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PALYNOLOGICAL STUDIES OF THE GENUS *COMBRETUM* LOEFL. FROM AKWA-IBOM STATE, NIGERIA

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ABSTRACT

Variations in pollen morphology were investigated in nine (9) common species of *Combretum* found in Uyo metropolis, Akwa-Ibom State, Nigeria. The species included *Combretum bracteatum*, *C. calobotrys*, *C. dolichopetalum*, *C. hispidum*, *C. mannii*, *C. mooreanum*, *C. platypterum*, *C. racemosum* and *C. zenkeri*. The pollen samples were acetolysed and examined under light microscope. The investigation showed that the pollen grains were all heterocolpate and circular in shape. Sculpturing was psilate or scabrate and pollen shape class was either sub-prolate, oblate-spheroidal or prolate spheroidal. Among the 9 species of *Combretum*, maximum polar dimension (34.41-44.40 μm) was found in *C. platypterum* whereas minimum polar dimension (21.09-38.85 μm) was noted in *C. racemosum* and *C. zenkeri*. The equatorial dimensions were highest (29.97-34.12 μm) in *C. mooreanum* while the lowest equatorial dimension (17.76-31.08 μm) was observed in *C. zenkeri*. Pore diameters were highest (8.88-15.54) in *C. calobotrys* and *C. platypterum* and least (5.55-13.32) in *C. bracteatum*, *C. racemosum* and *C. zenkeri*. There was no significant difference in exine thickness among the species.

Keywords: *Combretum*, Uyo metropolis, Pollen morphology

INTRODUCTION

Combretaceae consists of trees, shrubs and lianas, with up to 23 genera currently recognised (Stace, 2007). In West Africa, the family Combretaceae is represented by 9 genera with 72 species and the genus *Combretum* Loefl. is the largest genus with 48 species and 8 imperfectly known species (Gill, 1988). In Nigeria, the genus *Combretum* Loefl. is represented by 25 species which are mainly straggling shrubs or lianas (Keay, 1989). *Combretum* Loefl. occurs in tropical and subtropical regions (America, Africa, Madagascar, India, Asia, Malaysia, Australia), but is absent from the Pacific Islands (Stace, 2007). Three subgenera of *Combretum* are recognized by Exell and Stace; they are *Combretum*, *Cacoucia* (Aubl.) Exell & Stace and the monotypic Asian subgenus, *Apetalantum* Exell & Stace. On a worldwide scale, the subgenera *Combretum* and *Cacoucia* are separable with certainty only on the character of the presence of either scales (Subgen. *Combretum*) or microscopic stalked glandular hairs (Subgen. *Cacoucia*) (Wickens, 1973). In South Africa only three subspecies are currently recognized namely *C. subsp. gazense*, *C. subsp. suluense* and *C. subsp. taborense* (Carr, 1988). Erdtman (1966) stated that pollen grains of Combretaceae are tricolporate with pseudocolpi, prolate to prolate-spheroidal in shape, with the sexine evidently thinner than the nexine. Patel *et al.* (1984) also recognized the Combretaceae pollen to be isopolar, radially symmetrical, tricolpate or heterocolpate with three colpi alternating with three subsidiary colpi and subsidiary colpi united or separated at the polar area, spheroidal to subprolate shape in equatorial view.

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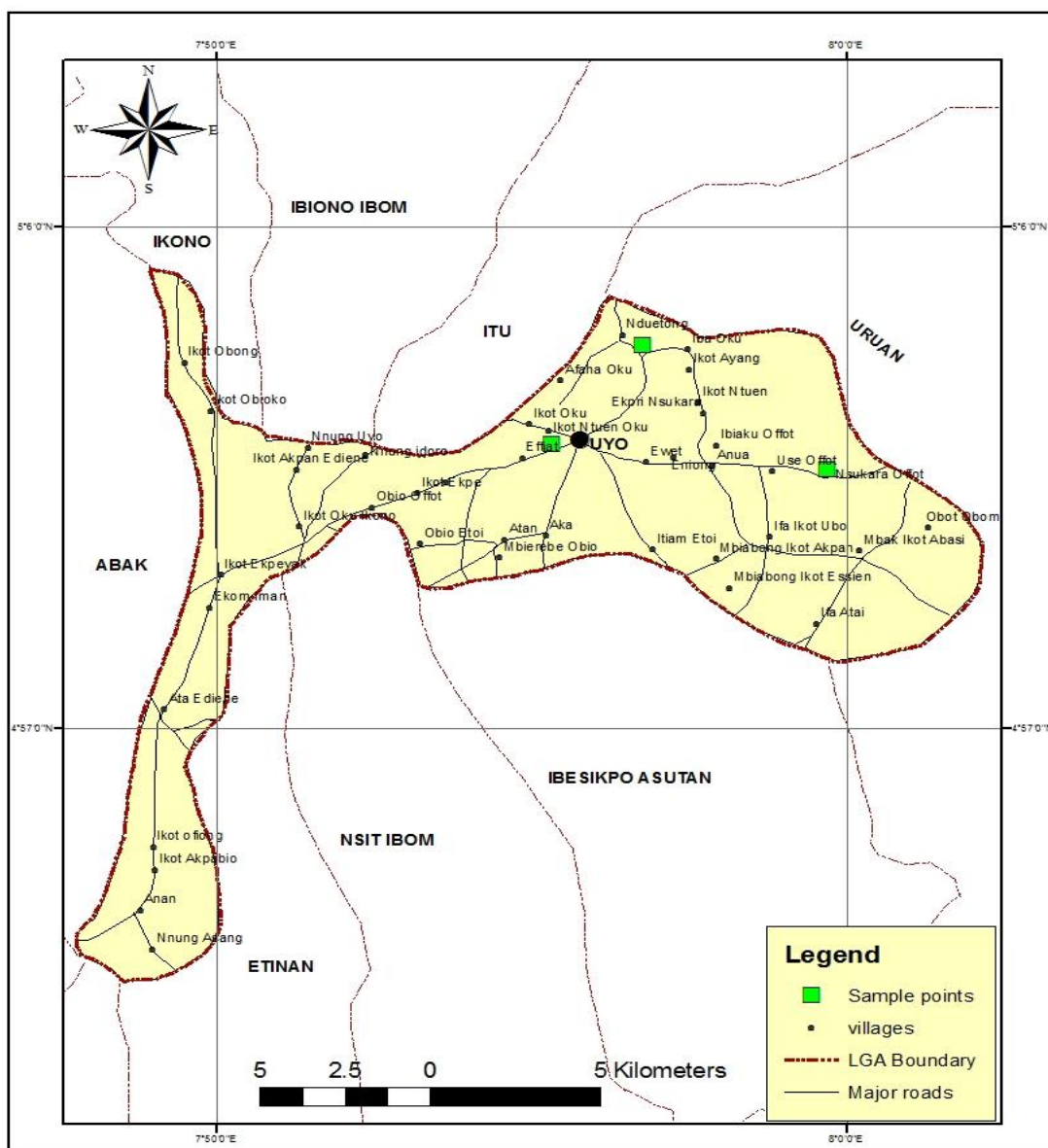


Figure 1. Map showing Uyo Local Government Area

MATERIALS AND METHODS

Flower specimens were collected from live plants of nine *Combretum* species in Uyo Metropolis, Akwa-Ibom State, Nigeria. The plants collected were taxonomically identified by Prof. (Mrs.) M. E. Bassey and Voucher specimens were deposited in the University of Uyo Herbarium (UUH).

Pollen samples were acetolysed following the method of Erdtman (1969) as modified by Nyananyo, (1992). Anthers were crushed gently using small vials with glass rods in a small quantity of dilute detergent as a wetting agent. The mixture was passed through a 180 μm sieve into centrifuge tubes. A few drops of lactic acid was added to prevent further expansion of the grains. The mixture was then centrifuged at "mark 4" of a bench centrifuge for five minutes and the supernatant decanted. Glacial acetic acid was added to the pollen pellet, mixed thoroughly and centrifuged and the supernatant was decanted. Acetolysis mixture was freshly prepared from acetic anhydride and concentrated sulphuric acid in a ratio of 9:1. Ten (10) ml of acetolysis mixture was then added to the pollen pellet mixture and maintained at boiling point in a water bath at 100⁰C for 3-5 minutes. The tubes were then centrifuged at "mark 4" for five minutes and the supernatant decanted. Glacial acetic acid was added to the pollen pellet which was then disaggregated, mixed thoroughly and centrifuged to stop the action of the Acetolysis mixture; the supernatant was decanted. The pollen pellet was then suspended in distilled water and centrifuged for five minutes. The recovered pollen pellet was stored in a plastic vial in glycerine and ethanol solution. Fresh mount of the pollen grains in glycerol was examined using a light microscope at x100 objective. All the measurements were taken in μm using a calibrated ocular micrometer. These include the dimensions of the polar axis, equatorial axis, the exine thickness and the diameters of the pores. Photographs were taken using a Motic Micrograph Unit.

RESULTS

Table 1 summarises the measurements of pollen grains of the species examined. Light micrographs of *Combretum* species are presented in Figures 2, 3 and 4.

Polar Dimension: The polar dimension was least (21.09 – 38.85 μm) in *Combretum racemosum* and *C. zenkeri* while the highest (34.41 – 44.40 μm) was observed in *C. platypterum*.

Equatorial Dimension: The equatorial dimension was least (17.76 – 31.08 μm) in *C. zenkeri* and highest (29.97 – 34.41 μm) in *C. mooreanum*.

Exine Thickness: Exine thickness was thin (3.33 – 4.44 μm) and similar in *C. hispidum*, *C. mannii*, *C. mooreanum*, *C. racemosum* and *C. zenkeri*. The thickest exine was observed in *C. calobotrys* (3.33 – 5.55 μm).

Pore Diameter: Pore diameter varied between 5.55 – 13.32 μm in *C. bracteatum*, *C. racemosum* and *C. zenkeri* up to 16.65 μm in *C. mooreanum*.

Sculpturing Type: The sculpturing was psilate in 4 species which include *C. bracteatum*, *C. calobotrys*, *C. hispidum* and *C. zenkeri*; it was scabrate in 5 other species which include *C. dolichopetalum*, *C. mannii*, *C. mooreanum*, *C. platypterum* and *C. racemosum*.

APERTURE TYPE: The apertures of the pollen grains of all the samples were heterocolpate.

POLLEN SHAPE CLASS: The pollen shape class was sub-prolate in 6 species which include *C. bracteatum*, *C. dolichopetalum*, *C. hispidum*, *C. mooreanum* and *C. platypterum*; oblate spheroidal in 2 species (*C. calobotrys* and *C. mannii*) and prolate spheroidal in *C. zenkeri*.

Table I. Pollen morphological features of *Combretum* species

Species	Polar dimension (μm)	Equatorial dimension (μm)	Exine thickness (μm)	Pore diameter (μm)	Sculpturing type	Aperture type	Pollen class (P/E)
<i>Combretum bracteatum</i>	32.19 – 43.29	25.53 – 33.30	3.33 – 4.99	5.55 – 13.32	Psilate	Hetero-colpate	Sub-prolate (1.27)
<i>Combretum calobotrys</i>	31.08 – 33.30	27.75 – 33.30	3.33 – 5.55	8.88 – 15.54	Psilate	Hetero-colpate	Oblate Spheroidal (1.06)
<i>Combretum dolichopetalum</i>	29.97 – 37.74	18.87 – 31.08	3.10 – 4.44	7.54 – 11.10	Scabrate	Hetero-colpate	Sub-prolate (1.30)
<i>Combretum hispidum</i>	29.97 – 35.52	24.43 – 29.97	3.33 – 4.44	7.77 – 13.32	Psilate	Hetero-colpate	Sub-prolate (1.19)
<i>Combretum mannii</i>	27.75 – 34.41	27.75 – 32.19	3.33 – 4.44	7.77 – 14.43	Scabrate	Hetero-colpate	Oblate spheroidal (1.01)
<i>Combretum mooreanum</i>	30.30 – 42.18	29.97 – 34.41	3.33 – 4.44	6.66 – 16.65	Scabrate	Hetero-colpate	Sub-prolate (1.23)
<i>Combretum platypterum</i>	34.41 – 44.40	25.53 – 34.41	2.77 – 3.88	8.88 – 15.54	Scabrate	Hetero-colpate	Sub-prolate (1.30)
<i>Combretum racemosum</i>	21.09 – 38.85	18.87 – 22.20	3.33 – 4.44	5.55 – 13.32	Scabrate	Hetero-colpate	Sub-prolate (1.32)
<i>Combretum zenkeri</i>	21.09 – 38.85	17.76 – 31.08	3.33 – 4.44	5.55 – 13.32	Psilate	Hetero-colpate	Prolate spheroidal (1.11)

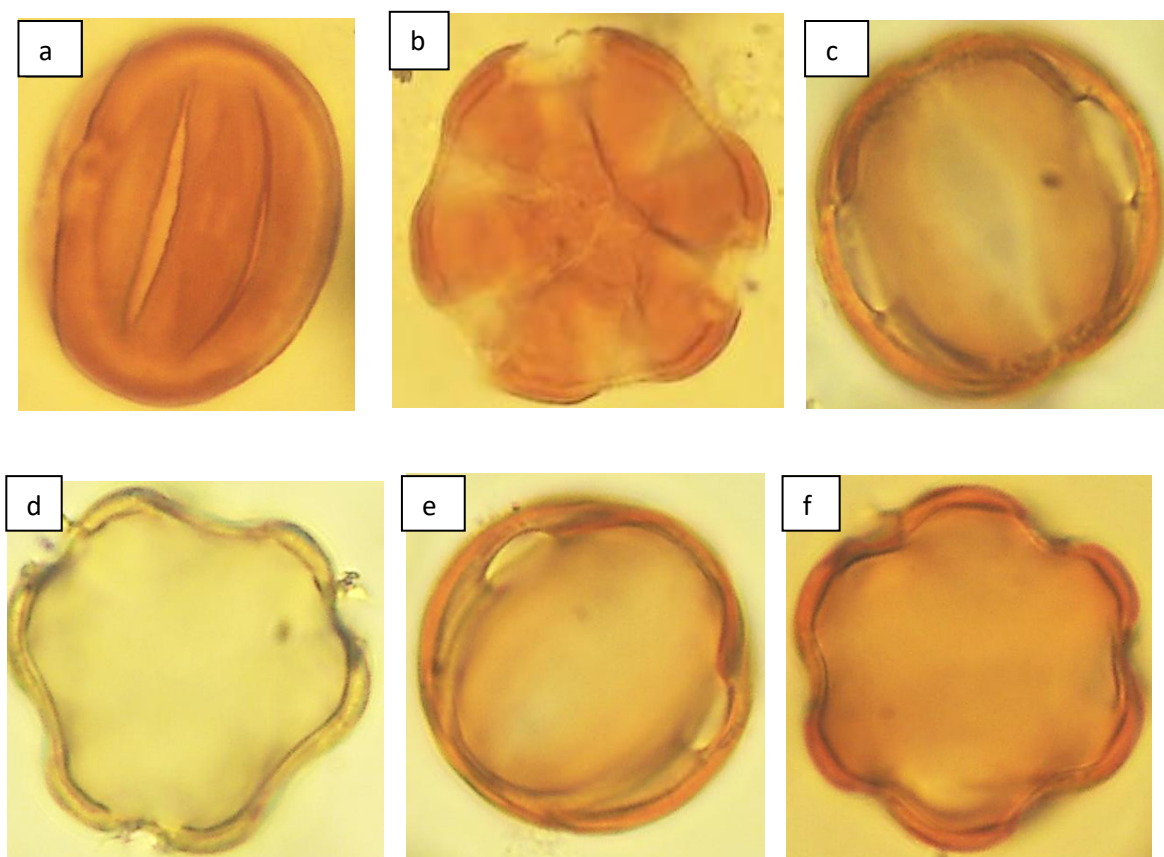


Figure 2: Equatorial and Polar views of pollen grains of *Combretum* species (a) Equatorial view of *Combretum bracteatum* (Laws) Engl. & Diels; (b) Polar view of *C. bracteatum* (Laws) Engl. & Diels; (c) Equatorial view of *C. calobotrys* Engl. and Diels; (d) Polar view of *C. calobotrys* Engl. and Diels; (e) Equatorial view of *C. dolichopetalum* Engl. & Diels; (f) Polar view of *C. dolichopetalum* Engl. & Diels.

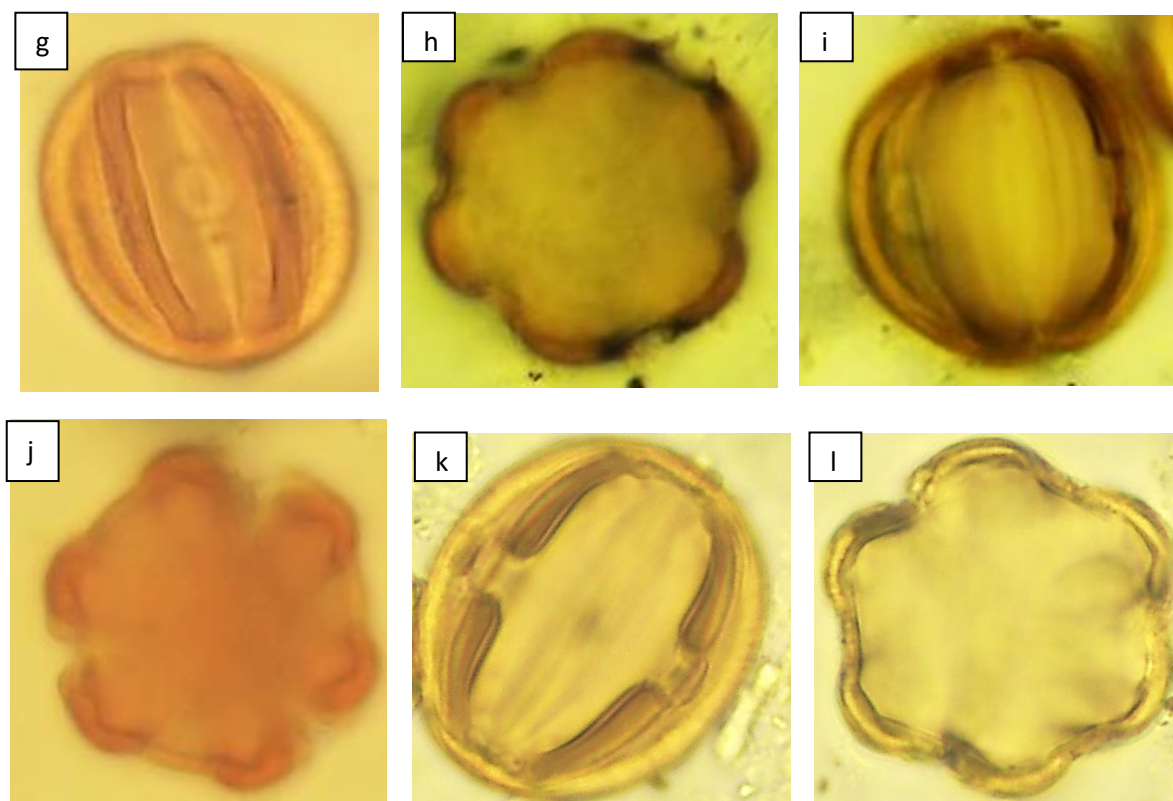


Figure 3: Equatorial and Polar views of pollen grains of *Combretum* species (g) Equatorial view of *C. hispidum* Laws.; (h) Polar view of *C. hispidum* Laws.; (i) Equatorial view of *C. mannii* Engl. & Diels; (j) Polar view of *Combretum mannii* Engl. & Diels (k) Equatorial view of *C. mooreanum* Exell (l) Polar view of *C. mooreanum* Exell.

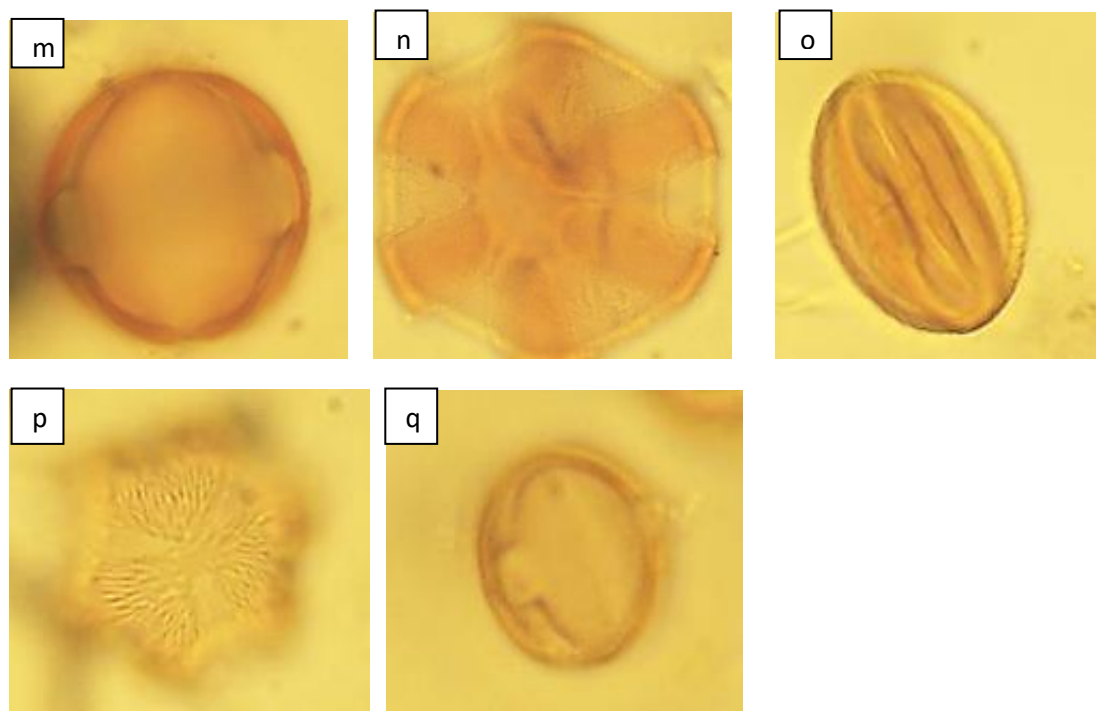


Figure 4: Equatorial and Polar views of pollen grains of *Combretum* species (m) Equatorial view of *C. platypterum* (Welw) Hutch. & Dalz. (n) Polar view of *C. platypterum* (Welw) Hutch. & Dalz. (o) Equatorial view of *C. racemosum* P. Beauv. (p) Polar view of *C. racemosum* P. Beauv. (q) Equatorial view of *C. zenkeri* Engl. & Diels

Light Micrographs (LMs) of the 'NPC' (Number, Position and Character of the apertures) of the

- i. Equatorial and Polar views of pollen grains of *Combretum zenkeri* Engl and Diels, Figure 4, o and p, shows that the pollen grains are tricoplate and
- ii. Polar view of *Combretum racemosum* Palisot de Beauvois, Figure 4, q shows that the pollen grains are tricoplate.

DISCUSSION

The study showed that the pollen grains of *Combretum* have a uniform aperture type and that all the species studied were heterocolpate, psilate, scabrate, sub-prolate, oblate- spheroidal and prolate-spheroidal. This agrees with the work of Puntiwat and Pimwadee (2015). Based on sculpturing type, aperture type, pollen shape class, range of polar dimension, size of equatorial dimension, exine thickness and pore diameter, *C. mooreanum* and *C. platypterum* apparently are more closely related than to the other species. One of the prominent and interesting features using Light Microscope is the presence of striate sculpturing type in the exine of *C. racemosum* which is of diagnostic value in the species (Figure 4p).

In conclusion, the data obtained from pollen morphological studies of *Combretum* species in Uyo Metropolis, Akwa-Ibom State, Nigeria has provided useful taxonomic information in Nigeria.

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