NJB, Volume 36 (2), 165-184, Dec., 2023

# TAXONOMIC SIGNIFICANCE OF THE POLLEN MORPHOLOGY OF FAMILY COMBRETACEAE R.BR. (MYRTALES) FROM NIGERIA

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Received 11th September, 2023; accepted 14th October, 2023

## ABSTRACT

A comparative study of pollen morphological characters of 19 species of Combretaceae from Nigeria was undertaken with a view to obtaining additional characters for adequate identification and classification of the taxa. Pollen samples were acetolysed and investigated under light microscope. Hierarchical cluster analysis was employed to show the similarities and affinity among the Combretaceae species based on pollen-morphological features. All the species studied were monads, small to medium sized, radially symmetrical, heterocolpate with three simple apertures alternating with three composites. The exine showed various sculpturing patterns in all the taxa studied, namely, micro-rugulate, reticulate, scabrate, striate and psilate. The pollen size ranged from 10 to 50 μm. The largest pollen size (42.68 × 38.17 μm) was recorded in *C. platypterum* and the smallest one (14.75×15.05 μm) in *C. sordidum*. The species had prolate, sub-prolate and oblate-spheroidal pollen shapes. The dendrogram and bi-plot revealed nested grouping of the *Quisqualis* species within *Combretum* sub-genus *Cacoucia*. Palynomorphological characters of the studied species are considered highly diagnostic at the generic and specific levels. Therefore, pollen morphological data provide diagnostic information for differentiating *Combretum platypterum* and *C. racemosum, C. zenkeri, C. smeathmannii, C. sordidum, Terminalia catappa* and *T. mantaly*, which are morphologically similar species.

**Key words:** Combretaceae; heterocolpate; palynology; taxonomy, Nigeria
https://dx.doi.org/10.4314/njbot.v36i2.6


## INTRODUCTION

Combretaceae also known as Almond family is one of the most diversified groups of Angiosperms in the Order Myrtales. The family includes 20 genera with over 500 species of trees, shrubs and lianas which are found across tropical and subtropical areas of the world. The greatest genetic diversity is inAfrica and Southeast Asia and very often in the savannah (Angiosperm Phylogeny Group, 2009). *Combretum* Loefl. is the largest genus in this family with about 250 species, while *Terminalia* (with about 200 species) is the second largest genus (Mudasiru *et al*., 2016). Several species of the Combretaceae have been reported as commercially important materials for food, herbal medicines, furniture, cosmetics, pharmaceutical, horticultural and silvicultural purposes. Many species in the family exhibit biological activities like anti-microbial, anti-haemorrhagic, anti-ulcer, anti-cancer activities and are in great demand world-wide for the extraction of phytochemicals like alkaloids, flavonoids, glycoside derivatives, pentacyclic triterpenes, tannins and other aromatic compounds (Fyhrquist, 2007).

Combretaceae was first named by Robert Brown in 1810 (Brown, 1810). He included nine genera for the first time, namely *Combretum*, *Cacoucia*, *Chuncoa*, *Quisqualis*, *Bucida*, *Terminalia*, *Conocarpus*, *Laguncularia* and *Getonia* but he did not classify them. The classification of Combretaceae has been unstable over the years because of the different positions authors adopted for the sub-families, tribes and genera (Tan *et al*., 2002; Maurin *et al*., 2010). The family is divided into two sub-families (Strephonematoideae Engl. et Diels and Combretoideae

Engl. and Diels). The first one contains the single genus *Strephonema* Hook f. while the latter comprises most of the genera. Combretoideae includes 19 genera (*Anogeissus*, *Buchenavia*, *Bucida*, *Calycopteris*, *Calopyxis*, *Combretum*, *Conocarpus*, *Dansiea*, *Getonia*, *Guiera*, *Gyrocarpus*, *Laguncularia*, *Lumnitzera*, *Meiostemon*, *Pteleopsis*, *Quisqualis*, *Terminalia*, *Terminaliopsis* and *Thiloa*) which are characterised by inferior ovary and seeds with small, folded or spirally twisted cotyledons (Chen and Turland, 2007). The Combretoideae genera are not sharply delimited and are difficult to classify based on their leaves, flowers, fruits and seeds, because of the morphological plasticity.

The classification of the sub-family Combretoideae within the family Combretaceae has been very ambiguous. Engler and Diels in 1899 grouped the sub-family into four tribes: Calycopterideae, Combreteae, Lagunculariеae and Terminalieae. Exell (1931) noted that the tribe Laguncularieae is a much more distinct group than the other three tribes and that it merits a different group. Within the Combretoideae, Stace (1966) emphasised that the tribe Laguncularieae is a very distinct one, characterised by having a pair of adnate bracteoles on the lower receptacle, and thus he considered the other three tribes to comprise the single tribe Combreteae. Tan *et al*. (2002) merged the three tribes (Combreteae, Terminalieae and Calycopterideae) into one tribe, Combreteae and retained the Laguncularieae as a distinct tribe. Exell and Stace (1966) published the Combretaceae classification, which was different from that of Stace (1966) in that 3 sub-tribes were recognised in the tribe Combreteae (containing seven genera: *Calopysis*, *Combretum*, *Guiera*, *Quisqualis* and *Thiloa*), Pteleopsidinae (containing only *Pteleopsis*) and Terminaliinae (comprising 5 genera: *Terminalia*, *Ramatuella* Kunth., *Bucida* Linn., *Buchenavia* Eichl., *Anogeissus, Finetia* Gagnep. and *Conocarpus*). The sub-tribe Pteleopsidinae is now inseparable from the subtribe Terminaliinae on any single character because they are very closely related. Shared morphological characters among *Pteleopsis*, *Combretum* and *Terminalia* formed the basis for the placement of *Pteleopsis* as a sub-tribe (Exell and Stace, 1966). *Pteleopsis* possesses morphological attributes of *Combretum* and *Terminalia* and is a tree or shrub that resembles both *Combretum* and *Terminalia*. The flowers have petals and resemble a *Combretum,* but there are male flowers as well as bisexual flowers in the same inflorescence as in *Terminalia*. Additional revisions on *Pteleopsis* by Van Vliet and Vollesen presented new evidence for merging the two sub-tribes, Terminaliinae and Pteleopsidinae (Vollesen, 1981). As a result, the circumscription and definition of the tribes and sub-tribes of this family are still unsettled and most local treatments have described species without reference to the affiliation of the sub-families (Jordaan, 2003).

The family Combretaceae is a taxonomically and phylogenetically complex group. Over the past years, many taxonomic problems remain within Combretaceae, which has never been satisfactorily classified into tribes, sub-tribes, genera and sections. Phylogenetic relationships within Combretaceae remain one of the biggest unresolved issues in the taxonomy of the family with most of the studies conducted on the tribe Combreteae so far being on the temperate taxa and a few representatives of some tropical taxa. Still, these studies rarely reflect the intricate pattern of character variation within a more robust group of tropical taxa in the family. The taxonomy of the family Combretaceae is very complex and challenging due to the lack of sufficient taxonomical characters and the existence of morphotypes. This family has been a subject of many academic researches both locally and internationally. The recent treatment of Combretaceae by Maurin *et al*. (2010) based on molecular analysis divided the tribe Combreteae into two sub-tribes but neither of them (Combretinae or Terminaliinae) considered morphological and anatomical characters. The only available but rather obsolete major taxonomic treatment of the Combretaceae in Nigeria by Hutchinson and Dalziel (1972) and Keay (1989) treated *Quisqualis* congeneric with *Combretum*. *Quisqualis* species are, for instance, difficult to separate from *Combretum*; *Ramatuella*, *Pteleopsis* and *Termiinaliopsis* are very close to *Terminalia*. Little is also known of the intricate pattern of character variation within *Pteleopsis* and *Terminalia*. *Pteleopsis* links the two biggest genera, *Combretum* and *Terminalia,* within the tribe Combreteae. On the inflorescence, the flowers of *Pteleopsis* species possess petals and resemble members of *Combretum* while there are male flowers as well as bisexual flowers in the same stalk as in *Terminalia*. Moreover, the Nigerian genera and species have not been studied in totality and comparatively in terms of basic research documentation. This study was conducted to determine and evaluate the qualitative and quantitative pollen morphological characteristics of Combretaceae with a view to improving the identification of the taxa and ascertain the phylogenetic relationships amongst members of the family in Nigeria.

## MATERIALS AND METHODS

### Study area and sample collection

All the pollen grains that were used in this study were collected from different locations within Nigeria (Table 1, Figure 1). Plant identification and authentication were done at the University of Ibadan Herbarium (UIH), Nigeria, where voucher specimens were also deposited for future reference. All collections were made in the field. The geo-coordinates (latitude/longitude) and elevation (altitudes) of the location for each specimen were determined with Global Positioning System (GPS). A complete list of taxa including date of collection, site of collection, collector's name, voucher number, GPS coordinates and altitudes are provided in Table 1.

### Preparation of pollen grains

The pollen grains of 19 species of the family Combretaceae were subjected to pollen analysis which included acetolysis at the Palynology Unit of Quaternary Laboratory, Birbal Sahni Institute of Palaeobotany (BSIP) Lucknow, India.

The pollen grains obtained from fresh flower buds containing anthers of the nineteen Combretaceae species were acetolysed using the Erdtman (1966) acetolysis method, as modified by Pandey and Minkley (2018). A mixture of acetic anhydride and concentrated sulphuric acid in the ratio of 9:1 (45 ml acetic anhydride and 5ml concentrated tetraoxosulphate VI ($H_2SO_4$)) was used. The flowers of the taxa collected were cut using a blade and put in 50 ml plastic centrifuge tubes clearly labelled. They were crushed with a glass rod in centrifuge tube. Three cubic centimetre (3 $cm^3$) of freshly prepared Acetolysis mixtures was added to the contents in the tubes. Each tube was equipped with a glass stirring rod and the contents were stirred at regular intervals. The contents were heated in a glass stirring rod and the contents were stirred at regular intervals. The contents were heated in a water bath from 70ºC up to boiling point for 5 minutes. The hot contents were centrifuged at 4000 rpm for 10 minutes and then the supernatant was decanted into "acetolysis waste bottle". Distilled water was added to the mixture in the tubes and then shaken thoroughly, centrifuged for 5 minutes at 3,000 rpm and the residue was decanted in the tube. This was to remove the excess acetolysis mixture. The washing with water and centrifuging were done 5 times. Finally, the samples were prepared in 50% glycerine solution for examination under the microscope.


Figure 1: Map of study area showing the geographical locations and distribution of the Nigerian species of Combretaceae

**Preparation of slides**

After preparation, each sample was mounted on a pair of slides and a drop of DPX (Dibutylphthalate Polystyrene Xylene) 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. All samples were studied under Olympus CH30 light microscopes with attached digital camera (3.2 MP LX) using both x40 and x100 objective lenses. A pre-calibrated microscope with micrometre eye piece was used for observation and measurements. Pollen grains were described based on the following characters: shape, size, exine pattern, apertural type number and character. Qualitative characters such as types and shapes of pollen grains were assessed and recorded for each species. The size of each pollen grain was measured based on the polar axis (PA) and equatorial diameter (ED) and the corresponding PA/ED ratio of the pollen recorded. All measurements of the pollen, and pollen characters were done in equatorial view and were presented in µm. The mean, range (minimum-maximum) and standard deviation values were calculated with the aid of micrometre for all the quantitative characters such as diameter of pollen grains, wall thickness of colpi, polar axis (P), equatorial diameter (E) of pollen grains and so on, based on twenty-five measurements. Descriptive terminologies and slide preparations of the pollen were based on

Erdtman (1966) and Ayodele (2005) procedures. All slides were submitted to the BSIP Herbarium, Lucknow, India and University of Ibadan Herbarium in Nigeria.

**Data analysis**

Both qualitative and quantitative pollen data obtained from this study were subjected to descriptive and multivariate statistical analyses. The mean values of all the quantitative data of Combretaceae were calculated with the range (minimum-maximum) and standard deviation. The pollen polar diameter, pollen equatorial diameter, colpi length, exine thickness, ora width, amb, exine pattern, pollen shape class, aperture and pollen size were scored and coded. Single linkage cluster analysis (SLCA) was performed using the Paleontological Statistical Software (PAST, version 4.13) package (Hammer *et al*., 2023) and Gower Distance to determine how similar the groups were, from which a dendrogram representing the phylogenetic relationships among the Combretaceae species was generated following Arogundade *et al.* (2019). Current sub-tribal and sectional classification of the family Combretaceae in the study were according to Alwan (1983) and APG III (2009).

Table 1: List of Combretaceae species collected from different geographical locations in Nigeria collected for this study

| S/N | Taxa | Voucher No. | Site of Collection | Date of Collection | Collector's Name | GPS Coordinates | | Elevation (m) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Latitude | Longitude | |
| 1. | *Anogeissus leiocarpus* (DC.) Guill. & Perr. | 22501 | Okomu National Park, Edo State | 06/04/2018 | Mudasiru, O.M. Ayodele A.E | N06° 20' 35.7" | E005° 21' 38.92" | 66 |
| 2. | *Combretum glutinosum* Perr. ex DC. | 22515 | Behind Central Mosque, Wikki Camp, Yankari Game Reserve, Bauchi State | 08/06/2018 | Mudasiru O. M. Ayodele A.E | N09° 49' 18.23" | E10° 19' 15.28" | 424 |
| 3. | *Combretum molle* R.Br. ex G.Don | 22610 | Kasuwa Goro Street, off Maiduguri Road, Ka State | 05/05/2018 | Mudasiru O. M. Ayodele A.E | N 11° 56' 44.7" | E008° 36' 46.87" | 471 |
| 4. | *C. paniculatum* Vent. | 22514 | Holiness Estate, Idi-Omo, Off Arulogun Road, Ibadan | 26/06/2022 | Mudasiru, O.M. Ayodele A.E | N07° 30' 16.99" | E003° 57' 9.04" | 244 |
| 5. | *C. platypterum* (Welw.) Hutch. & Dalz. | 22522 | Olode-Ijaye Village, Ibadan, Oyo State | 07/07/2018 | Mudasiru, O.M. Ayodele A.E | N07° 37' 52.21" | E003° 47' 51.04" | 237 |
| 6. | *C. racemosum* P. Beauv. | 22601 | Obudu Cattle Ranch, Ikwette, Cross River State | 11/02/2018 | Mudasiru, O.M. | N07° 24' 26.35" | E009° 23' 55.82" | 746 |
| 7. | *C. sordidum* Exell. | 22547 | Off PG School Road, University of Ibadan, Nigeria | 21/07/2018 | Mudasiru, O.M. | N07° 26' 49.02" | E003° 54' 5.47" | 225 |
| 8. | *C. smeathmannii* G.Don | 22462 | Along Jericho GRA, Ibadan | 22/07/2018 | Mudasiru, O.M. | N07° 25' 37.78" | E003° 54' 7.60" | 173 |
| 9. | *Combretum hispidum* Laws. | 22546 | Along Jericho Specialist Hospital, Ibadan | 20/07/2018 | Mudasiru, O.M. | N07° 24' 31.07" | E003° 53' 26.48" | 188 |
| 10. | *C. zenkeri* Engl. & Diels | 22510 | Sabongida Ora, Owan West, Edo State | 14/05/2018 | Mudasiru, O.M. Ayodele A.E | N06° 53' 34.26" | E005° 44' 40.81" | 53 |
| 11. | *Guiera senegalensis* J.F. Gmel. | 22676 | Off Gwadabawa Kasuwa, Gwadabawa Sokot State | 14/12/2017 | Mudasiru, O.M. Ayodele A.E | N13° 22' 38.24" | E005° 13' 18.59" | 262 |
| 12. | *Pteleopsis habeensis* Aubrev ex Keay | 22482 | Opposite Museum, Yankari Game Reserve, Bauchi | 08/06/2018 | Mudasiru, O.M. Ayodele A.E | N10° 19' 15.67" | E009° 49' 18.44" | 393 |
| 13. | *P. suberosa* Engl. & Diels | 22431 | Along Wawa-Babana Road, Wawa, Niger State | 19/12/2017 | Mudasiru, O.M. | N09° 54' 36.43" | E004° 24' 4.79" | 248 |
| 14. | *Quisqualis indica* Linn. | 22491 | Along Alabata Road, Abeokuta, Ogun State | 14/06/2018 | Mudasiru, O.M. | N07° 11' 48.62" | E003° 26' 20.26" | 51 |
| 15. | *Q. latialata* (Engl. ex Engl. & Diels) Exell | 22555 | Iguelaba Village, Sapoba Forest Reserve Community, Orhionmwon LGA, Edo State | 05/07/2018 | Mudasiru, O.M. Ayodele A.E | N06° 4' 32.44" | E005° 49' 8.94" | 74 |
| 16. | *Terminalia catappa* Linn. | 22552 | Department of Botany, University of Ibadan, Nigeria | 22/07/2018 | Mudasiru, O.M. | N07° 23' 53.02" | E003° 54' 55.01" | 228 |
| 17. | *T. glaucescens* Planch. ex Benth. | 22502 | Along Coulthard way, Yankari Game Reserve, Bauchi | 09/06/2018 | Mudasiru, O.M. Ayodele A.E | N09° 57' 3.96" | E10° 30' 47.23" | 337 |
| 18. | *T. mantaly* H. Perrier | 22465 | NIFOR Club, Nigerian Institute for Oil Palm Research, Benin City | 05/04/2018 | Mudasiru, O.M. Ayodele A.E | N06° 33' 25.78" | E005° 37' 20.82" | 53 |
| 19. | *T. superba* Engl. & Diels | 22416 | Oluwa Forest Reserve, Ondo State | 22/06/2018 | Mudasiru, O.M. | N06° 49' 27.01" | E004° 43' 16.43" | 34 |

GPS = Global Positioning System

## RESULTS

### Descriptions of Pollen Grains

Pollen morphology of 19 species from six Combretaceae genera was investigated using the light microscopy. The qualitative and quantitative characters obtained are listed in Table 2, while representative pollen grains are shown in Figure 2 A-Al. Pollen grains of the species studied were monads, radially symmetrical, isopolar, small to medium-sized and heterocolpate with three simple apertures alternating with three composite ones. The number of subsidiary colpi and intercolpar concavities within the family were the same as the apertures and alternates with them. Five main types of pollen grains sculpturing were recognised in the family. These include fine reticulate in *Combretum platypterum*, *C. racemosum*, *C. smeathmannii*, *Guiera senegalensis*, *Quisqualis indica*; micro-rugulate in *Anogeissus leiocarpus*, *Pteleopsis habeensis, P. suberosa*, *Combretum molle*, *Terminalia catappa, T. mantaly, T. superba*; psilate in *T. glaucescens*; reticulate in *Combretum paniculatum* and *C. hispidum*; scabrate in *Quisqualis latialata* and striate in *Combretum sordidum*, *C. glutinosum* and *C. zenkeri*. Micro-rugulate was the common type of tectum surface ornamentation among the species, since it occured in 7 species in 4 genera (Table 2). Hexacolpate and octacolpate pollen grains were observed in this study. Octacolpate characteristic features were only found on the polar axis of *Combretum zenkeri* (Figure 2 A-Al). On the same polar diameter, the amb outlines were characteristically hexagonal in all the taxa except *C. glutinosum* and *C. molle* which were circular in shape (Figure 2 A-C, Ae-Af). Based on pollen shape, many taxa of Combretaceae had prolate shape while a few possessed oblate and subprolate spheroidal types (Table 2, Figure 2 A-Al). Only *Quisqualis indica* and *Q. latialata* had oblate-spheroidal shape.

### Comparative Pollen Morphology and Morphometric Analysis of the Pollen Character States

The results demonstrated that all the species studied exhibited wide inter-specific variations with respect to the overall size of polar axes (P) and equatorial diameters (E), shape classes, the ratios of P/E, exine thickness and ora features. The largest size of pollen (42.68 × 38.17 µm) was recorded in *Combretum platypterum* and the smallest one (14.75 × 15.05 µm) in *C. sordidum* (table 2). The apertures of all the species had both colpi and lalongate ora (an aperture in the inner layer of the sporoderm). The colpi length ranged from 11.83 µm in *Combretum sp nov.* A to 36.22 µm in *C. platypterum* (Table 2). The ora width ranged from 1.2 µm in *Guiera senegalensis* to 7.5 µm in *C. platypterum*. The minimum and maximum exine thickness were recorded as 0.84 µm in *Combretum zenkeri* and 2.75 µm in *Q. indica* while the polar axis over equatorial diameter range from 0.87 (*Q. indica*) to 1.22 (*P. habeensis*). The cluster and principal component analyses revealed the extent of affinity among 19 species of the family Combretaceae (Figures 2 and 3). The cluster analysis of Combretaceae palynological data clearly separated the species and two major clusters were found in the cladogram constructed through cluster analysis (Figure 2). The first cluster consisted of only taxa of the genera *Combretum* Loefl. and *Quisqualis* Linn., namely *Combretum glutinosum*, *C. molle*, *C. paniculatum*, *C. platypterum*, *C. racemosum*, *Quisqualis indica*, *Q. latialata*, *Combretum zenkeri*, *C. sordidum*, *C. smeathmannii*, *C. hispidum*, while the second cluster comprised members of genera *Terminalia*, *Anogeissus*, *Pteleopsis* and *Guiera*. *T. catappa, T. glaucescens*, *T. superba*, *Terminalia mantaly, Anogeissus leiocarpus*, *Pteleopsis habeensis, P. suberosa* and *Guiera senegalensis* were found to be farther from all other taxa in the group (Figure 3). The PCA yielded four groups and the position of each species in the PCA showed evolutionary trend within the family Combretaceae (Figure 4). Amongst *Combretum paniculatum*, *C. racemosum*, *Quisqualis indica* and *Q. latialata*, only *Quisqualis indica* and *Q. latialata* were identified by the PCA as having a strong liking for one another in comparison to the other species (*C. paniculatum* and *C. racemosum*) in the PCA. Within the species in the scatter diagram, only *Q. indica* and *Q. latialata* had tricolpate characteristic feature and oblate-spheroidal pollen shape. The closeness of *C. racemosum* and *C. paniculatum* to *Q. indica* and *Q. latialata* in the group is attested by the characters of pollen size (medium) and exine wall thickness (Figure 4).

Table 2: Qualitative and quantitative pollen-grain morphological data of Combretaceae taxa

| S/No. | Taxa | Polar axis (P) (µm) | Equatorial diameter (E) (µm) | Colpi length (µm) | Exine thickness | Ora width (µm) | P/E | Amb | Exine pattern | Aperture | Shape class | Pollen Size |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Anogeissus leiocarpus* | 15.0-16.3 (15.84±0.89) | 14.7-15.1 (14.92±0.64) | 11.7-12.5 (12.24±1.86) | 1.2-1.3 (1.26±0.28) | 2.5 | 1.06 | 6-lobed | Micro-rugulate | Hexacolpate | Subprolate | Small |
| 2. | *Combretum glutinosum* | 27.6-31.5 (28.25±1.25) | 23.5-28.7 (26.95±0.32) | 20.0-22.5 (21.43±0.76) | 1.0-2.0 (1.70±0.31) | 2.5 | 1.05 | 6-circular | Striate | Hexacolpate | Prolate-spheroidal | Medium |
| 3. | *Combretum molle* | 25.4-27.9 (26.15±2.03) | 24.2-29.5 (27.08±1.17) | 21.0-24.5 (22.62±3.08) | 1.0-2.0 (1.45±0.03) | 2.5 | 1.09 | 6-circular | Faintly micro-rugulate | Hexacolpate | Subprolate | Medium |
| 4. | *C. paniculatum* | 22.7-28.9 (25.84±0.75) | 21.5-25.1 (23.28±0.19) | 20.5-24.5 (21.58±1.29) | 1.5-2.5 (2.13±0.38) | 2.5 | 1.11 | 6-angular | Reticulate | Hexacolpate | Subprolate | Medium |
| 5. | *C. platypterum* | 40.0-45.0 (42.68±1.83) | 35.5-40.2 (38.17±2.24) | 35.1-37.5 (36.22±1.08) | 2.5-2.8 (2.63±0.14) | 7.5 | 1.12 | 6-angular | Fine reticulate | Hexacolpate | Subprolate | Medium |
| 6. | *C. racemosum* | 25.1-30.5 (28.47±2.15) | 22.5-27.2 (25.50±0.97) | 20.5-25.3 (23.18±1.08) | 2.5-2.52 (2.51±0.47) | 2.5 | 1.12 | 6-angular | Fine reticulate | Hexacolpate | Subprolate | Medium |
| 7. | *C. sordidum* | 14.5-15.0 (14.75±0.23) | 15.0-15.2 (15.05±0.03) | 11.7-12.0 (11.83±0.05) | 1.2-1.3 (1.25±0.02) | 1.3 | 0.98 | 6-angular | Striate | Hexacolpate | Prolate-spheroidal | Small |
| 8. | *C. smeathmannii* | 26.5-31.5 (29.61±3.04) | 24.8-28.1 (26.93±2.18) | 20.5-23.0 (22.23±1.01) | 1.0-2.0 (1.83±0.25) | 1.3 | 1.10 | 6-angular | Fine reticulate | Hexacolpate | Prolate-spheroidal | Medium |
| 9. | *C. hispidum* | 17.5-20.0 (18.56±2.08) | 15.0-17.5 (16.48±1.32) | 15.0-16.0 (15.50±0.03) | 2.5-2.6 (2.55±0.02) | 2.5 | 1.13 | 6-angular | Reticulate | Hexacolpate | Subprolate | Small |
| 10. | *C. zenkeri* | 17.5-18.5 (17.94±2.15) | 15.7-17.5 (16.88±0.76) | 12.5-15.0 (13.50±0.83) | 0.7-1.0 (0.84±0.03) | 1.3 | 1.06 | 8-angular | Striate | Octacolpate | Prolate-spheroidal | Small |
| 11. | *Guiera senegalensis* | 12.5-17.5 (16.67±1.04) | 12.0-15.0 (13.81±0.96) | 10.0-13.5 (12.50±2.18) | 1.2-1.3 (1.25±0.03) | 1.2 | 1.21 | 6-angular | Fine reticulate | Hexacolpate | Prolate-spheroidal | Small |
| 12. | *Pteleopsis habeensis* | 22.9-28.6 (24.81±1.27) | 18.8-22.5 (20.35±0.92) | 22.0-25.5 (23.57±4.14) | 1.0-2.0 (1.50±0.22) | 2.5 | 1.22 | 6-lobed | Micro-rugulate | Hexacolpate | Subprolate | Small |
| 13. | *P. suberosa* | 18.5-23.5 (19.65±0.84) | 15.0-17.5 (16.08±1.04) | 17.5-22.5 (19.22±3.07) | 1.5-2.0 (1.68±0.19) | 2.5 | 1.14 | 6-lobed | Micro-rugulate | Hexacolpate | Subprolate | Small |
| 14. | *Quisqualis indica* | 37.0-38.5 (37.76±2.09) | 42.3-45.9 (43.38±1.05) | 28.5-34.5 (31.58±1.75) | 2.7-2.8 (2.75±0.02) | 5 | 0.87 | 3-angular | Fine reticulate | Tricolpate | Oblate-spheroidal | Medium |
| 15. | *Q. latialata* | 27.0-32.7 (29.47±3.28) | 22.3-27.9 (25.28±1.94) | 19.5-25.5 (24.50±1.40) | 2.0-2.2 (2.05±0.17) | 5 | 1.17 | 3-angular | Scabrate | Tricolpate | Oblate-spheroidal | Medium |
| 16. | *Terminalia catappa* | 20.0-22.5 (21.35±2.17) | 20.0-20.5 (20.25±0.84) | 18.7-19.5 (19.24±1.05) | 2.0-2.2 (2.10±0.07) | 2.5 | 1.05 | 6-lobed | Perforate micro-rugulate | Hexacolpate | Prolate-spheroidal | Small |
| 17. | *T. glaucescens* | 20.0-21.5 (20.50±0.89) | 17.0-17.5 (17.20±0.68) | 17.5-18.5 (18.25±3.17) | 1.2-1.3 (1.28±0.04) | 2.5 | 1.19 | 6-lobed | Psilate | Hexacolpate | Prolate-spheroidal | Small |
| 18. | *T. mantaly* | 16.2-17.5 (16.84±2.02) | 14.5-15.0 (14.75±1.04) | 14.5-15.2 (14.88±0.87) | 1.2-1.3 (1.26±0.12) | 2.5 | 1.14 | 6-lobed | Micro-rugulate | Hexacolpate | Subprolate | Small |
| 19. | *T. superba* | 17.8-23.5 (21.93±0.86) | 17.0-19.5 (18.76±0.62) | 17.0-21.5 (19.57±1.65) | 1.5-2.0 (1.78±0.25) | 2.5 | 1.17 | 6-lobed | Perforate micro-rugulate | Hexacolpate | Prolate-spheroidal | Small |

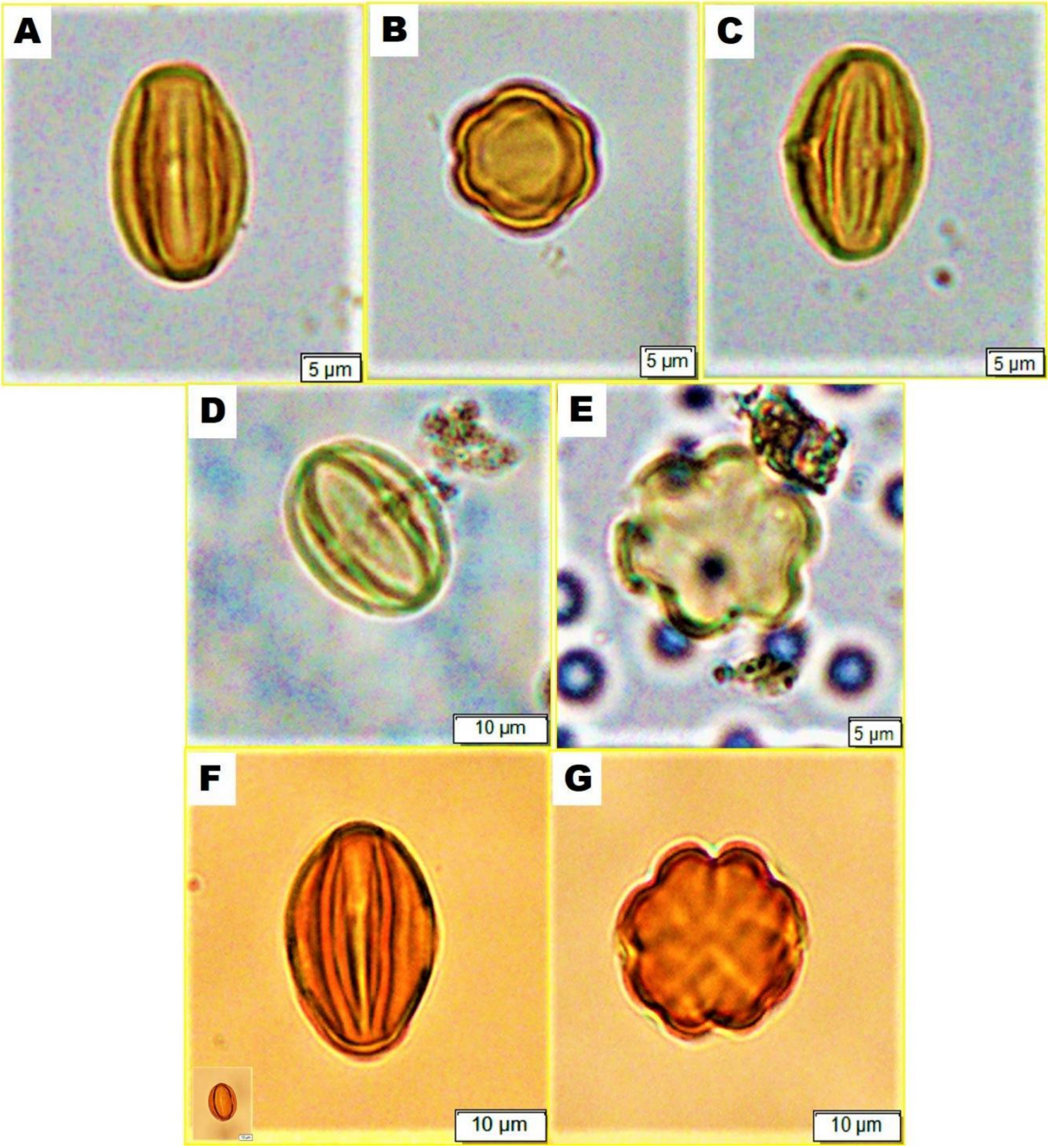

Figure 2 (A-G): Photo-micrographs of pollen grains of some Combretaceae species

A: *Combretum glutinosum* showing colpi at equatorial view
B: *Combretum glutinosum* showing circular/hexagonal amb at the polar view
C: *Combretum glutinosum* showing striate exine pattern at equatorial view
D: *Combretum paniculatum* showing two colpi at equatorial view
E: *Combretum paniculatum* showing 6-angular amb and heterocolpate pollen at the polar

view
F: *Combretum zenkeri* showing two colpi at the equatorial view
G: *Combretum zenkeri* showing heterocolpate pollen with 8-angular amb at the polar view

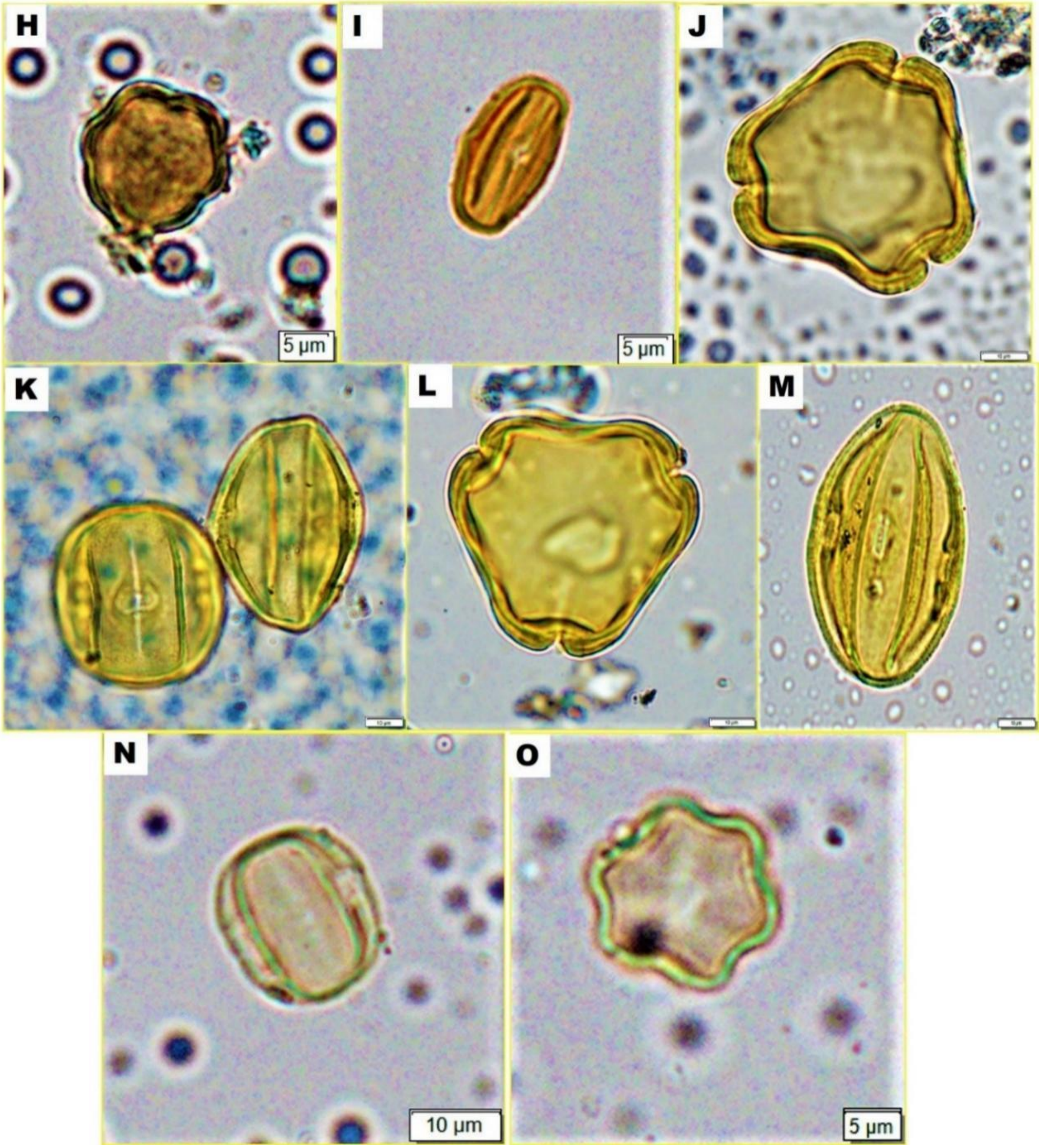


Figure 2 (H-O): Photo-micrographs of pollen grains of some Combretaceae species

H: *Guiera senegalensis* showing heterocolpate pollen grain at the polar view
I: *Pteleopsis habeensis* showing colpi at equatorial view
J: *Quisqualis indica* showing tricolpate pollen type with 3-aperture at the polar view
K: *Quisqualis indica* showing colpi at equatorial view
L: *Quisqualis latialata* showing tricolpate pollen grain with 3-aperture at the polar view
M: *Quisqualis latialata* showing colpi at equatorial view
N: *Terminalia superba* at equatorial view
O: *Terminalia superba* showing heterocolpate pollen grain at the polar view

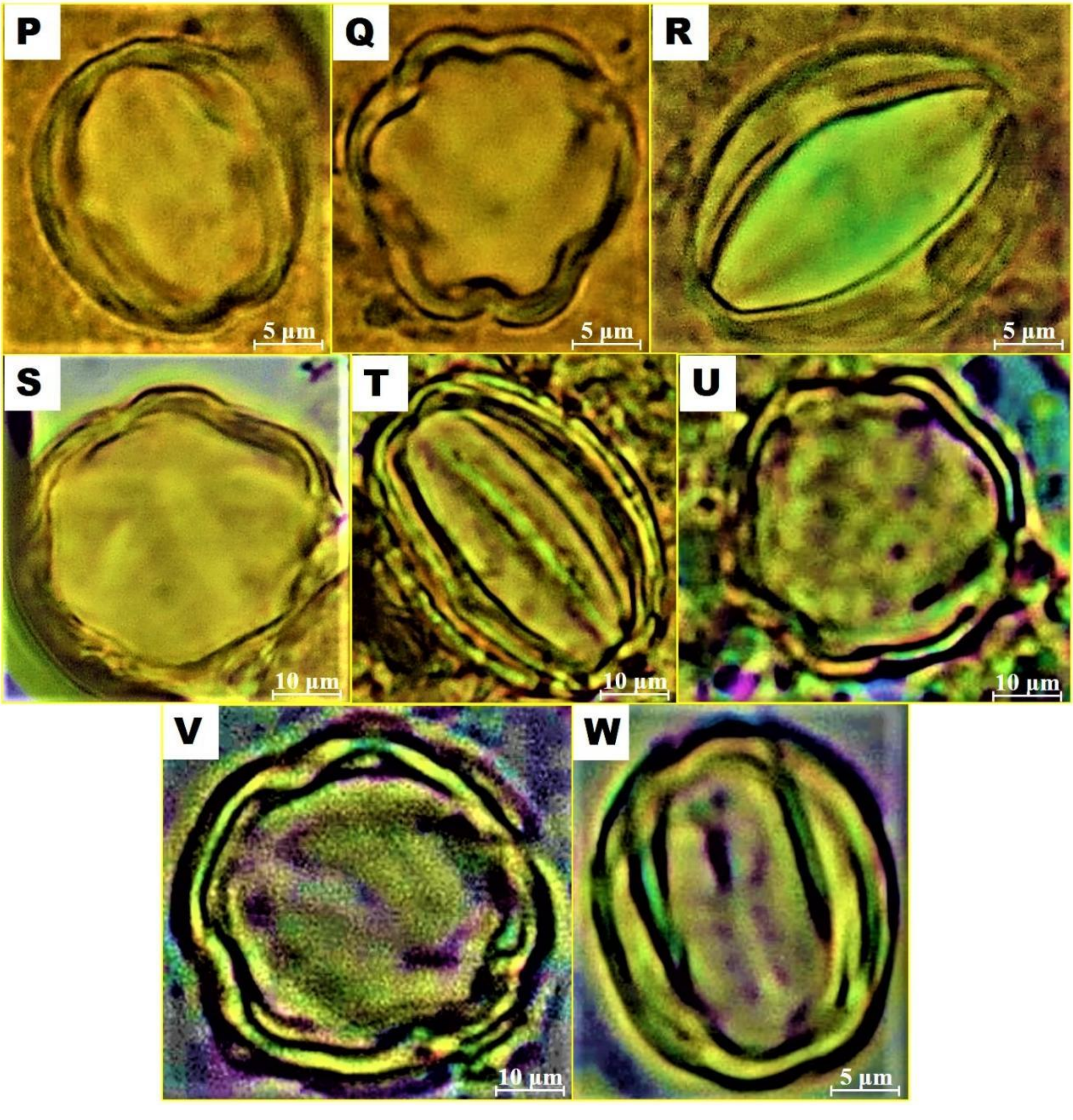


Figure 2 (P-W): Photo-micrographs of pollen grains of some Combretaceae species

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P: *Terminalia glaucescens* showing colpi at equatorial view
Q: *Terminalia glaucescens* showing hexacolpate pollen type at the polar view
R: *Terminalia mantaly* at equatorial view
S: *Terminalia mantaly* showing hexacolpate pollen at the polar view
T: *Terminalia catappa* showing colpi at equatorial view
U: *Terminalia catappa* showing hexacolpate pollen type at the polar view
V: *Anogeissus leiocarpus* showing a sub-prolate pollen shape at the polar view
W: *Anogeissus leiocarpus* showing one aperture and ora at equatorial view

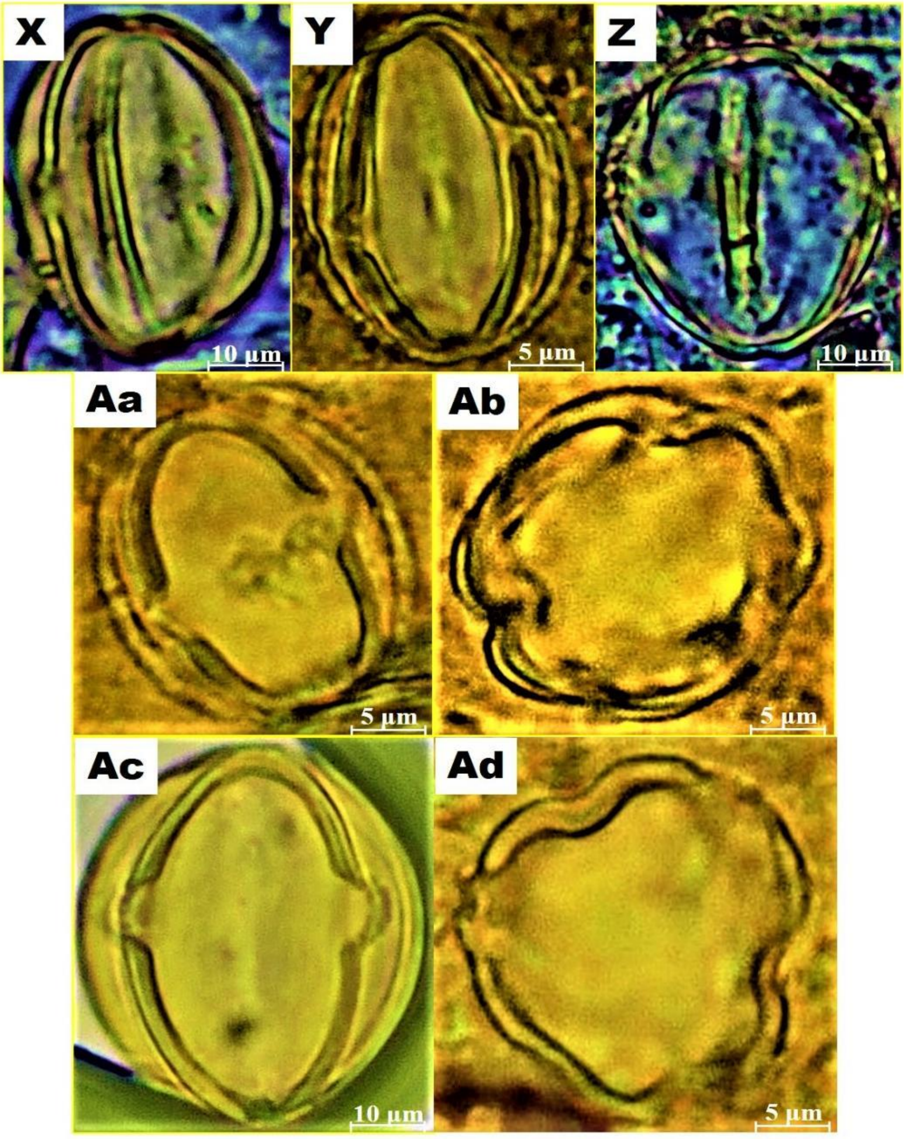


Figure 2 (X-Ad): Photo-micrographs of pollen grains of some Combretaceae species

geriaX: *Combretum platypterum* showing one clear aperture at equatorial view
Y: Pollen of *C. platypterum* showing two apertures
Z: Pollen of *C. platypterum* showing central aperture with distinct os
Aa: Pollen of *Combretum racemosum* showing two colpi
Ab: Pollen of *Combretum racemosum* showing a hexacolpate pollen type at the polar view
Ac: Pollen of *Combretum hispidum* displaying two colpi
Ad: Pollen of *Combretum hispidum* displaying hexacolpate pollen at the polar view

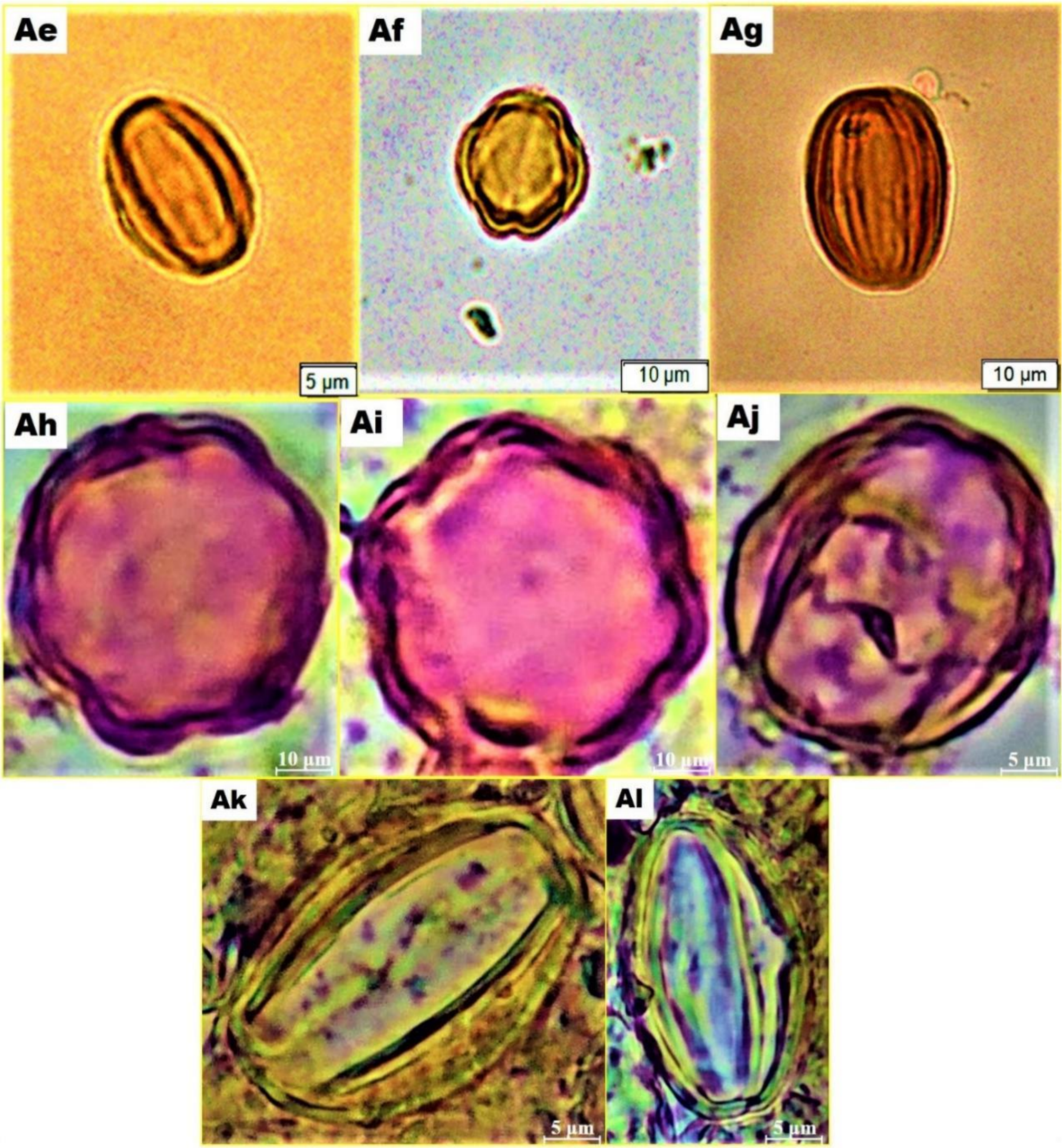


Figure 2 (Ae-Al): Photo-micrographs of pollen grains of some Combretaceae species

Ae: *Combretum molle* showing colpi at equatorial view
Af: *Combretum molle* showing circular/hexagonal amb at the polar view
Ag: *Pteleopsis suberosa* displaying colpi at equatorial view
Ah & Ai: Polar view of *Combretum sordidum* showing hexacolpate pollen grain
Aj: *Combretum sordidum* showing one colpos at equatorial view
Ak: Polar view of *Combretum smeathmannii* displaying three colpi
A**l**: Pollen of *Combretum smeathmannii* displaying central colpos

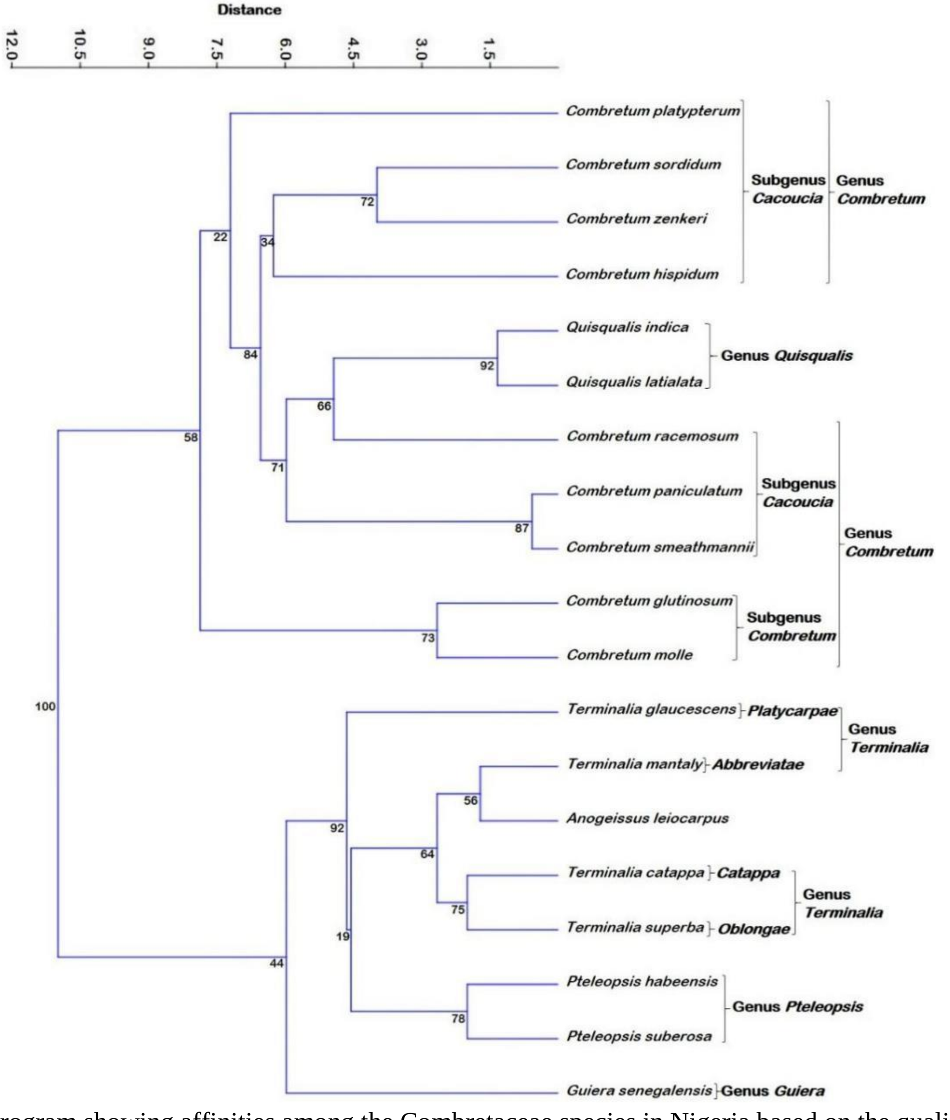


Figure 3: Dendrogram showing affinities among the Combretaceae species in Nigeria based on the qualitative and quantitative pollen morphological data
(Note: Numbers under branches indicate bootstrap percentages (%) derived from 1000 replicates).
Current sub-tribal (APG III, 2009) and sectional (Alwan, 1983) classifications are shown on the right side

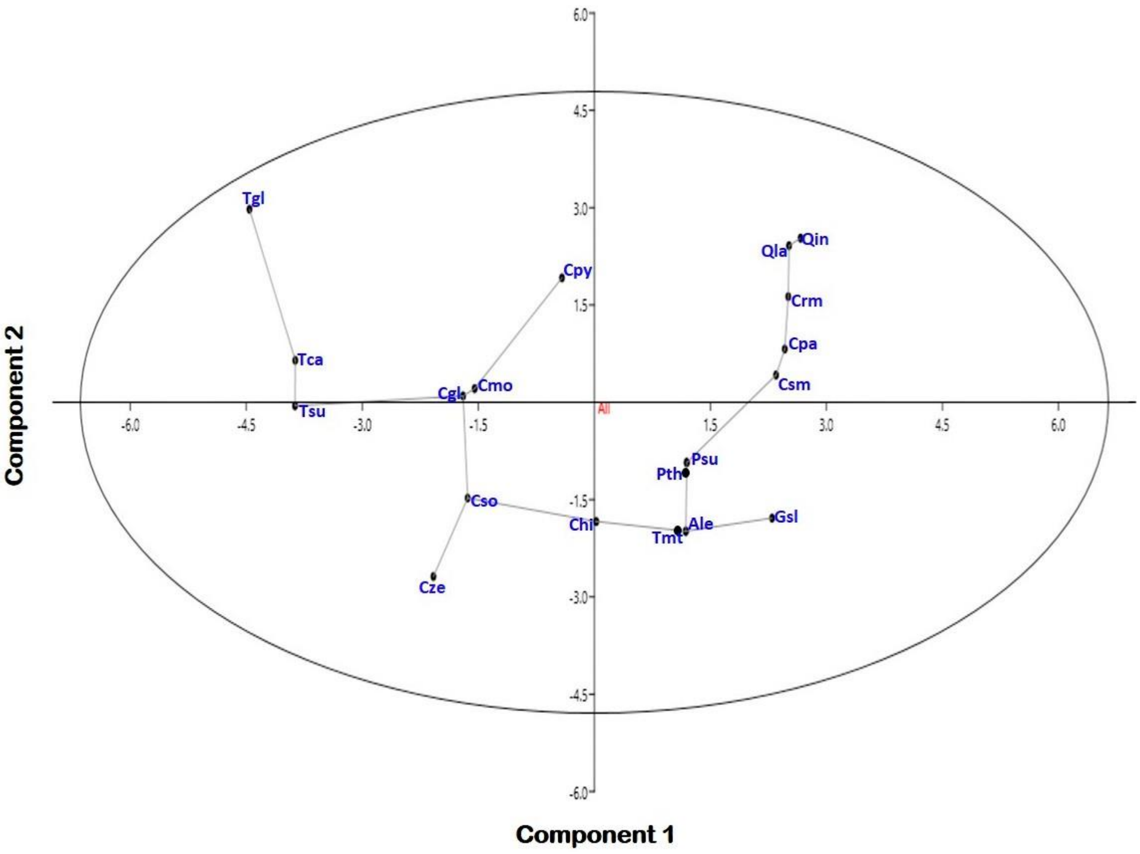


Figure 4: Component plot (scattered diagram) for the nineteen (19) Combretaceae species based on the pollen morphological characters

**Legend:**

Ale– *Anogeissus leiocarpus*, Cgl– *Combretum glutinosum*, Cmo– *Combretum molle*, Cpa– *Combretum paniculatum*, Cpy– *Combretum platypterum*, Crm– *Combretum racemosum*, Cso– *Combretum sordidum*, C*sm*– *Combretum smeathmannii*, Chi– *Combretum hispidum*, Cze– *Combretum zenkeri*., Gsl– *Guiera senegalensis*; Pth– *Pteleopsis habeensis*, Psu– *Pteleopsis suberosa*, Qin– *Quisqualis indica*, Qla– *Quisqualis latialata*, Tca– *Terminalia catappa*, Tgl– *Terminalia glaucescens*, Tmt– *Terminalia mantaly*, Tsu– *Terminalia superba*

## DISCUSSION

Palynologically, pollen grains are most conservative plant structures and their parent genus or even species can be recognised on the basis of morphology as each plant is unique for its pollen. Palynological features are used in taxonomy as additional diagnostic characters, and these could be used in delineating angiospermic species. Pollen morphology is of great application in taxa identification, delimitation and interpretation of relationships amongst different taxonomic levels. The findings from this study revealed that Nigerian Combretaceae pollen grains are highly heterogenous and exhibit a wide inter-specific variation with respect to the overall sizes and shape classes (Table 2, Figure 2 A-Al). Except for some characters like amb, pollen size, colpi length as well as shape of aperture, ora width and ratios of P/E, which are significant in the taxonomy of the genera in the family Combretaceae, Krachai and Pornpongrungrueng (2015) used some pollen morphological features to define Thailand species in Combretaceae. Numerous studies have shown the value of pollen morphology in the taxonomical classification of angiosperms, and to propose relationship between taxa within families and to solve issues at the familial, sub-familial and generic levels (El-Ghazali *et al*., 1998; Krachai and Pornpongrungrueng, 2015; El-Ghazali, 2016; Ibrahim and Ayodele, 2017).

The pollen grains of the members of Combretaceae as recorded in this study are shed in monads and are radially symmetrical, isopolar, small to medium-sized, heterocolpate with 6-8 apertures and colpus 17-36 μm in length. Pollen grains with more colpi were found. Tricolporate grains were not observed in this study, which is in line with findings on the general characteristics of Combretaceae pollen (El-Ghazali *et al*.,1998). This study also showed that the pollen grains of the Combretaceae species are eurypalynous, far from being uniform and do not exemplify natural groups. Combretaceae has a more advanced pollen type with colpi, isopolar and three or four aperturate than monocolpate and non-aperturate pollen grains (primitive type). The presence of a higher number of colpi in any group of plants has been attributed to recent evolutionary advancement in such a group of plants (Arogundade *et al*., 2019). The line of advancement of pollen types in this study is from hexacolpate versus octacolpate.

Pollen size and shape varied among the species studied. The size of pollen grains varied from small to medium. The species of the sub-tribe Terminaliinae have only small sized-grains whereas the sub-tribe Combretinae has small to medium sized-grains. The largest pollen size (42.68 × 38.17 μm) was recorded in *Combretum platypterum* and the smallest one (14.75 × 15.05 μm) in *Combretum sordidum.* However, the size of pollen grains was slightly different in *Anogeissus*, *Combretum*, *Pteleopsis*, *Terminalia* and *Quisqualis* species, indicating that size variation may be used at generic level. The pollen shape in equatorial view varied among species. Similarly, the minimum and maximum exine thickness was recorded as 0.84 μm in *C. zenkeri* and 2.75 μm in *Q. indica,* whereas the polar axis over axis of the equatorial ranged from 0.87 in *Q. indica* to 1.22 in *P. habeensis*.

The phylogenetic relationships among the selected members of Combretaceae were presented through cluster analysis, while more taxonomic information on classification of the Combretaceae taxa was revealed using the PCA (Figure 4). All taxa were delineated into two clusters (Figure 3). The first cluster comprises eleven taxa, *viz: Combretum platypterum*, *C. sordidum*, *C. zenkeri*, *C. hispidum*, *Quisqualis indica*, *Q. latialata*, *C. smeathmannii*, *C. paniculatum*, *C. racemosum*, *C. glutinosum* and *C. molle*. The second cluster contains eight taxa, namely *Terminalia glaucescens*, *T. mantaly*, *T. catappa*, *T. superba*, *Anogeissus leiocarpus*, *Pteleopsis habeensis* and *P. suberosa*. *Guiera senegalensis* was found to be the most distant from all the other taxa in the group (Figure 4).

In the first cluster, *Combretum zenkeri* was grouped with *C. sordidum* and *C. hispidum*, *C. racemosum* with *Quisqualis indica* and *Q. latialata; C. smeathmannii* with *C. paniculatum*, indicating that the members of each of these three groups were closer to each other than they were to the members of the sub-genus *Combretum* (*C. glutinosum* and *C. molle*). The closeness between *Combretum zenkeri* and *C. sordidum* was supported by their exine pattern (striate) and pollen shape class (prolate-spheroidal). The close relationship between *Quisqualis indica* and *Q. latialata* in the sub-genus *Cacoucia* group was supported by their pollen shape class (oblate-spheroidal), type (tricolpate), size (medium), amb (6-angular) and ora width (5 μm). These similarities were inconsistent with the findings of Akinsulire *et al*. (2018), who reported a remote link between *Q. indica* and members of *Combretum* while studying macro-morphology of selected Combretaceae species. The sub-cluster of *Combretum glutinosum* and *C. molle* was cornfirmed by their colpi length (ca. 22 µm), ora width (2.5 µm), amb (6-circular) and medium pollen size. Thus, there is the need for further study particularly on the *Combretum* and *Quisqualis*.

In the second major cluster, three distinct sub-clusters were recorded. The first one consists of *Pteleopsis habeensis* and *P. suberosa*. The common characteristics shared by these taxa comprise sub-prolate pollen shape, small pollen size, amb (6-lobed) and exine sculpturing (micro-rugulate) with exine thickness (1.5-1.7 µm). In the second sub-cluster, *Terminalia catappa* and *T. superba* were observed to be closer to each other than they were to *T. glaucescens*, *T. mantaly* and *Anogeissus leiocarpus*. The close relationship between *T. catappa* and *T. superba* was evidenced by their exine pattern perforate micro-rugulate and colpi length (19 µm). *Anogeissus leiocarpus* and *T. mantaly* form the third sub-cluster and their close association was evidenced by exine thickness (ca. 1.26 µm), amb (6-lobed) and prolate spheroidal pollen shape. Within the second major cluster, *Terminalia glaucescens, T. mantaly*, *T. catappa*, *T. superba*, *Anogeissus leiocarpus*, *Pteleopsis habeensis* and *P. suberosa* were found to share common traits with the highest bootstrap support (92%). This finding is in line with Maurin *et al*. (2010), who suggested that members of the genera *Pteleopsis* and *Anogeissus* should be placed in the same sub-tribe (Terminaliinae) based on the molecular studies. They also sugested that *Pteleopsis* and *Anogeissus* should be placed within the genus *Terminalia* and that all genera of the sub-tribe Terminaliinae, should be encompassed in an expanded circumscription of *Terminalia*, with the exception of *Conocarpus*. Results of this study confirmed anatomical and macro-morphological data (Mudasiru, 2023). *Guiera senegalensis* has been reported to be morphologically most distant from all other species examined in the cladogram. The first two component of the Principal Component Analysis (PCA) accounted for 89% of the variation among the species studied. Based on component 2, pollen polar diameter, pollen equatorial diameter, colpi length, exine thickness, ora width, pollen size, amb and aperture were responsible for the variation observed among the Combretaceae species. Based on component 2, only exine pattern and pollen shape class were accountable for the variation observed among the species (Figure 4). The scatter plot yielded four groups and the position of each species in the PCA showed the evolutionary trend within Combretaceae. The results of the single linkage cluster analysis and PCA as earlier reported by Ayodele (2005) and Soladoye *et al*. (2010) indicate that the species in the same group are to some extent, related morphologically, indicatng that they have similar ancestral origin with a common gene pool. This study has shown how numerical taxonomy justifies the classification of the genus using morphological characteristics as suggested by different authors (Ayodele, 2005; Soladoye *et al.*, 2011; Sonibare *et al.*, 2014).

## CONCLUSION

Pollen morphology has proven to be an important tool for taxonomic identification of species within the family Combretaceae. This study showed that Nigerian Combretaceae pollen grains are largely heterogenous. The species can be identified based on morphometric and morphological pollen characters, particularly overall size of polar axes (P) and equatorial diameters (E), shape classes, the ratios of P/E, exine thickness and apertural features. The size of pollen grains was slightly different in the members of all the six genera (*Anogeissus*, *Pteleopsis, Terminalia*, *Combretum*, *Guiera* and *Quisqualis*), indicating that size variation may be used at the generic level in this family. Also, Combretaceae taxa were observed to be eurypalynous, with four shape classes, six exine sculpturing patterns and eight aperture facets. The cluster analyses and PCA plots showed nested grouping of the *Quisqualis* species within *Combretum,* with high bootstrap support (84%). The cluster analysis revealed congruence in the number of main clusters resolved. Palynomorphological data have confirmed the existence of diversity and relationships among the nineteen species of Combretaceae, in order to improve the identification of the taxa and serve as additional source of taxonomic information for the family. The data obtained in this study not only supported the information already known about the family Combretaceae but can also be used in combination with other characteristics to distinguish between the various species that make up the family.

**ACKNOWLEDGEMENTS**

The authors are grateful to The World Academy of Sciences (TWAS), Trieste, Italy and CSIR, New Delhi, Government of India for joint award of Ph.D. Fellowship to Mudasiru, O.M. at CSIR-NBRI, Lucknow, India under which this study was carried out. We thank the authorities of the University of Ibadan, Ibadan, Nigeria for permitting Mudasiru, O.M. to take up the fellowship. Special thanks to the Prof. Saroj. K. Barik, ex-Director of the CSIR-National Botanical Research Institute in Lucknow, Uttar Pradesh, India, for providing facilities used for the study.

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