen grains could be an evolutionary attribute used to determine the mode of pollination. Similarly, it has been observed that where pollen grain is longer than wide, it could be a structural adaptation for effective dispersal by wind [13]. In light of the above, species with prolate shape as recorded in this present study are said to be highly prolific and as such, are always seen growing together in groups in any habitat where they occur.

4.3 Surface pattern/exine ornamentation
The diversity of exine ornamentation as revealed in this present study appears to be related to the diversity of pollinator [30]. This report concurred with those of Furness and [31]; [10], who also posited that pollen grains do not differ within most families and as such could be of great value in establishing taxonomic affinity. The exine sculpture type for *Canarium schweinfurthii* was in line with the findings of [32], [33] was able separate the tribe Bombaceae from the family Malvaceae into the present family called Bombacaceae on the basis of the reticulate exine possessed by its members which is different from other members of the Malvaceae family possessing spinose exine. [22]; [25] also reported similar exine sculpture for *Buahinia purpurea* as recorded in this study.

4.4 Pollen size
Pollen grain of the studied taxa varied greatly in size among the different species. Pollen grains according to [26] are grouped based on their sizes into very small pollens, small pollins, medium pollens (diameter 25-50 μm), large pollens, very large pollens and giant pollens. This classification reveals that pollen grains of the studied taxa ranged from small to medium. As observed in this study, the pollen size of *Bombax ceiba* and *Adansonia digitata*, corresponded with the reports of [34] and [35]. Pollen grains of most of the studied taxa are considered to be among the largest among Angiosperms, especially for members of mimosoidae and Malvaceae [36]. According to [23] pollen size is of useful taxonomic character, particularly at tribal level. [30], suggested that the large to very large compound grains found in Mimosoideae encourage agents of pollination (insects, birds, bats). Pollen size is regarded as a tertiary character in phylogenetic studies which does not possess much phylogenetic significance [34]. However, [18] describes pollen size as a useful taxonomic tool at the tribal level.

4.5 Grains arrangement
[37] reported that Monads are considered the simplest in the evolutionary line while polyads are the most advanced. According to [38] 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 by species with polyad grains. Although monads arrangement is considered the basic pollen unit for other angiosperms grain arrangement, polyad pollen grains increases the reproductive capacity of species [39]. The result of this study therefore revealed that the ten taxa investigated with monad grains arrangement are the primitive angiosperms in the evolutionary scale.
5.0 Conclusion
The variations and similarities observed in the pollen characters of the studies taxa gives clue of the importance of palynology in separation and delineation of taxa.

Acknowledgment
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REFERENCES


