Pollen Morphology of Some *Phyllanthus* Species in Nigeria

Wahab, Olasumbo Monsurat[1] and Ayodele, Abiodun Emmanuel[2]

[1.] Department of Crop Production Technology, Federal College of Forestry, Ibadan. Nigeria
[2.] Department of Botany, University of Ibadan, Ibadan. Nigeria
olasumbowahab@yahoo.com

Abstract: Circumscription of the genus *Phyllanthus* has been a cause of much confusion and disagreement. The fact that many herbaceous *Phyllanthus* species grow in similar habitats and share common vernacular names in Nigeria give rise to misidentifications. Field and Herbarium observations of some *Phyllanthus* species show that there are similarities of highly conspicuous morphological features, making identification of the species difficult. The pollen grain morphology of 18 field specimens comprising 10 *Phyllanthus* species using light microscope was therefore analysed in the present study with the aim of providing additional information on their taxonomy. The pollen type of the species have 3 – colporate, finely reticulate pollen without much ornamentation. Pollens were prolate, subprolate in shape in all taxa except *P. muellerianus* which was oblate–spheroidal. The pollen grains ranged in size from small in *P. amarus, P. muellerianus, P. maderaspatensis, P. pentandrus* and *P. reticulatus* to medium in *P. maderaspatensis, P. capillaris, P. niruroides, P. odontadenius* and *P. urinaria*. The smallest pollen size was observed in *P. muellerianus* being 12.4µm by 13.0µm while the largest pollen size was observed in *P. capillaris* being 31.5µm by 23.25µm. The colpi length ranged from 12.2µm in *P. muellerianus* to 26.75µm in *P. urinaria* while the percentage polar over equatorial axis ranged from 95.4% in *P. muellerianus* to 145.8% in *P. niruroides*. Information obtained from the study do not only corroborate the existing information on the genus, they can be used in conjunction with other characters to delimit the species in the genus.
[Wahab, Olasumbo Monsurat and Ayodele, Abiodun Emmanuel. **Pollen Morphology of Some *Phyllanthus* Species in Nigeria.** *N Y Sci J* 2018;11(10):40-45]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 6. doi:10.7537/marsnys111018.06.

Keywords: *Phyllanthus* species; pollen characters; taxonomic implications; Nigeria

1.Introduction

Phyllanthus is one of the 79 genera of Phyllanthaeceae (http://www.the plantlist.org) which include *Margaritaria* L.f., *Flueggea* Willd, *Securinega* Comm.ex Juss, *Antidesma* Burm, *Bridelia* Willd, *Cicca* Baill, *Hymenocardia* Walli ex Lindi, *Uapaca* Baill. All of these genera are found in Nigeria. *Margaritaria* is sister to all genera of Phyllanthaceae with phyllantoid branching (Samuel *et al.,* 2005). The genus *Phyllanthus* has a diversity of growth forms including terrestrial or floating aquatics, pachycaulous succulents, trees, shrubs, climbers, annual and perennial herbs. Some species have flattened leaf-like stems or modified branchlets called phylloclades. All these growth forms are distributed in all tropical and subtropical regions of both hemispheres (Webster, 1994). They are found in open and shaded conditions in rocky areas, waste grounds, roadsides, on termitaria, cultivated fields and swamps in different vegetational zones including the grassland, derived savanna and rainforest. According to Webster (1994) and Silva (2009), despite the variety of growth forms, almost all *Phyllanthus* species express a specific type of growth called "phyllanthoid branching" in which the leaves on the main (vertical) plant axes are reduced to cataphylls while leaves on the plagiotropic (horizontal) axes are deciduous and floriferous. Indeed, leaf flower is the common name for all *Phyllanthus* species and '*Phyllanthus'* means 'leaf and flower' because the flowers as well as the fruits are associated with the leaf (Cabiesses 1993). Govaerts *et al.,* (2000) reported that with increasing knowledge from molecular phylogenetic studies and more genera being embedded in the genus *Phyllanthus,* the species number has increased tremendously making it a giant and heterogeneous genus.

The circumscription of the genus has been a cause of much confusion and disagreement. The fact that many herbaceous *Phyllanthus* species grow in similar habitats and share common vernacular names in Nigeria give rise to misidentifications. Field and Herbarium observations of some *Phyllanthus* species show that there are similarities of highly conspicuous morphological features, making identification of the species difficult.

The heterogeneity in plant architecture in association with pollen grain ornamentation were proposed as the main taxonomic characters to establish the sections within the genus (Webster, 1956, 1957, 1958, 1988, 1994a, 1999, 2002; Webster & Airy Shaw, 1971; Webster & Proctor, 1984). These structures were shown to be an important tool in understanding the evolution of *Phyllanthus* species at section and subsection levels.

The pollen grain morphology of *Phyllanthus* species using light microscope was therefore analysed in the present study with the aim of providing additional information on their taxonomy.

2. Materials and Methods

The method of acetolysis and slide preparation after Erdtman (1952) and terminologies used in description of the pollens were based on that of Erdtman (1952) and Moore *et al*., (1991). To each numbered plastic centrifuge tube containing pollen sample from flower buds of 18 field specimens and preserved in 50% ethanol was added 5ml of acetolysis mixture (9 parts acetic anhydride to 1 part concentrated Sulphuric acid) and heated in a water bath from 70^{o}C to boiling point, stirring occasionally. This was left in the boiling water for 5 minutes. Acetolysis mixture left over was then poured into the special bottle labeled 'Acetolysis waste'. It was centrifuged while still hot and decanted into the special bottle, distilled water was added and shaken vigorously with a mixer, centrifuged and decanted. This was repeated four times to rinse off the acetolysis mixture. Fifty percent aqueous glycerol was added and then left for at least 30 minutes, later vigorously mixed on the mixer, centrifuged for 10 minutes and then decanted. It was mixed thoroughly and stored in well labeled vials from where aliquots of it were mounted on slides.

Mounting of slides

Slides were cleaned with cotton wool soaked in ethanol and labeled appropriately, a drop or two of the aliquot were then placed at the center of the slide and covered with a cover slip for it to spread evenly. The sides of the cover slip were sealed with nail varnish and then studied under the light microscope, Photomicrographs of the specimens were taken using Leica CME with a digital microscope eyepiece attached and photo explorer 8.0 SE basic software. All slides were deposited in the herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria.

3. Results

The qualitative and quantitative pollen morphological characters of some of the species in the genus *Phyllanthus* are presented in Table 1. The pollen type of the species studied have 3 – colporate, finely reticulate pollen without much ornamentation. Pollens were prolate, subprolate in shape in all taxa studied except *P. muellerianus* which was oblate–spheroidal (Table 1, Plate 1d). The pollen grains ranged in size from small in *P. amarus, P. muellerianus, P. maderaspatensis, P. pentandrus* and *P. reticulatus* to medium in *P. maderaspatensis, P. capillaris, P. niruroides, P. odontadenius* and *P. urinaria* (Table 1). The smallest pollen size was observed in *P. muellerianus* being 12.4µm by 13.0µm while the largest pollen size was observed in *P. capillaris* being 31.5µm by 23.25µm. The colpi length ranged from 12.2µm in *P. muellerianus* to 26.75µm in *P. urinaria* (Table 1) while the percentage polar over equatorial axis ranged from 95.4% in *P. muellerianus* to 145.8% in *P. niruroides*.

Table 1: Qualitative and Quantitative pollen characters of *Phyllanthus* species in Nigeria

Taxa	Polar axis (P) (µm)	Equatorial axis (E) (µm)	Colpi length (µm)	Pollen class	P/E (%)	Pollen size
Phyllanthus amarus	17.5(20.75±2.06)22.5	15.0(16.75±1.21)17.5	15.0(18.5±1.75)20.0	Subprolate	123.9	Small
P. capillaris	25.0(31.5±3.16)35.0	20.0(23.25±1.69)25.0	22.5(25.75±2.65)30.0	Prolate	135.5	Medium
P. maderaspatensis	20.0(23.0±3.07)27.5	12.5(18.25±2.65)22	20.0(21.0±1.29)22.5	Subprolate	126.0	Small
P. muellerianus	11.0(12.4±0.84)14.0	12.0(13.0±0.67)14.0	11.0(12.2±0.79)13.0	Oblate-spheroidal	95.4	Small
P. niruri	25.0(30.75±4.42)37.5	17.5(22.25±3.22)27.5	17.5(24.0±4.12)30.0	Prolate	138.2	Medium
p. niruroides	25.0(26.25±1.32)27.5	15.0(18.0±1.97)20.0	22.5(24.5±1.97)27.5	Prolate	145.8	Medium
P. odontadenius	22.5(28.25±3.34)32.5	17.5(22.2±2.49)25.0	20.0(23.0±1.97)25.0	Subprolate	127.0	Medium
P. pentandrus	22.5(24.0±1.29)25.0	17.5(18.25±1.21)20.0	20.0(21.5±1.29)22.5	Subprolate	131.5	Small
P. reticulatus	15.0(16.5±1.75)20.0	12.5(14.0±1.29)15.0	15.0(16.25±1.32)17.5	Subprolate	117.9	Small
P. urinaria	22.5(25.5±2.84)30.0	12.5(18.25±2.37)20.0	22.5(26.75±2.90)30.0	Prolate	139.7	Medium

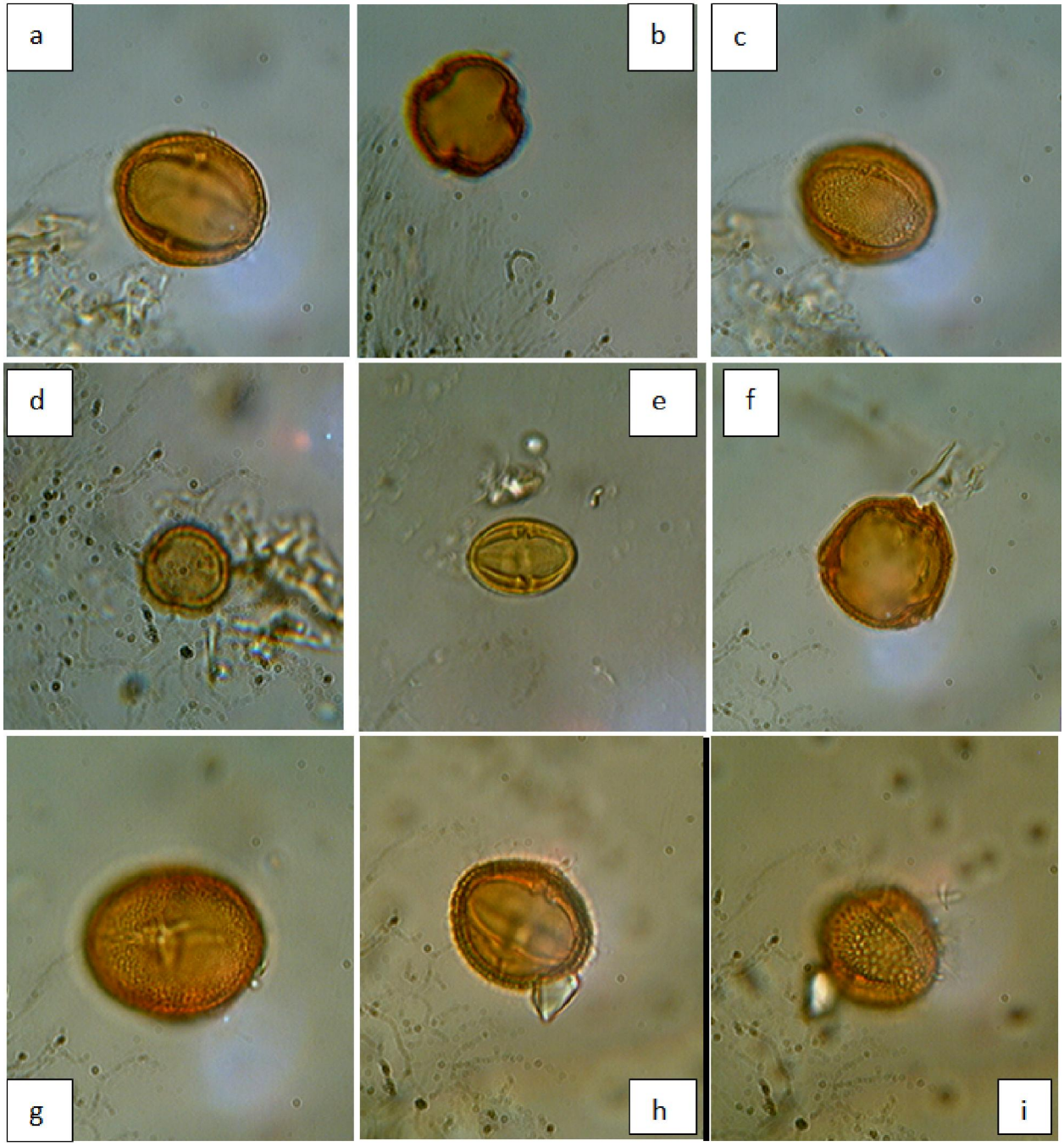

Plate 1: Photomicrographs of pollen grains of *Phyllanthus* species in Nigeria.

a: Equatorial view of *Phyllanthus amarus*
b: Polar view of *Phyllanthus amarus*
c: Equatorial view of *Phyllanthus amarus* showing reticulate pattern
d: Polar view of *Phyllanthus muellerianus*
e: Equatorial view of *Phyllanthus niruri*
f: Polar view of *Phyllanthus niruri*
g: Equatorial view of *Phyllanthus niruri* showing reticulate pattern
h: Equatorial view of *Phyllanthus pentandrus*
i: Polar view of *Phyllanthus pentandrus* showing reticulate pattern

Plate 2: Photomicrographs of pollen grains of *Phyllanthus* species in Nigeria.

j: Equatorial view of *Phyllanthus odontadenius*
k: Equatorial view of *Phyllanthus odontadenius* showing reticulate pattern
l: Equatorial view of *Phyllanthus urinaria*
m: Equatorial view of *Phyllanthus capillaris*
n: Equatorial view of *Phyllanthus capillaris*

4. Discussion and Conclusion

Pollen morphology and evolutionary characteristics in the genus *Phyllanthus* have been extensively studied (Punt, 1962, 1967, 1972, 1980; Punt and Rentrop, 1974; Bor, 1979; Meewis and Punt, 1983; Lobreau-Callen *et al*., 1988; Webster, 1994a, 1994b). Webster (1956) reported pollen morphology and architectural pattern of the species to be considered the main characteristics useful in *Phyllanthus* taxonomy. He reported that a large number of *Phyllanthus* species showed 3–colporate and reticulate pollen grains which have been reported in the primitive tribes of Phyllanthoideae. These structures were shown to be an important tool in understanding the evolution of *Phyllanthus* species at section and sub-section levels. This led Webster (1956, 1957 & 1958) to propose species of the section *Phyllanthus* as the ancestors of section *Chroretropsis*. In the present study, pollens were reported to be prolate or subprolate in shape except *P. muellerianus* which was found to be oblate-spheroidal (Table 1, Plate 1b and 1d). This could be regarded as a diagnostic feature to distinguish *P. muellerianus* from other taxa studied.

Webster (1956-1958) accumulated much phytomorphological, cytotaxonomical (Webster and Ellis, 1962) and other taxonomical data that some species were considered primitive and others appeared to be advanced. Hence Punt (1967) reported that pollen morphology might give additional support for

the position of Webster's primitive and advanced taxa and that if their pollen morphology and taxonomy would arrive at the same conclusion, it would mean a real advance in the subdivision of the genus. Webster (1956) suggested that there is a relationship between *Phyllanthus* and the presumably primitive genera *Securinega, Andrachne* and *Savia* of the same family having found out that some species of *Phyllanthus* e.g *Phyllanthus maderaspatensis* (also documented for Nigeria) which show relationships with their genera are the most primitive ones in the genus. Thus, Punt (1962); Webster (1956) and Köhler (1965) logically concluded that the characteristics (tricolporate, distinctly prolate, reticulate and an elongated endoaperture) which the pollen grains of their taxa exhibit are also primitive. In addition, Punt (1967) also recognized that the so called 'primitive' pollen grains of the genus *Phyllanthus* are already 'advanced' in comparison to the other pollen types in the plant kingdom. A number of evolutionary trends within the genus *Phyllanthus* have already been presented by Punt (1967) and due to more recent and extensive investigations, a reconsideration with regard to the evolutionary trends within *Phyllanthus* was presented by Meewis and Punt (1983) where *P. amarus* type was reported to include only one species *P. pentandrus* with many more species (such as *P. odontadenius* from Africa and *P. fraternus* from other continents) from the subgenus *Phyllanthus*. Hence the information obtained from the present study on the pollen morphological characters of the *Phyllanthus* species do not only corroborate the existing information on the genus, they can be used in conjunction with other characters to delimit the species in the genus. In addition, the reported presence of identical pollens can only be resolved when other lines of evidence are examined.

Corresponding author
Wahab Olasumbo Monsurat
Department of Crop Production Technology,
Federal College of Forestry, Ibadan. Nigeria
Telephone: +234-802 3516 870
E-mail: olasumbowahab@yahoo.com

References

1. The Plant List. Version 1. Published on the internet, 2010; http://www.the plantlist.org/ (accessed 8/8/2016)
2. Samuel, R., Kathriarachchi, H., Hoffmann, P., Barfuss, M., Wurdack, K.J. and Chase, M.W. Molecular phylogenetics of *Phyllanthaceae*: Evidence from plastid *matK* and nuclear *PHYC* sequences. American Journal of Botany 2005; 92: 132–141.
3. Webster, G.L. Classification of the Euphorbiaceae. Annals of the Missouri Botanical Garden 1994a; 81: 3-32.
4. Webster, G.L. Synopsis of the genera and suprageneric taxa of Euphorbiaceae Annals of the Missouri Botanical Garden 1994b; 81: 33–144.
5. Silva, M.J. Neotropical Phyllanthaceae. In: Milliken, W. Klitgard, B. & Baracat, A. (2009) Onwards Neotripikey-Interactive key and information resources for flowery plants of the Neotropics htt://www.kew.org/ science/tropamerica/ neotropikey/families/Phyllanthaceae.htm 2009.
6. Cabieses, F. Apuntes de medicina traditional. La Racionalizcion de lo lrracional. 'Notes on Traditional Medicine.'Consejo Nacional de Ciencia Y Technologia CONCYTEC Lima- Peru 1993; pp 414.
7. Govearts, R., Frodin, D.G. and Radcliffe-Smith, A. World checklist and Bibliography of Euphorbiaceae. 2000; 4 vols. Royal Botanic Gardens, Kew.
8. Webster, G.L. Studies on the Euphorbiaceae, Phyllanthoideae II. The American species of *Phyllanthus* described by Linnaeus. J. Arnold Arb 1956; 37(1): 1-14.
9. ———, G.L. A monograpgic study of the West Indian species of *Phyllanthus.* Journal of the Arnold Arboretum 1956-1958; 37: 91-122, 217-268, 340-359; 38: 51-80, 170-198, 295-373; 39: 49-100, 111-212.
10. ———, G.L. A revision of *Phyllanthus* (Euphorbiaceae) in eastern Melanesia. Pacific Science 1988; 40: 88–105.
11. ———, G.L. *Phyllanthus*. In: Berry, P.E, Yatskievych K, Holst B.K, eds. Flora of the Venezuelan Guayana 1999; Vol.5. St Louis: Missouri Botanical Garden Press, 191-205.
12. ———, G.L. A synopsis of the Brazillian taxa of *Phyllanthus* section *Phyllanthus* (Ephorbiaceae). Lundellia 2002; 5:1-26.
13. ———, G. L. and Airy-Shaw, K. H. A provisional synopsis of the New Guinea taxa of *Phyllanthus* (Euphorbiaceae). Kew Bull. 1971; 26(1): 85-109.
14. ———, G.L and Proctor G.R. A new species of *Phyllanthus* (Euphorbiaceae) from the Cayman Island. Rhodora 1984; 86: 121–125.
15. Erdtman, G. *Pollen morphology and Plant taxonomy: angiosperms.* Chronica Botanica Co. Waltham, Massi USA. 1952; 539p.
16. Moore, P.D., Webb, J.A and Collinson, M. *Pollen analysis*. Blackwell, London, 1991; pp 226.

17. Punt, W. Pollen morphology of Euphorbiaceae with special reference to taxonomy. Wentia 1962; **7:** 1-116.
18. _____, W. Pollen morphology of the genus *Phyllanthus* (Euphorbiaceae). Review of Palaeobotany and Palynology 1967; 3: 141-150.
19. _____, W. Pollen morphology and taxonomy of section of *Ceramanthus* Baillon *s. l.* of the genus *Phyllanthus* (Euphorbiaceae). Review of Palaeobotany and Palynology 1972; 13: 213-228.
20. _____ , W. Pollen morphology of the *Phyllanthus* species (Euphorbiaceae) occurring in Guinea. Review of Palaeobotany and Palynology 1980; 31: 155-177.
21. _____, W. and Rentrop, J. Pollen morphology of the *Phyllanthus* species occurring in the continental United States. Review of Palaeobotany and Palynology 1974; 16: 243-261.
22. Bor, J. Pollen morphology and the bi- reticulate exine of the *Phyllanthus* species (Euphorbiaceae) from Mauritius and Reunion. Review of Paleobotany and Palynology 1979; 27: 149-172.
23. Meewis, B and Punt, W. Pollen morphology and taxonomy of the subgenus *Kirganelia* (Jussieu) Webster (genus *Phyllanthus*, Euphorbiaceae) from Africa. Review of Palaeobotany and Palynology 1983; 39: 131-160.
24. Lobreau-Callen, D., Punt, W. and Schmid, M. Pollen morphology and taxonomy of the *Phyllanthus* species (Euphorbiaceae) native to New Caledonia. Review of Palaeobotany and Palynology 1988; 54: 283-304.
25. Webster, G.L and Ellis, J.R. Cytotaxonomic studies in the Euphorbiaceae subtribe Phyllanthinae. American Journal of Botany 1962; 49: 14-18.
26. Köhler. E. Die Pollen morphologie der biovulaten Euphorbiaceae and ihre Bedeutung füdie *Taxonomie*. Grana Palynologica. 1965; 6: 26-120.

10/25/2018