Original Research Article

Morphological, Anatomical, and Palynological Studied of *Lasianthera africana* P. Beauv (Stemonuraceae) from some parts of Southern Nigeria

ABSTRACT

This study examined *Lasianthera africana* P. Beauv based on, anatomical and palynological parameters. The anatomical studies were carried out with freehand sectioning. The species is hypostomatic, the epidermal cells are are irregular, with undulating anticlinal walls. The stomata types are anomocytic, and anisocytic. The midrib has bicollateral vascular bundles and the petiolar vascular bundle is arced with rib traces on both sides of the wings. Calcium oxalate and tannin occurred in the lamina, midrib, petiole and stem. The pollen grains are smooth, intectate, with thick exine, with tricolporate aperture, and with an equatorial diameter of 21.5-25.8 µm. These features are diagnostic and confirm the placement of this species in Stemonuraceae.

Keywords: Calcium oxalate, *Lasianthera africana,* taxonomy, pollen grain.

1. INTRODUCTION

The family Stemonuraceae Kårehed comprised of evergreen trees or shrubs [1-3] in 12 genera (*Cantleya, Codiocarpus, Discophora, Gastrolepis, Gomphandra, Grisollea, Hartleya, Irvingbaileya, Lasianthera, Medusanthera, Stemonurus*, and *Whitmorea*), with about 90 species, distributed mainly in the tropics [1]. *Lasianthera* P. Beauv. is a monotypic species occurring in West Africa [1, 4-6]. *Lasianthera africana* P. Beauv. has two variaties namely *L. africana* P. Beauv. var. *africana* and *L. africana* var. *microphylla* Pellegr. ex Villiers [7-9], and a native of Nigeria to Western Central Tropical Africa [4]. It formally in the family Icacinaceae [5] but now transferred to Stemonuraceae due to its close affinity to the members of this family [7 -9]. *Lasianthera africana* natively called "Editan" in Akwa Ibom state is a perennial shrub of about 4m in height [10]. It occurs in tropical rain-forest such as guinea-Congolan African, certain species occur in the coastal. *Lasianthera africana* has one species and two varieties in tropical Africa and among the Ibibios, four local varieties distinguished by their taste, leaf colour and ecological distribution are known [11].

Anatomical and palynological characters play relevant role in plant identification. For instance, anatomical characteristics are important for the formulation of phylogenetic and phonetic groups [1,

6, 12-14]. The use of information of anatomy such as pollen, petiole, stem, and leaf in taxonomic delimitation however restricted, has been documented [15 -18]. Estimable taxonomic evidence has been acquired from the pollen, leaf, stem, epidermis, and stomata. Some of these anatomical characteristics are so diagnostic that they are now usually utilized in routine plant identification, rather than being confined to a used in phylogeny or classification or the identification of fragment of a plant [19]. The relevance of palynological information has been stressed by several researchers in the family Cruciferae. The pollen exine ornamental plays important role in the delimitation of some closely related taxa in Cruciferae [17]. This study examines anatomy and pollen morghology of the different variants of *L. africana* found in Akwa-Ibom State, Nigeria.

MATERIALS AND METHODS

2.1 Source of plant materials: Collections of different plants were made from wild and cultivated locations in Akwa Ibom State, Nigeria (Table 1 and Figure 1). The samples were authenticated at the University of Port Harcourt herbarium and anatomical studies and palynological studies.

Table 1: Location of areas of Sample Collection

S/N	Collection No.	Location	Date	Geographical coordinates
1	Okon 001	Aya, Ikot Ekpene, Akwa Ibom State	02/06/2017	Lat. 5.18° North Long. 7.71° East
2	Okon 002	Oku Iboku, Itu L. G. A., Akwa Ibom State	03/06/2017	Lat.5.24^0 North Long. 7.44^0 East
3	Okon 003	Ibiono	04/06/2017	Lat. 5°12’25.34’’ North Long. 7.53’35.12’’ East
4	Okon 004	Ikot Abasi	06/06/2017	Lat.4°36’20.92’’ North Long. 7°37’27.84’’ East

2.2 Stem, petiole, and midrib anatomical studies: Collected samples of the stem, petiole, and midrib of about 10cm in length and 6mm in diameter were cut and fixed in formalin acetic acid and alcohol (FAA) of 1ml: 1ml: 3ml respectively for 24hours. After this, samples were passed through a series of different alcoholic concentrations of 50% and 70% [20] for 3hours and 24hours respectively. Samples were infiltrated, embedded in paraffin wax and a section was made. The transverse sections obtained were stained with Alcian blue, countered stained with Safranin, and mounted on glycerine. All slides were examined under a microscope.

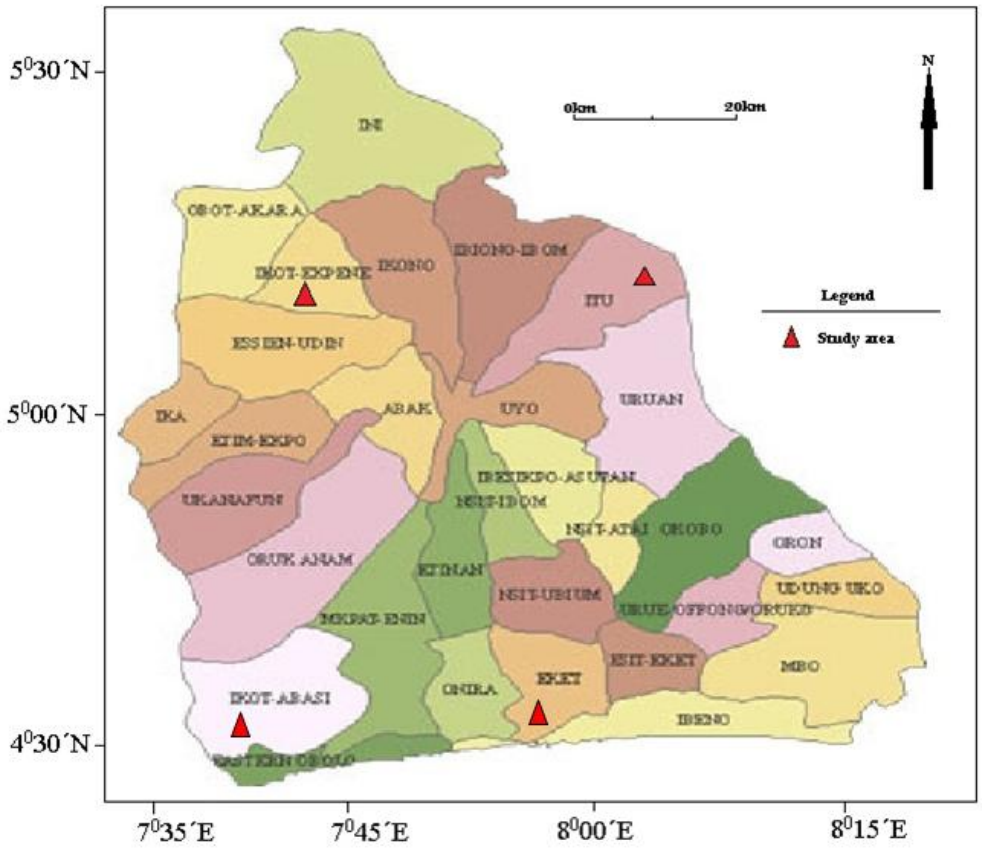


Figure 1: Map of Akwa Ibom State showing the Local Government Areas sampled.

2.3 Epidermal peels: The fresh leaves of both abaxial (lower) and adaxial (upper) part of *L. africana* were obtained by holding portions of the leaf between the left thumb and index finger on both hands and making angular tear across the lamina of the exposing epidermal peels of both surfaces. The translucent epidermal peels of about 4mm× 5mm were cut off and fixed in a separate bottle containing 95% ethanol for 24hours for a clear epidermis, and these samples were rinsed with distilled water and stained with safranin. The stained samples were mounted on a slide containing a drop of glycerine. Photomicrographs were taken from Leiz-Habolux-12-microscope filled with a Wild-MPS camera.

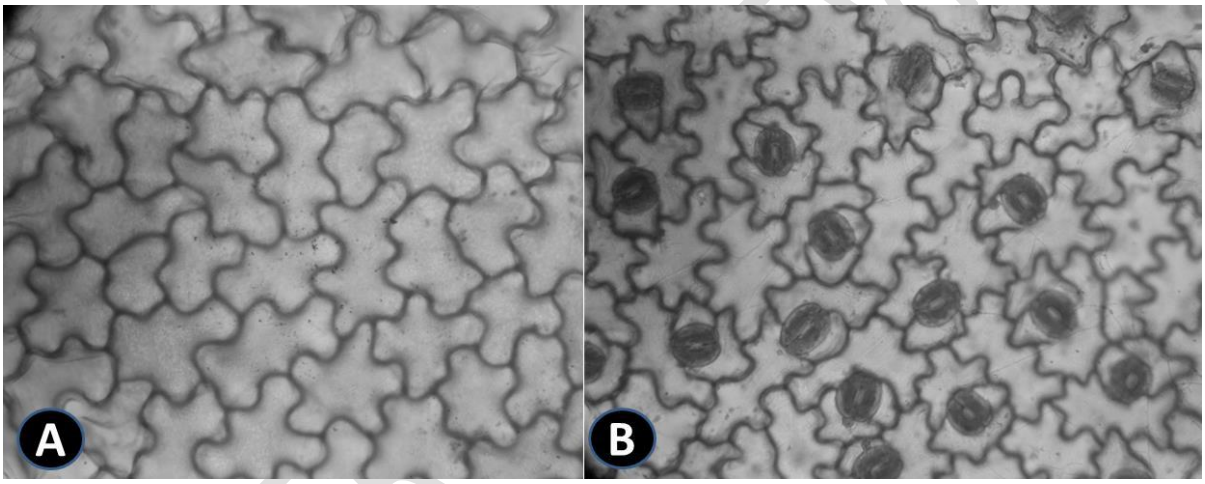
2.4 Palynological studies: Fresh mature flower buds were fixed in ethyl alcohol (96% ethanol) to separate the other flower parts which could be separated inside distilled water. Flower buds were transferred into test tubes; the pollens were dried in a thermostat and wetted with an acetolysis mixture (acetic anhydride and concentrated sulphuric acid in a 9:1 ratio) repeatedly. After this, the test tubes were placed together with granules and acetolysis mixture in a 70^{o}C water bath (for 5seconds). The granules (pollen) were centrifuged and cleaned with distilled water several times.

The pollens were placed on a slide that contains a drop of glycerine and observed using a microscope.

2. RESULTS

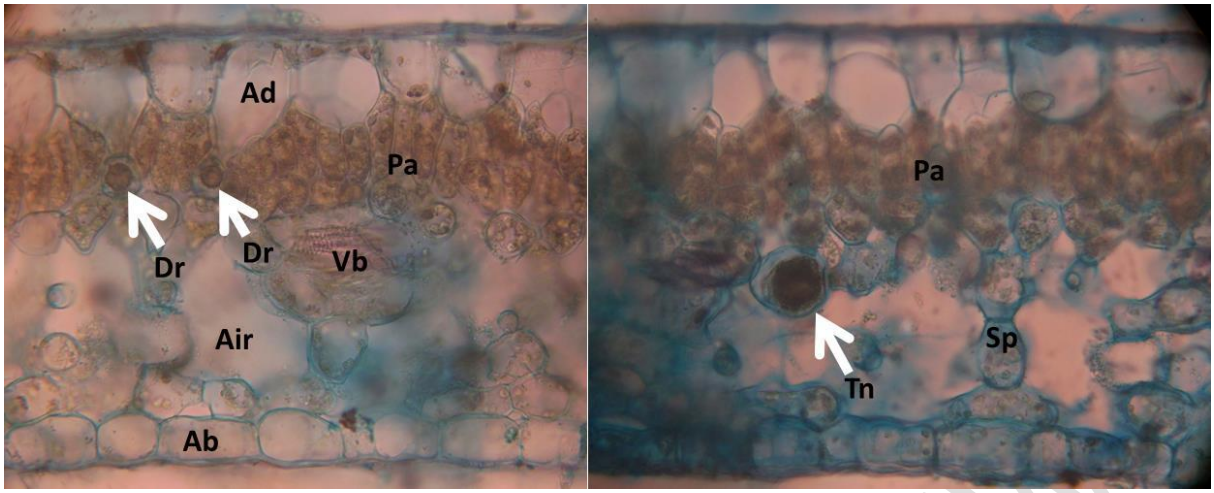
Based of the leaf morphology, two variants of *L. africana* were collected (Table 1). Results of the anatomical studies of the stem, petiole, and leaf including pollen morphology of *L. africana* are shown on (Figs. 2 – 7).

3.1 Epidermal features: The leaf of this plant species is hypostomatic (stomata are only on the abaxial and surfaces). The abaxial and adaxial cells are irregular with undulating anticlinal walls (Fig. 2). The stomata on the abaxial leaf surfaces are anisocytic, and anomocytic. The predominant stomata type is the anisocytic type while the other two are few and the anomocytic stomata occurred mainly on the leaf veins. The stomatal guard cells are oval.



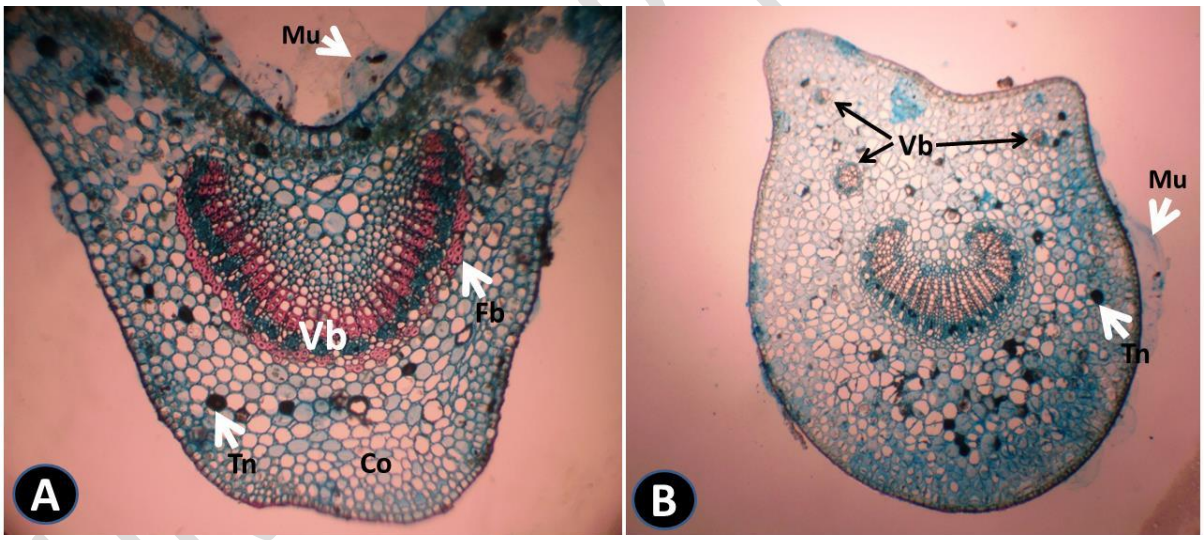
Figures 2: Epidermal peels of *L. africana* (A) abaxial epidermis and (B) adaxial epidermis

3.2 Lamina: The cross-section of *L. africana* leaf lamina showed a layer of oval and anticlinal elongated adaxial epidermal cells with cylindrical and periclinal elongated abaxial epidermal cells (Fig. 4). The palisade mesophyll comprised 2 – 3-layers while the spongy mesophyll has 3 – 4-layers with intercellular air spaces.



Figures 3: Transverse section of *L. africana* leaf lamina, Vb –vascular bundles, Ad – adaxial epidermis, Tn – tannin, Ab – abaxial epidermis, Sp – spongy mesophyll, Pa – palisade mesophyll, Dr – druses, Air – intercellular air spaces

3.3 Midrib: *L. africana* midrib transverse section showed a V-shaped adaxial outline and a circular or U-shaped abaxial outline (Fig. 4A). The vascular bundle is collateral, U- or V-shaped, with a continuous layer of fibre or sclerenchymatous cells of the outer region. The adaxial cortex has 5 – 13-layers of parenchymatous cell while the abaxial cortex has 7 – 12-layers.



Figures 4: Transverse section of *L. africana* (4) midrib and (5) petiole: Vb –vascular bundles, Co – cortex, Ad – adaxial epidermis, Tn – tannin, Fb – fibre, Mu – mucilage.

3.4 Petiole: *L. africana* petiolar transverse section showed a semicircular vascular bundle with rib traces on both sides of the main vascular bundle (Fig. 4B). The adaxial outline is concave while the abaxial outline is circular. The cortex is made up of mainly multiseriate parenchymatous cells, which are mainly oval but partly semicircular.

3.5 Stem: *L. africana* stem section is oval with two protrusions and uniseriate epidermis supported by about 8 – 12-layers of the parenchymatous cortex. The pith is narrow and consists of angular to isodiametric cells (Fig. 5). The vessels are solitary, with imperforate tracheary, and diffused cells.

3.6 Calcium oxalate, mucilage, and tannin distribution: The occurrence and distribution of calcium oxalate crystal and tannin varied in the different parts of the plant. Also, only druse crystals occurred in *L. africana* and were observed in the palisade mesophyll (Fig. 3). Mucilage was observed in the epidermis of the petiole and midrib (Figs. 4). Tannin occurred in the midrib, petiole, and stem. In the midrib, it was observed in the abaxial cortical cells and adaxial palisade and epidermal cells (Fig. 4A), in the cortical and phloem cells of the petiole (Fig. 4B), and pith and phloem cells in the stem (Fig. 5B).

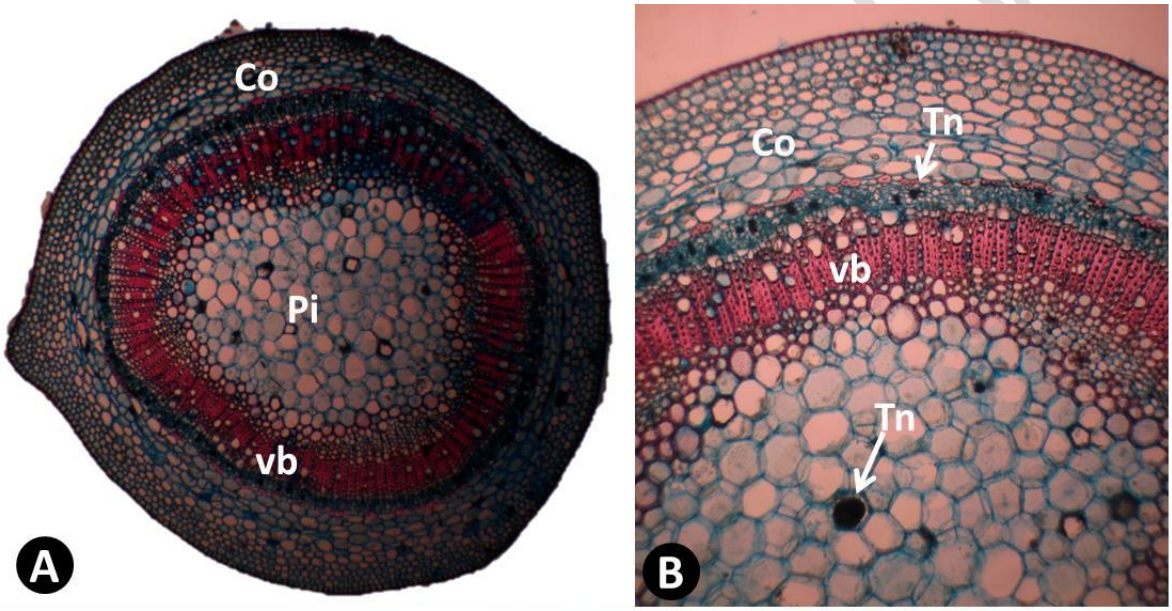


Figure 5: Stem anatomical features of *L. africana*: Pi – pith, Vb – vascular bundles, Tn – tannin, and Co – cortex.

3.7 Pollen morphology: The morphology of the pollen of *L. africana* showed a smooth, intectate thin exine. The aperture type is tricolporate. These species discharge their pollen by a non-explosive mechanism. The pollination is either by insects or wind. From the equatorial view, the species are oblate in shape, while from the polar view is circular. The surface pattern is reticulate. The walls of the pollen are sculptured. The grains are present as a monad. The equatorial diameter is 21.5 - 25.8µm (Fig. 6).

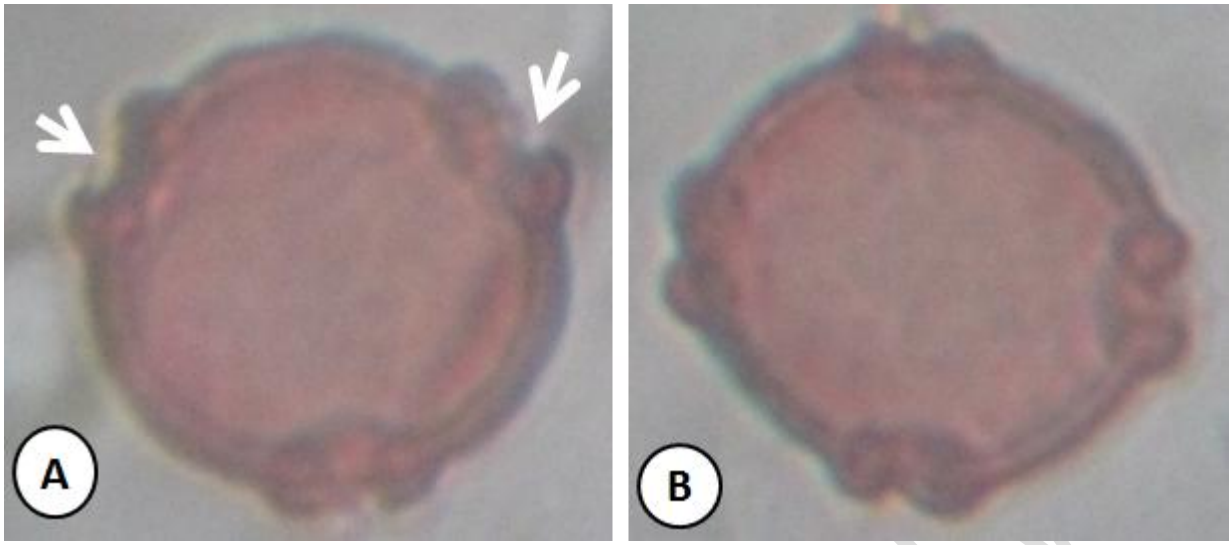


Figure 6: Pollen of *L. africana* (A) Tricoporate and (B) Pantocolporate, arrows show aperture

3. DISCUSSION

Morphological features and leaf epidermal characters are of taxonomic importance and have been commonly used for the classification of taxa in different plant families [21-23]. Watson and Dallwitz [24] reported that the leaf of members of Stemonuraceae have hydathodes, mucilaginous epidermis, anomocytic and anisocytic stomata, and hairs of assorted unicellular and multicellular forms. Also, the lamina has secretory cavities containing latex. The mesophyll with sclerenchymatous idioblasts, or without sclerenchymatous idioblasts. Also, reports on Stemonuraceae showed that members of the family (*Discophora, Gastrolepis, Lasianthera, Medusanthera* and *Stemonurus*) have rhombic crystals. Also, other species such as *Cantleya corniculata* and *Stemonurus malaccensis* contain tannin crystals which are scattered in the mesophyll and ground tissue of the midrib [25]. Anisocytic stomata has been reported in *L. africana* [26]. In *L. africana,* Bassey and Sunday [27] reported undulating anticlinal walls in the forest variants and while straight to wavy anticlinal walls in the variants that grow in the riverine areas. In our study, on *L. africana* from some parts of Akwa Ibom State we recorded anomocytic, and anisocytic stomata, with anisocytic type being more frequent. All the species studied are hypostomatic and the shape of the epidermal cells are irregular with undulating anticlinal walls. Our findings corroborate the previous reports on this species and other members of this family.

The differences in shape, size, aperture, polar unit, symmetry, and wall sculpture of pollen have been utilized by numerous authors in the delimitation of various taxa [28]. These pollen features are also of taxonomic value. Olowokudejo and Nyananyo [29] also use the seed coat morphology and other palynological characters of *Talinum* and *Calandrinia* to produce a more acceptable

classification among these taxa. Edeoga and Ikem [30] also proved that *Boerhavia coccinea* is characterized by tricolporate pollen grains while *Boerhavia erecta* and *Boerhavia diffusa* have alcopate pollen grains. This means that *B. coccinea* could be distinguished from other collections of *Boerhavia* in Nigeria based on pollen features. Nyananyo [31] and Mbagwu and Edeoga [32] have utilized pollen features to confirm true evidence of the relationship among certain groups of flowering plants in Nigeria. Pollen characteristics have been important in the identification of plant families (28-33, 35). Schori and Furness [33] reported among Stemonuraceae family, the pollen grains are typically triporate and triangular in polar view. They further noted that the pollens colud have 4–5 pores but rarely up to 9. Their size varies from 6 x 19 mm in *Stemonurus* to 21 x 32 mm in *Gomphandra*. Polar to equatorial quota more than 0.9mm. Also, exines in other genera vary from striate in *Gomphandra* and *Whitmorea* to regulate in *Gastrolepis, Gomphandra, Cantleya;* fossulate-verrucate in *Stemonurus*; microgemmate in *Irvingbaileya*, or microechinate in *Lasianthera* [33, 35]. Irregular grains are common in certain species of *Gomphandra* and *Gastrolepis*. Pollen sterility [33, 35] has been recorded for both normal and irregular grains in *Gomphandra*. The result of palynological studies showed that the morphology of the pollen of *L. africana* is tectate, with thick exine, tricolporate aperture, and the equatorial diameter is 21.5-25.8 μm. These species discharge their pollen by a non-explosive mechanism. The pollination is either by insects or wind. Results from these lines of evidence confirm the placement of this species in Icacinaceae.

In the genera, *Hartleya* and *Stemonurus* **(**Stemonuraceae) growth ring boundaries are generally absent and indistinct with the wood diffuse-porous [34, 35]. The vessels range from 4 – 50 per mm^2 and are solitary in *Cantleya* and *Lasianthera*, or solitary and in radial and/or tangential multiples of 2–4 in *Discophora* [34]. Watson and Dallwitz [24] also reported diffuse porous wood with very small to medium vessels; solitary, or radially paired, or in radial multiples, or clustered, or in tangential arcs some members of Stemonuraceae. Watson and Dallwitz [24] noted that the primary vascular tissues in members of this family forms a cylinder, without separate bundles, or comprising a ring of bundles, or comprising two or more rings of bundles, collateral, or bicollateral and internal phloem are present. Also, they medullary bundles present, and secondary thickening occur in the cambial ring. We observed narrow pith with angular to isodiametric cells ; solitary vessels, with imperforate tracheary, and diffused cells in the *L. africana* we studied.

REFERENCES

1. Kårehed, J. (2001). Multiple origin of the tropical forest tree family Icacinaceae. American Journal of Botany 88: 2259-2274.

2. Winkworth, R.C., Lundberg, J., Donoghue, M. J. 2008. Toward a resolution of Campanulid phylogeny, with special reference to the placement of Dipsacales. Taxon 57: 53–65.
3. Tank, D. C. & Donoghue, M. J. (in press). Phylogeny and phylogenetic nomenclature of the Campanulidae based on an expanded sample of genes and taxa. Systematic Botany
4. Kew Science (2021). Plants of the World Online, *Lasianthera* P.Beauv. Retrieved 16 May 2021.
5. Hutchinson J, Dalziel JM. Flora of West Tropical Africa. *Grown agents of overseas Government and Administration.* 1973; 2(1):638*.*
6. Davis PH, Heywood VH. Principles of angiosperm taxonomy. *Robert E. Krieger publishing Comp, New York* . 1973; *558pp*
7. Stevens, Peter F. (2001). "Angiosperm Phylogeny Website – Aquifoliales – Stemonuraceae". Version 12, July 2012 with updates. Retrieved 7 August 2013.
8. The Angiosperm Phylogeny Group (2009). "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III". Botanical Journal of the Linnean Society. 161 (2): 105–121. doi:10.1111/j.1095-8339.2009.00996.x. ISSN 1095-8339. See APG III system.
9. Lebrun J.P. & A. Stork (2010). Buxaceae-Simaroubaceae Trop. Afr. Flow. Plants (Ecol. Distr.) 5: 156
10. Burkill, H.M., 2000. The useful plants of West Tropical Africa. 2nd Edition. Volume 5, Families S - Z, Addenda. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 686 pp.
11. Utteridge, T.M.A., Schori, M. 2009. Stemonurus corrugatus (Stemonuraceae, Icacinaceae s.l.) a new species from Sarawak. *Kew Bull.* 64: 327–331.
12. Melanie S, Carol AF. Pollen diversity in Aquifolia less Botanical Burnal of the Linnean Society. 2014; 175(11):169-190.
13. Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. Hypertens. Res. 2011; 34, 431–440. doi: 10.1038/hr.2010.264
14. Walters SJ, Olmstead RG. A survey of tricolpate (eudicot) phylogenetic relationships. American Journal of Botany, 2004; 91 (10): 1627-1644*.*
15. Esaus K. Anatomy of seed plant. Wiley and Sons *New York 2nd. Ed.* 1971; *540pp.*
16. Opeyemi PA, Oladipo T, Akinwumi JA, Herber CI. Structure, distribution and taxonomic significance of leaf and petiole anatomical characters in five species of *Terminalia* (L) (Combretaceae: Magnoliopsida). Brazillian Journal of Biological Science*.* 2018; 5 (10): 515-528.
17. Khalik KA, Maesen LJG, Kpoman WJM, Berg RG. Numerical Taxonomic study of some tribe of Brassicaceae from Egypt. Plant Syst. Evd. 2002; 233: 207-221.

18. Grant M, Blackmore C, Morton C. Pollen morphology of the subfamily Aurantiodeae (Ruthceae) Grana. 2000; 39:8.20.

19. Clement UO, Chisom F, Alex LI, Nkumah CO, Chibuike IO, Nyananyo BL. Comparative systematic leaf and petiole anatomical studies of genus *Starchytarpheta*. Journal of medicinal plant studies. 2015; 3(4):92.

20. Johansen DA. 1940. *Plant microtechnique*. Mcgraw-Hill, New York. 532p.

21. Stace CA. The taxonomic importance of the leaf surface in current concepts in plant taxonomy (eds). Heywood SVH, Moore DM. Academic Press, London. 1984.

22. Ahmad K, Khan MA, Ahmad M, Shaheen N, Nazir A. Taxonomic diversity in Epidermal cells of some subtropical plant species Int. J. Agri. Bio. 2010; 12(1); 115–118

23. Al-Edany T, Saadi AL.Taxonomic significance of anatomical characters in some species of the family Myrtaceae. Am J. plant Sci. 2012; 3:572-581.

24. Watson, L., and Dallwitz, MJ. 1992 onwards. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 15th November 2021

25. Teo, S.P., Haron, N.W. 1999. Anatomical studies in West Malaysian Icacinaceae. Austr. Syst. Bot. 11: 729–738.

26. Potgieter MJ, van Wyk AE. Leaf anatomy of the southern African Icacinaceae and its taxonomic significance. S. Afr. J. Bot. 1999; 65(2): 153- 162

27. Bassey ME, Etuk RU, Obop IE. Chemotaxonomic study of *Lasianthera africana* (Icacinaceae) in Akwa Ibom State of Nigeria. *Nigerian Journal of Botany,* 2006; 19 (1) 99-102.

28. Agwu, COC. and Uwakwe, G. O. Melissopalynological studies of honey from Abia and Imo States of Nigeria. Nig J Bot 1992; 5: 85 – 91

29. Nyananyo BL, Olowokudejo JD. Taxonomic Studies in the Genus *Talinum* (Portulacaceae) in Nigeria Willdenowia, 1986; 455-463.

30. Edeoga HO, Ikem IC. Structural morphology of the pollen grains in three Nigeria species of Boerhavia. New Bot. 2002; 29:89-95.

31. Nyananyo BL. Tribal and generic relationship in Portulacacae (Centrospermae) Feddes Repport. 1990; 101:23-24. Doi: 10./002/fedr.19901010504.

32. Mgbagwu FN, Edeoga HO. Leaf anatomy of some Nigeria species of *Vigna savi* (Leguminosae – Papilioniodae), Agric J. 2006; 1:5-7.

33. Schori, M., Utteridge, T.M.A. 2010. Three new species and a new name in Southeast Asian *Gomphandra* (Stemonuraceae/Icacinaceae s.l.). Blumea 55: 189–195.

34. Lens, F., Karehed, J., Baas, P., Jansen, S., Rabaey, D., Huysmans, S., Hamann, T., Smets, E. 2008. The wood anatomy of the polyphyletic Icacinaceae s.l., and their relationships within asterids. Taxon 57: 525–552.
35. Potgieter MJ, Schori M, Utteridge TMA. Stemonuraceae Amer. J. Bot., 2001; 88: 367-376.