International Journal of Plant & Soil Science

20(1): 1-7, 2017; Article no.IJPSS.37478
ISSN: 2320-7035

Pollen Morphology as a Useful Taxonomic Tool in Delimiting the Species of Loranthaceae in Nigeria

J. A. Ibrahim[1*] and A. E. Ayodele[2]

[1]Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, P.M.B. 21, Garki, Abuja, Nigeria.
[2]Department of Botany, University of Ibadan, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author JAI conceptualized the study, carried out field work, bench work, analysis of result and writing of manuscript. Author AEA participated in field work, analysis of the result and review of manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/37478
Editor(s):
(1) Hon H. Ho, Biology, State University of New York, New York, USA
Reviewers:
(1) Kamal I. Mohamed, State University of New York at Oswego, USA.
(2) Marina Gotelli, Universidad de Buenos Aires, Argentina.
(3) Ortrud Monika Barth, Instituto Oswaldo Cruz, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/21875

Short Research Article

Received 18th October 2017
Accepted 9th November 2017
Published 13th November 2017

ABSTRACT

Background and Objective: Pollen grains shape, size, aperture and wall ornamentation have been useful in taxonomic studies of plants especially in resolving relationship or determining variation in a taxa. The Loranthaceae (Mistletoes) are parasitic family with known taxonomic problem of poorly identified species in Nigeria. Pollen grains of fourteen species which spread across six genera of Nigerian Loranthaceae was studied with a view of generating characters that would further help in their identification.
Methods: Samples were subjected to standard acetolysis for palynological sample preparation and analysis.
Results: Pollen grains are mainly triangular or three-armed in nature with percentage of polar to equatorial axis ranging from 90.69% to 100%. All pollen grains were oblate-spheroidal except the pollen of *Helixanthera mannii* that was prolate-spheroidal and all the genera had tri-snycolporate pollen grains except pollen grains of *Tapinanthus* which were tri-colporate. The smallest grains are found in the genus *Helixanthera* while others range from medium to large in size with *H. spathulata*

**Corresponding author: E-mail: sadiqoyene04@yahoo.com, sadiqoyene@yahoo.com;*

having the smallest size of 20.75 µm by 20.75 µm and the largest of 49.00 µm by 52.50 µm in *Globimetula mannii.*

Conclusion: The information obtained from this study do not only add to the already existing information on the family, but the diagnostic characters obtained can be used in conjunction with other characters like morphology and anatomical characters to delimit the species in the family.

Keywords: Palynology; mistletoes; Nigeria; identification; Loranthaceae.

1. INTRODUCTION

The parasitic Mistletoes of Nigeria are found mainly in the Loranthaceae family with about fifteen documented species [1]. Species of Loranthaceae are with known history of misidentifications not only in Nigeria but all over the world as a result of overlapping delimitation characters between species or variations among individuals of same species and also lack of sufficient information on the taxonomy of the family [2,3,4,1]. Several parameters have been employed by researchers to solve taxonomic problems and one of such parameter is the use of pollen morphological characters.

Pollen grain morphological data in terms of shape, size, apertural configuration and surface ornamentation are believed to be very useful taxonomically [5]. They have been used at all levels of taxonomic hierarchy either in suggesting relationship or to determine variation within taxa. Mueller [6] used pollen grain characters to suggest relationship in *Sonneratis* Linn. f. and Stuessy [7] also used it to determine variation within a species and below species level. West African species of Polygonaceae were also grouped into three based on pollen grain characters which agree with the morphological delimitation of the genera in the family [8]. Pollen grains of some selected species of the genus *Arceuthobium*, family Loranthaceae have been independently studied by several authors [5,9,10,11], but Hawksworth and Wiens [12] made the first comprehensive study of the pollen grains of all the species known then in the genus. Han et al. [13] also studied the pollen grains morphology of thirty-eight taxa of Loranthaceae from China.

Grimsson et al. [14] carried out a study of pollen grain morphology of some species of Loranthaceae and with addition of data of some species extracted from published literatures, evaluated the correlation of pollen grain morphology and phylogenetic relationships within Loranthaceae as inferred from molecular sequence data using fifty-five species. None of the fifteen species found in Nigeria were among the fifty-five species used for this study suggesting that information is unavailable on the pollen grains of Nigerian species of Loranthaceae.

The present study is a part of series of studies [15,16,17] aimed at investigating the extent to which microscopic characters could contribute to the elucidation of relationships in the family Loranthaceae for easy identification and delimitation of the taxa. This particular study focuses on the pollen morphological features.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preservation

Specimens used for the study were collected during field trips [1] and well – authenticated Herbarium specimens for species that were not collected during the field trips. Flower buds of field collections were preserved in 50% ethanol while dry specimen were kept in sealed envelopes until ready to be used. List of taxa studied are presented in Table 1.

2.2 Sample Preparation and Analysis

Erdtman's [9,18] acetolysis method was used. The flower buds were crushed with a glass rod in centrifuge tubes. Three milliliter of freshly prepared acetolysis mixture (9 parts acetic anhydride to 1 part concentrated Tetraoxosulphate VI acid) was added to the content in the tubes. The content was heated in a water bath from 70℃ to boiling point, stirring. The centrifuge tubes and content were left in boiling water for 3 minutes and then centrifuged at 4000 r.p.m. for 5 minutes while still hot. The supernatant was decanted into an Acetolysis waste bottle. About 2 ml distilled water was then added to the sediments in the tubes and shaken vigorously using a whirl mixer. Few drops of methylated spirit were added to remove the foam formed and centrifuged again. The supernatant was decanted. The washing with water and centrifuging was repeated four times. Fifty

percent glycerine was added and left standing for two hours. The tubes were shaken vigorously using a whirl mixer and centrifuged at 4000 r.p.m. for 10 minutes. The supernatant was then decanted, inverted over filter paper and left overnight. One hundred percent glycerol was added to the tubes and shaken. This was then poured into labeled storage vials. Slide preparations was done by placing a drop of the content of the vials on a slide and covered with a cover slip and sealed with nail vanish.

Table 1. List of Loranthaceae species studied

Genera	Species
Agelanthus	*Agelanthus brunneus* (Engl.) Balle & Halle *Agelanthus dodoneifolius* (DC.) Polh. &Wiens Syn. *Tapinanthus dodoneifolius* (DC.) Danser *Agelanthus heteromorphus* (A. Rich.) Polh. & Wiens
Englerina	*Englerina gabonensis* (Engl.) Balle
Globimetula	*Globimetula braunii* (Engl.) Danse *Globimetula oreophila* (Oliv.) Danser
Helixanthera	*Helixanthera mannii* (Oliv.) Danser *Helixanthera spathulata* Wiens & Polh.
Phragmanthera	*Phragmanthera nigritana* (Hook. F. ex Benth.) Balle *Phragmanthera talbotiorum* (Sprague) Balle *Phragmanthera capitata* (Sprengel) Balle
Tapinanthus	*Tapinanthus bangwensis* (Engl. & K. Krause) Danser *Tapinanthus cordifolius* Polh. & Wiens. Syn. *T. sessilifolius* (P. Beauv) Tieghem *Tapinanthus globiferus* (A. Rich.) Tieghem

The slides were observed under x40 and x100 magnification using bright – field Microscope. Photomicrographs were taken using Leica CM E with Digital Microscope Eyepiece attachment and Photo Explorer 8.0 SE Basic software. Mean, range and Standard Error were calculated for all the quantitative variables based on ten measurements. Terminologies used were based on Moore et al. [19] and Erdtman [9]. All slides were deposited in the University of Ibadan Herbarium (UIH).

3. RESULTS AND DISCUSSION

The qualitative and quantitative characters of pollen grains of Loranthaceae are shown in Table 2, Table 3 and photomicrographs in Plate 1.

The pollen grain morphological characters of Loranthaceae obtained from this study show that some of the characters are very useful in delimiting the species while others are useful in showing affinity in the family. The general appearances of the pollen grains (AMB) were all similar in the family i.e being triangular or 3-armed in nature, although the degree of their concaveness varies (Table 2 and Plate 1). Metcalfe and Chalk [20] observed the triangular or 3-armed AMB pollen in the species of Loranthaceae and the snycolporate nature (Plate 1 and Table 2) of the pollen grains observed in the Nigerian Loranthaceae were also in corroboration with their work [20]. Han et al. [13] also observed the tri-syncolporate and tri-colpate pollen type in china species of Loranthaceae.

The pollen grains in *Globimetula braunnii, G. oreophila, Helixanthera mannii, H. spathulata Phragmanthera talbotiorum, Tapinanthus bangwensis, T cordifolius, T. globiferus* were slightly concave or straight at the edges while the pollen grains in the remaining six species were deeply concave or almost 3-armed (Plate 1 and Table 2).

The deeply constricted base of the arms of the pollen grains of *Agelanthus brunneus,* the characteristic wide pore-like feature of *Helixanthera spathulata,* the broader in the middle of the arm of pollen grains of *Englerina gabonensis* and the thinner middle of the arm of pollen of *Tapinanthus globiferus* are all diagnostic features of these species (Plate 1 and Table 2). The small pollen grain sizes of 21.5 µm x 22.25 µm in *Helixanthera mannii* and 20.75 µm x 20.75 µm in *Helixanthera spathulata* readily distinguished the species from the other species which are more than 25.0 µm x 25.0 µm in sizes (Table 3). Also, the tri-colporate pollen grains types in *Tapinanthus* readily separate the pollen of these species from all other species which have tri-syncolporate pollen grains. The exine patterns or sculpturing of all the pollens were mainly finely reticulate to reticulate viewed under the bright – field microscope were not very

distinct (Plate 1). Grimsson et al. [14] documented that the sculpturing of the Loranthaceae pollen grains studied are typically minute in nature even under the Scanning Electron Microscope.

The significance of pollen grain morphology in the taxonomy of angiosperms have been exemplified in several works where it was used either to suggest relationship in the family, to determine variation at the specific level or to solve problems at the generic or sub-generic levels [6,7,8,21,22]. Similarities and dissimilarities observed among the taxa of the group when used in conjunction with characters from previous studies [1,15,16,17] would help in delimitating the genera and species of Loranthaceae in Nigeria.

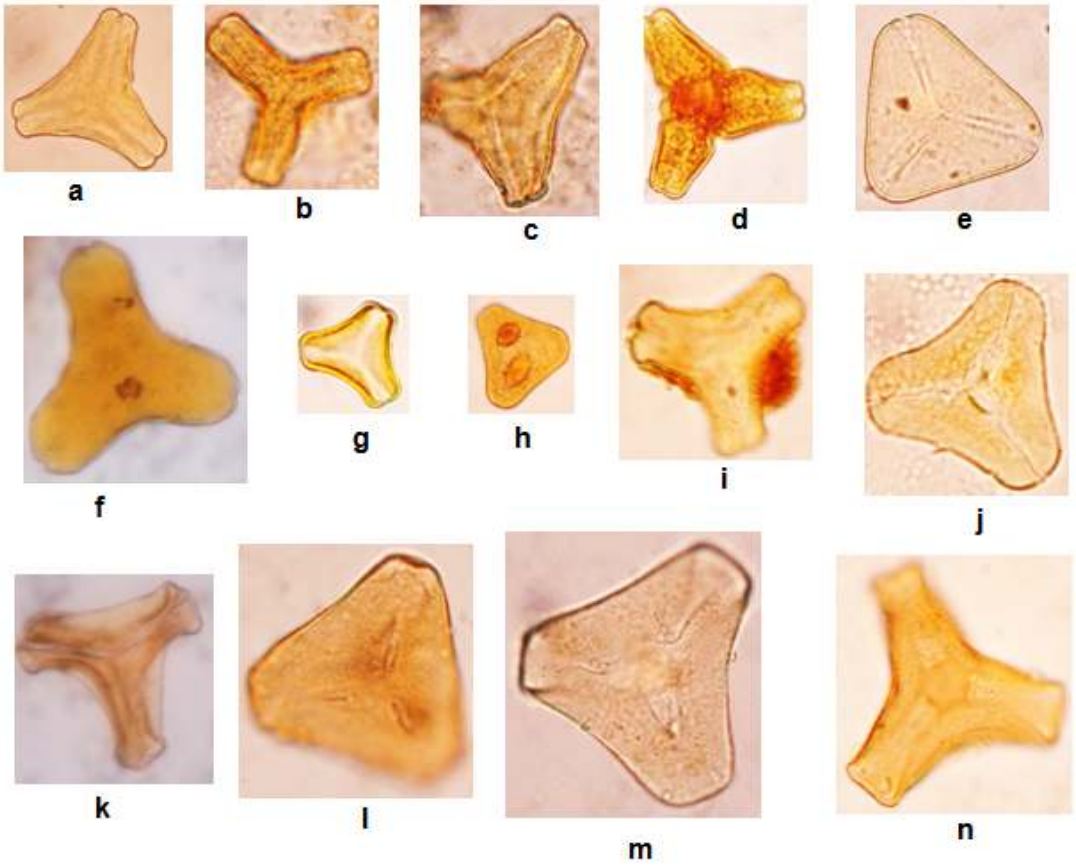

Plate 1. Pollen grains of the family Loranthaceae in Nigeria

a: Pollen grain of Agelanthus bruneus showing trisyncolporate grains
b: Pollen grain of Agelanthus dodoneifolius showing trisyncolporate grains
c: Pollen grain of Agelanthus heteromorpus showing trisyncolporate grains
d: Pollen grain of Englerina gabonensis showing trisyncolporate grains
e: Pollen grain of Globimetula braunii showing trisyncolporate grains
f: Pollen grain of Globimetula oreophila showing trisyncolporate grains
g: Pollen grain of Helixanthera mannii showing trisyncolporate grains
h: Pollen grain of Helixanthera sapthulata showing peculiar type pollen with two large pore-like structure
i: Pollen grain of Phragmanthera capitata showing trisyncolporate grains
j: Pollen grain of Phragmanthera talbotiorum showing trisyncolporate grains
k: Pollen grain of Phragmanthera nigritana showing trisyncolporate grains
l: Pollen grain of Tapinanthus bangwensis showing tricolporate grains
m: Pollen grain of Tapinanthus cordifolius showing tricolporate grains
n: Pollen of Tapinanthus globiferus showing tricolporate grains

Table 2. Qualitative Pollen characters of Nigerian species of Loranthaceae

Taxa	AMB	Shape class	Pollen size	Types
Agelanthus brunneus	Triangular-slightly concave	Oblate-spheroidal	Medium	Tri- syncolporate
Agelanthus dodoneifolius	Triangular –deeply concave	Oblate-spheroidal	Rather large	Tri- snycolporate (Colpi faint)
Agelanthus heteromorphus	Triangular – concave	Oblate-spheroidal	Medium	Tri- syncolporate
Englerina gabonensis	Triangular- concave; base of arm deeply constricted	Oblate-spheroidal	Medium	Tri- syncolporate
Globimetula braunnii	Triangular- straight	Oblate-spheroidal	Large	Tri- syncolporate
Globimetula oreophila	Triangular. concave	Oblate-spheroidal	Large	Tri-syncolporate
Helixanthera mannii	Triangular- concave	Oblate-spheroidal	Small	Tri- syncolporate
Helixanthera spathulata	Triangular – slightly concave	Prolate-spheroidal	Small	Peculiar type
Phargmanthera capitata	Triangular- deeply concave	Oblate-spheroidal	Rather large	Tri- syncolporate
Phragmanthera nigritana	Triangular- deeply concave; tip of arm wider	Oblate-spheroidal	Rather large	Tri- syncolporate
Phragmanthera talbotiorum	Triangular- slightly concave	Oblate-spheroidal	Medium	Tri- syncolporate
Tapinanthus bangwensis	Triangular- almost straight	Oblate-spheroidal	Rather large	Tri- colporate
Tapinanthus cordifolius	Triangular – concave	Oblate-spheroidal	Large	Tri- colporate
Tapinanthus globiferus	Triangular – deeply concave	Oblate-spheroidal	Rather large	Tri- colporate

AMB – General appearance of pollen

Table 3. Quantitative Pollen characters of Nigerian species of Loranthaceae

Taxa	Polar axis (P) (µm)	Equatorial axis (E) (µm)	Exine thickness (µm)	P/E (%)
Agelanthus brunneus	27.3 (33.5 ±0.78) 37.5	27.5 (34.00 ±0.55) 37.5	1.0 (1.58 ±0.12) 2.0	98.53
Agelanthus dodoneifolius	45.0 (46.25 ±0.42) 47.5	45.0 (47.75 ±0.79) 52.5	0.75 (0.95 ±0.08) 1.25	96.86
Agelanthus heteromorphus	30.0 (34.25 ±0.84) 37.5	35.0 (36.75 ±0.84) 40.0	1.0 (1.23 ±0.06) 1.5	93.20
Englerina gabonensis	35.0 (36.0 ±0.41) 37.5	37.5 (38.75 ±0.56) 42.5	0.5 (0.68 ±0.08) 1.0	92.90
Globimetula braunnii	45.5 (49.0 ±0.41) 50.0	50.0 (52.50 ±0.53) 55.0	0.5 (0.93 ±0.08) 1.25	93.33
Globimetula oreophila	45.0 (49.5 ±1.04) 55.0	42.0 (49.75 ±1.02) 55.0	1.0 (1.35 ±0.07) 1.75	99.50
Helixanthera mannii	20.0 (21.5 ±0.55) 25.0	20.0 (22.25 ±0.25) 22.5	0.5 (0.55 ±0.03) 0.75	96.63
Helixanthera spathulata	17.5 (20.75 ±0.53) 22.5	17.5 (20.75 ±0.53) 22.5	0.5 (0.53 ±0.03) 0.75	100.0
Phargmanthera capitata	37.5 (39.75 ±0.87) 45.0	37.0 (43.25 ±0.84) 47.5	0.5 (0.63 ±0.03) 0.75	91.91
Phragmanthera nigritana	35.0 (40.75 ±1.49) 52.5	35.0 (42.25 ±1.37) 50.0	0.5 (0.68 ±0.05)1.0	96.45
Phragmanthera talbotiorum	35.0 (38.75 ±0.93) 40.0	35.0 (39.25 ±1.06) 42.5	0.5 (1.0 ±0.08) 1.25	98.73
Tapinanthus bangwensis	37.5 (39.5 ±0.62) 42.5	40.0 (43.50 ±0.55) 45.0	0.5 (0.61 ±0.03) 0.75	90.81
Tapinanthus cordifolius	42.5 (46.25 ±0.56) 45.5	45.5 (51.0 0±0.76) 52.5	2.0 (2.33 ±0.05) 2.5	90.69
Tapinanthus globiferus	40.0 (42.75 ±0.58) 45.0	42.5 (45.00 ±0.65) 47.5	0.5 (0.7 ±0.44) 0.75	95.0

Measurements = Minimum (Mean ± standard error) maximum

4. CONCLUSION

The information obtained from this study based on the morphological characters of pollen grains of the Loranthaceae have added not only to the existing information on the family, but also can be used in conjunction with other characters from previous studies to delimit the species in the family.

ACKNOWLEDGEMENT

The following people are acknowledged for rendering assistance during field trips for specimen collection: Dr. Florence Tarfa, Dr. Theresa Omara-Achong, Oyepeju M. K. O., Baba Nafi, Pastor Frank, Dr. Colman Goji, Muazzam Ibrahim, Tanko Garba, Mrs. Sumbo Wahab and Mr. Owolabi. We are also grateful to Dr. E. A. Orijemie of Archaeology and Anthropology Department, University of Ibadan for his assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ibrahim JA, Ayodele AE. Taxonomic revision of Loranthaceae in Nigeria. Nigerian Journal of Botany. 2011;24(1): 153–188.
2. Hutchinson J, Dalziel JM. Flora of West Tropical Africa. Grown Agents for Overseas Government and Administration, London. 1954;1:658–665.
3. Burkill HM. The useful plants of West Tropical Africa. 2nd ed. Families J-L. Royal Botanic Gardens, Kew, London. 1995;3: 857.
4. Polhill RM, Wiens D. Mistletoes of Africa. The Royal Botanic Gardens, Kew, London. 1998;370.
5. Gill LS. *Arceuthobium* in the United States. Connecticut Academy of Arts and Science Transactions. 1935;32:111–245.
6. Mueller J. New observations on pollen morphology and fossil distribution of the genus *Sonneratia* (Sonneratiaceae). Rev. Paleobot. Palynol. 1978;26:277-300.
7. Stuessy TF. Plant taxonomy. The systematic evaluation of comparative data. Columbia University Press, New York. 1990;514.
8. Ayodele AE. The morphology and taxonomic significance of pollen in the West-African Polygonaceae. Thaiszia Journal of Botany. 2005;15:143-153.
9. Erdtman G. Pollen morphology and plant taxonomy. Chronica Botanica Co. Waltham, Massi, USA. 1952;539.
10. Whitehead DR. Northern" elements in the Pleistocene flora of the Southeast. Ecology. 1963;44:403–406.
11. Bhandari NN, Nanda K. Studies in the Viscaceae. 1. Morphology and embryology of the Indian dwarf mistletoe, *Arceuthobium minutissimum* Hook. f. Phytomorphology. 1968;18:435-450.
12. Hawksworth GF, Wiens D. Biology and classification of dwarf mistletoes (*Arceuthobium*). Agriculture Handbook 401. Washington, DC; U.S. Department of Agriculture, Forest Service. 1972;234.
13. Han R, Zhang D, Hao G. Pollen morphology of the Loranthaceae from China. Journal of Systematics and Evolution, Acta Phytotaxonomica Sinica. 2004;42(5):436–456.
14. Grímsson F, Grimm GW, Zetter R. Evolution of pollen morphology in Loranthaceae, Grana; 2017.
(Accessed 18th July, 2017)
DOI: 10.1080/00173134.2016.1261939
Available:http://dx.doi.org/10.1080/00173134.2016.1261939
15. Ibrahim JA, Ayodele AE, Okhale SE, Jegede AI, Kunle OF. The taxonomic significance of *Agelanthus dodoneifolius* (DC.) Polh. & Wiens in relation to its hosts. Nigerian Journal of Botany. 2009;22(1):89–101.
16. Ibrahim JA, Ayodele AE. Taxonomic significance of leaf epidermal characters of the family Lorantaceae in Nigeria. World Applied Sciences Journal. 2013;24(9): 1172–1179.
17. Ibrahim JA, Kunle OF, Ayodele AE. Anatomical Features of the Transverse Sections of the Leaves of Loranthaceae in Nigeria. International Journal of Pharmacognosy and Phytochemical Research. 2015;7(3):489-501.
18. Erdtman G. The acetolysis method- a revised description. Svensk Bot. Tidskr. 1960;54:561-564.
19. Moore PD, Webb JA, Collinson ME. Pollen analysis. 2nd Ed. Black Well Scientific Publications. 1991;216.

20. Metcalfe CR, Chalk L. Anatomy of the dicotyledons. Oxford, Clarendon Press. 1950;2.
21. Blackmore S. Palynology and intergeneric relationships in subtribe Hyoseridinae (Compositae: Lactuceae). Botanical Journal of Linnean Society. 1981;82:1–13.
22. Guinet PH. Geographic patterns of the main pollen characters in genus *Acacia* (Leguminosae) with particular reference to subgenus Phyllodineae. In: Pollen and Spores: Form and Function, Blackmore S, Ferguson IK, (Eds). Academic Press, London. 1986;297–311.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21875