

Pollen characterization of *Rhizophora* species in Nigeria

Abstract

Pollen characterization of mangrove species in Nigeria Niger Delta was conducted in permanent plots in Koko, Ogidighen (Delta State), Akakumama, Nembe (Bayelsa State), Olupiri-Epelema, Ugwede (Rivers State), Ikwe, Upenekang (Akwa Ibom State), Adiabo Ukanabio and Esighi (Cross River State) between 2013 - 2016. A total of three hundred and sixty four (364) *Rhizophora* pollen samples were obtained from the sea water-land interfaces to 604 metres inland (maximum *Rhizophora* occurrence landward). The samples were prepared using standard Erdtmanian methods. The result showed the presence of five different shapes of f tricolporate pollen. The exine sculptures were baculate, rugulate, striate and reticulate while the pollen shapes were either sub prolate, prolate or oblate. The polar shapes were circular in Operational Taxonomic Units (OTUs) 1 and 4, triangular in OTUs 2 and 3 and trilobate in OTU5. The grain arrangements for all five OTUs were monad. When this data was normalized and converted to numerical taxonomy using Euclidean distance, a loose relationship was observed between OTUs 1 and 2 suggesting distinct species. Although, OTUs 3, 4 and 5 showed statistical difference (0.05 confidence limit) among themselves, analysis revealed no statistical difference to OTU 1 and 2, implying them as subtypes of either OTUs. The finding is in contrast to the widely held notion that only three putative *Rhizophora* species exist in Nigeria. Edaphic and genetic research of the two inferred species and three subtypes should be conducted.

Key words: *Rhizophora*, subtypes, *harisonii*, *mangle*, *Nembe*, *Unical*

Background

Mangrove forests are known for their high productivity. They offer various ecosystem services such as shoreline stabilization ([Teas, 1977](#); [Field, 1996](#)), habitat, nursery and breeding ground for many fish species and other fauna ([Teas, 1977](#); [Collete, 1983](#); [Ahmad, 1984](#); [Kurian, 1984](#); [Robertson and Duke, 1987](#); [Ngoile and Shunula, 1992](#); [Sasekumar et al., 1992](#); wood for fuel wood, timber, poles, boats ([Ahmad, 1984](#); [Burbidge, 1984](#); [Fredericks and Lampe, 1984](#); [Aksornkoae, 1987](#); [Dahdouh-Guebas et al., 2000](#); [Bosire et al., 2003](#)), establishment of restrictive impounds that offer protection for maturing offspring, filtering and assimilating pollutants from upland run-off and stabilization of bottom sediments ([Saenger and Bellan, 1995](#)) among other products. The common characteristics they all possess is tolerance to salt and brackish waters. It confers an excellent sense of place, aesthetic grandeur and serenity value to the inhabitants. They have been shown as excellent candidates for carbon capture and sequestration ([de Groot et al., 2002](#)). Mangrove habitat is found along the coastlines of Nigeria. It straddles such states as Lagos, Ondo, Delta, Bayelsa, Rivers, Akwa Ibom and Cross River. Hamilton and Casey, 2016 placed Nigeria mangrove habitat as the eight largest in the world. There are five indicator genera of the mangrove environment in Nigeria. *Rhizophora*, *Avicinnia*, *Laguncularia*, *Conocarpus* and lately but regrettably, the invasive *Nypa*. Of them all, *Rhizophora* is the embodiment of the mangrove environment in Nigeria. Its root system, height and hanging roots make it easily distinguishable. The pore space it creates in the soil makes it an excellent keystone engineering species that houses the hermit crabs and lobsters. In turn, the presence of

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these invertebrates is attraction for varieties of *Mona* and *Cercopithecus* taxa. The inevitable role of *Rhizophora* in shoreline protection is better appreciated where and when the coastlines are inadvertently cleared of it. Coastline embankment costing millions of dollars has been spent in such instances. The efficiency and life cycle of such artificial embankments is incomparable to the natural *Rhizophora* species.

The functionality of an organism is not generic, rather specific. It is a trite that species is the only tangible unit of life. It is therefore intuitive to suggest that the ecological niche of one *Rhizophora* species just like other genera (–for instance *Irvingia gabonensis* versus *Irvingia Wombolu*, *Vernonia colarata* versus *Vernonia amygdalina*) would differ albeit how little, from the other.

More so, the alarming rate of mangrove conversion in the country calls for urgent and species specific studies. FAO 2000 put the size of the Nigeria mangrove as 997,700 ha prior to 2000 as against the current size of 240,400ha in 2015 (Hamilton and Casey 2016). Worst still, the high rate of speciation in the tropics makes frequent species characterization inevitable.

Viewed against these backdrops, this study aims to characterize the various *Rhizophora* species in Nigeria using pollen information.

Methodology

Pollen Collection: Three hundred and sixty four (364) pollen samples were collected in ten permanent plots spread across five Niger Delta States (Fig. 1) over a four year period (2013-2016) as shown in Table 1.

Fig 1 Study Area

Table 1 Pollen sample collection

S/N	Sample Number	Location	State	Coordinates		Year of Collection
				N	E	
1	KOK 1-34	Koko	Delta	05° 58' 33" - 5° 59' 03"	005° 23' 119" - 005° 24' 09"	2013
2	OGD 35-69	Ogidigben		05° 23' 47" - 05° 24' 29"	005° 39' 42" - 005° 41' 13"	2013
3	OBN 70-104	Akakumama	Bayelsa	04° 36' 29" - 04° 37' 16"	006° 10' 35" - 006° 11' 18"	2013
4	NEM 105-144	Nembe		04° 37' 55" - 04° 38' 29"	006° 14' 46" - 006° 15' 28"	2013
5	OPM145-179	Olupiri-Epelema	Rivers	04° 43' 35" - 04° 44' 48"	007° 18' 49" - 007° 19' 27"	2014
6	UGD180-214	Ugwede		04° 40' 05" - 04° 41' 33"	007° 22' 16" - 007° 23' 30"	2014
7	IKW215-249	Ikwe	Akwa	04° 32' 08.6" -	007° 54'	2015

Comment [MS1]: Still this question is not answered: why only pollen information?

Comment [MS2]: Which GPS was used to measure the coordinates, is missing in material and methods, further values of altitude is missing, needs to be added.

			Ibom	04° 33' 02.6"	24.0" - 007° 54' 56.7"	
8	UNK250-294	Opolom		04° 32' 37" - 04° 33' 09"	007° 55' 5" - 007° 55' 34"	2015
9	AUB 295-329	Adiabo Ukanabio	Cross River	05° 02' 59" - 05° 02' 33"	008° 16' 36" - 008° 17' 07"	2016
10	ESG 330-364	Esighi		004° 54' 20" - 004° 55' 43"	008° 26' 43" - 008° 27' 16"	2016

At each location, five samples were collected each at 1- 75m, 76-150m, and 151- 225m, 226-300m, 301-375m, 376-450m and 451-525m from the shoreline to the inland. No *Rhizophora* species was observed beyond the 525m distance except in Nembe where it was observed at 604m (hence an extra five samples were collected between 526-604m).

Pollen Collection & Storage

Collected pollen samples were labeled and stored in vials/sample bottles containing glacial acetic acid (GAA) for preservation prior to laboratory analysis [\(reference is missing\)](#).

POLLEN SAMPLE PREPARATION

The widely accepted method of pollen analysis by Erdtman (1960) as adopted by Moore *et al.* (1991) was used. The obtained anthers were crushed with a glass rod, and the debris removed with a needle to release the pollen grains. Glacial acetic acid (GAA) was used to transfer the crushed anthers into plastic test tubes and centrifuged for about 15 minutes at 5,000 revolution per minute (RPM) [at what temperature?](#) The centrifuged samples were decanted. The residues were washed three times with distilled water, [each time, centrifuged and decanted](#). Samples were acetolysed [according to as per](#) Erdtman (1960). The acetolysed mixture (9 part acetic anhydride and 1 part sulphuric acid) was added to the samples, and water bathed at 84°C for 10 minutes. The heated samples were centrifuged and washed with distilled water three times, each decanted to remove the acetolysed mixture. The residues were transferred into sterile vials. Glycerine jelly was added to the prepared samples giving a ratio of 50 part sample: 50 part glycerine.

Mounting and Photomicrography

Comment [MS3]: Why all words were written in upper case???

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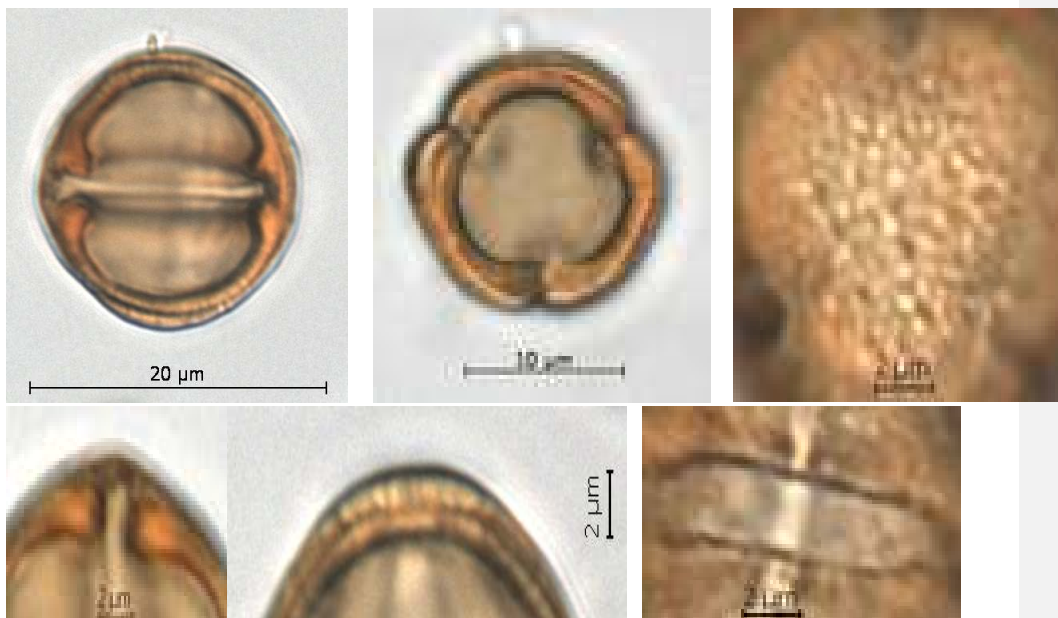
The prepared samples were pipette into a clean glass slides, covered with slid and sealed using a transparent nail hardener. The prepared pollen samples were properly examined under light microscope (AmScope microscope with X100 magnification). Photograph of the prepared pollen samples were taken with the aid of AmScope MA1000 camera with an in-built micrometer for measurement. Permanent slides of the prepared pollen samples were deposited in the Department of Botany, University of Calabar-Calabar.

Various quality assurance protocols were observed.

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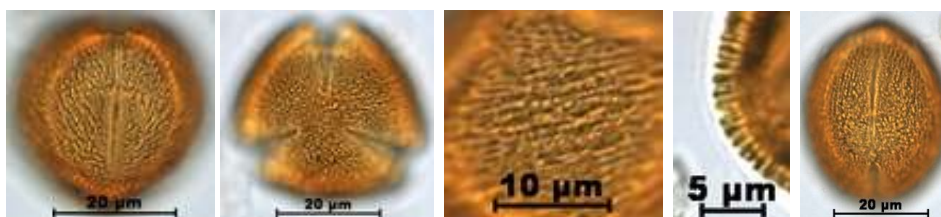
Result

Five different shapes of the tricolporate pollen were observed as shown in plates 1-5 and table 2



Equatorial, polar, surface ornamentation, special features, wall, aperture

Plate 1:- Pollen characters for group 1



Equatorial View

Polar View

Surface Pattern

Wall

Aperture



Unique Features

Plate 2: Pollen Characters of Group 2



Equatorial View
Aperture

Polar view

Surface Pattern

Wall



Unique Features

Plate 3: Pollen Characters of Group 3



Plate 4: Pollen Characters of Group 4



Plate 5:- Pollen Characters characters of Group-group 5

Table 2:- Summary of Pollen-pollen characters in the Genus-genus Rhizophora

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OTU	Pollen Morphology	Surface Pattern	Equatorial Size			Polar Size			Equatorial Shape	Polar Shape	Arrangement
			Min	Max	Mean	Min	Max	Mean			
1	Tricolporate	Reticulate	17.2	19.3	16.4	14.7	16.2	15.5	Sub prolate	Circular	Monad
2	Tricolporate	Baculate	32.9	38.6	34.86	28.1	31.6	29.15	Prolate	Triangular (convex)	Monad
3	Tricolporate	Striate, Rugulate	23.5	27.5	25.0	21.0	25.0	24.0	Oblate	Triangular	Monad
4	Tricolporate	Gemmate	27.2	34.3	30.2	22.8	27.8	24.6	Oblate	Circular	Monad
5	Tricolporate	Reticulate	18.6	20.2	15.4	26.4	29.2	27.9	Prolate	Trilobate	Monad

As evident across plate 1-5 and table 2, the basic pollen type in the genus is Tricolporate. However four different surface patterns were observed. They are reticulate, baculate, striate rugulate and gemmate. The equatorial shape on the other hand ranged from sub prolate to prolate to oblate as against triangular, circular and trilobate for the polar shape. The grain arrangement across the samples was uniform, Monad.

Discussion

Table 3 shows the set of pollen data to be transformed.

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Table 3:- Pollen data for transformation

Species	1	2	3	4	5
Pollen Character					
Polar size (µm)	15.5	29.15	24.0	24.6	27.9
Equatorial size (µm)	16.4	34.86	25.00	30.2	28.1
P/E ratio (µm)	0.95	0.84	0.96	0.81	0.99
Number of Apertures	3	3	3	3	3

Normalization of the data in table 3 was carried out as shown in table 4 preparatory to application of cluster analysis.

Table 4:- Data Standardization-standardization

Species/Pollen character	1	2	3	4	5
Polar	1.19	1.46	1.38	1.39	1.45
Equatorial	1.21	1.54	1.40	1.48	1.45

P/E	-0.02	-0.08	-0.02	-0.09	-0.01
Aperture	0.48	0.48	0.48	0.48	0.48

As could be seen in table 4, the transformed values ranged from negativity in the P/E ratio to positivity in the other three parameters. Similarity and distance among the five operational taxonomic units was obtained using Euclidean index. The result is as shown in table Table 5.

Table 5:- Euclidean index of Similarity and Distances among Five OTUs

Taxonomic Units	OTU 1	OTU 2	OTU 3	OTU 4	OTU 5
OTU 1	0	0.43052	0.26382	0.33963	0.34666
OTU 2	0.43052	0	0.17703	0.097803	0.11924
OTU 3	0.26382	0.17703	0	0.11088	0.083857
OTU 4	0.33963	0.097803	0.11088	0	0.10753
OTU 5	0.34666	0.11924	0.083857	0.10753	0

Principal Component Analysis to depict the relationship of the five OTUs is shown in fig-Fig. 2

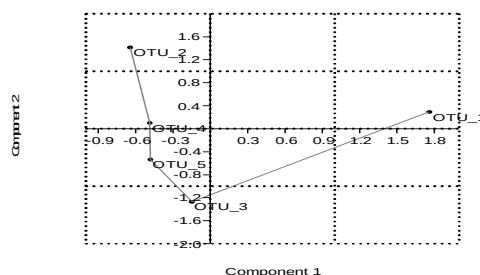


Fig 2:- Principal Component Analysis

As shown in table-Table 5 and exemplified in fig-Fig 2, the summed distance between OTU 1 and the other OTUs was 1.3807, as against 0.8245, 0.6356, 0.6559 and 0.6570 for OTUS 2, 3, 4 and 5 respectively. The percentage dissimilarity among the OTUs are shown in table 6.

Table 6 :- Percentage Dissimilarity Index among Rhizophora OTUs

OTUs	% Dissimilarity
1 & 2	55.62
1 & 3	74.51
1 & 4	72.48
1 & 5	72.37
2 & 3	18.89
2 & 4	16.86
2 & 5	16.75
3 & 4	2.03
3 & 5	2.14
4 & 5	0.11

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Statistical ~~analysis using~~ analysis using WINKS SDA 6 revealed a $p < 0.001$ at 0.05 confidence interval. The analysis further revealed;

- Significant differences between OTUs 1 and 2, OTUs 3 and 4, OTUs 3 and 5 and OTUs 4 and 5.
- No significant difference between OTUs 1 and 3, OTUs 1 and 4 and OTUs 1 and 5.
- No significant difference between OTUs 2 and 3, OTUs 2 and 4 and OTUs 2 and 5.

These findings are graphically shown in fig 3

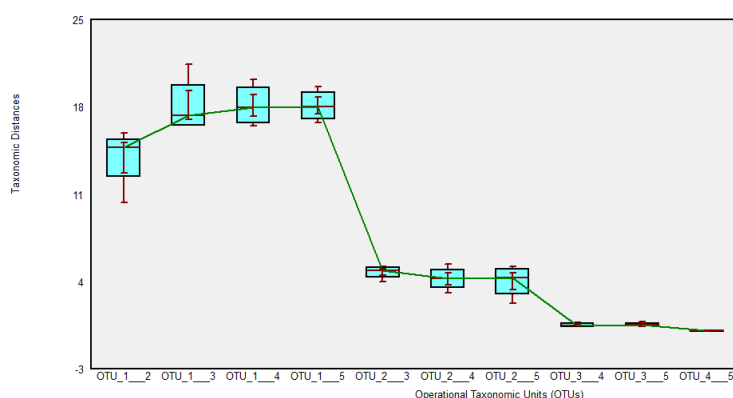
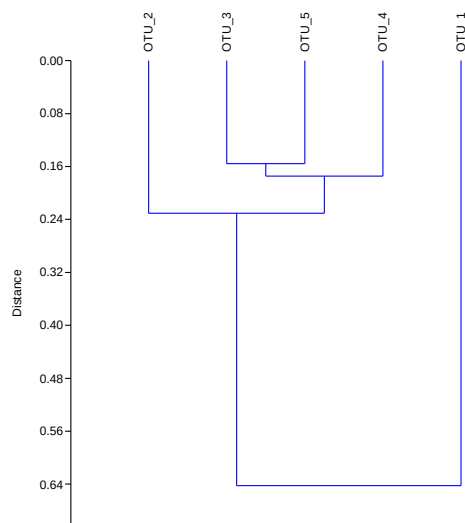


Fig 3.÷ Graphical ~~illustrations-illustrations~~ of Taxonomic-taxonomic Relationships-relationships among ~~Studied-studied~~ OTUs

Based on this study, four ~~possible-deductions~~ possible deductions could be made. Subject to other taxonomic lines of evidence, OTU 1 may represent a distinct species so do OTU 2. OTU 3, 4 and 5 though distinct from each other could represent subtypes of either OTU 1 or OTU 2. This suggestion was further strengthened by result of the cluster analysis shown in fig 4

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Fig 4: Cluster Analysis (from using Past Software (version?))

Lewis, 2000 reported controversies on the mangrove types in Nigeria suggesting the possible existence of more than three that are often quoted but confirmed the presence of *racemosa* and *mangle* and to a little extent *harisonii*. Albert and Ekine, 2012 and Ebigwai and Akomaye, 2014 confirmed the presence of the three species in Rivers state but gave an indication of species type with distance from shoreline. While the report situated *racemosa* at the fringes of the water bodies, *harisonii* at the middle and *mangle* at the upper landward part. Hartoungh 1996 reported differences in the species along soil types. The report reserved the exclusive presence of *racemosa* on the silty shoreline, *harisonii* and *mangle* on the peaty soil and the saline soil supports the growth of scrubby *racemosa* and *harisonii*. Onwugbta-Enyi *et al.*, 2008 agreed with this categorization. It is inferred from the above reports that *Rhizophora* types differ with distances away from the shoreline as do soil types. This position strengthens the functionality of salinity differentials as a major criterion for *Rhizophora* species types. The findings of *harisonii* on peaty soil and also on saline soil need re examination. Peter and Sivasothi 2001 equated peaty soil with the different loamy soils in the textural triangle. In this study, three morphological indistinct *Rhizophora* samples were obtained on peaty-loamy soils as shown in plate 1.

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(A)

(B)

(C)

Comment [MS9]: Merge these photographs into a single plate

Plate 1:- *Rhizophora* in ~~Loamy loamy Soil soil~~ across the ~~Study study Areas areas~~ in Niger Delta

In other mangroves of the world, loamy or peaty soil as it may be called has been shown to support the presence of other types of *Rhizophora* species (Peter and Sivasothi 2001, Chandral et al 2011 and Galung et al., 2003).

Conclusion

It is ~~arguable~~ ~~from arguable from~~ this study that there may be two distinct *Rhizophora* species with three subtypes in Niger Delta.

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Recommendation

Samples and the permanent plots established for this study should be further examined for genetic, ~~anatomical, anatomical~~, morphological and phytochemical analysis. Such studies may reveal more interesting ~~finds findings~~. Soil analysis is also required as complimentary evidence.

Acknowledgement: is still missing

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Comment [MS11]: Use reference manager such as endnote or mendely for uniform reference formatting or format all the reference uniformly as per journals instructions

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