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Pollen Album of *Rhizophora* Members in Nigeria and Its Taxonomic Implications

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Authors' contributions

This work was carried out in collaboration between all authors. Author JKE designed the study, authenticated the pollen and wrote the manuscript. Author AAE collected the samples, author BLN proof read the final manuscript and author BAN performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Pollen samples of *Rhizophora* individuals in Nigeria Niger Delta were conducted to generate a pollen album. Samples were collected in permanent plots in Koko, Ogidigben (Delta State), Akakumama, Nembe (Bayelsa State), Olupiri-Epelema, Ugwede (Rivers State), Ikwe, Opolom (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 meters inland (maximum *Rhizophora* occurrence landward). The samples were prepared using standard Erdtmanian methods. The result showed the presence of five different shapes of 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

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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 OTU. 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.

Keywords: Rhizophora; taxonomy; mangroves; mangle; Niger Delta; Unical.

1. BACKGROUND

Mangroves as excellent candidates of productivity had long been established. They offer various ecosystem services such as shoreline stabilization [1,2], habitat, nursery and breeding ground for many fish species and other fauna [1,3-6]; 2001 [7,8]; wood for fuel wood, timber, poles, boats [4,9,10-13]. Mangroves also aids in the establishment of restrictive impounds that offer protection for maturing offspring, filtering and assimilating pollutants from upland run-off and stabilization of bottom sediments [14] among other products. The common characteristics they all posses 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 [15]. Mangrove habitat is found along the coastlines of Nigeria. It straddles such states as Lagos, Ondo, Delta, Bayelsa, Rivers, Akwa Ibom and Cross River. [16] 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. Classed in the family *Rhizophoraceae*, 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 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. The size of the Nigeria mangrove was 997,700 ha prior to 2000 as against the current size of 240,400 ha in 2015 [16]. Worst still, the high rate of speciation in the tropics makes frequent species characterization inevitable. Creation of a pollen album is one of the first steps for further research in *Rhizophora* characterization.

Viewed against these backdrops, the enlarged research is aimed at characterizing *Rhizophora* species in Nigeria using anatomical, phytochemical, molecular, serological, morphological, and cytological and pollen information. However, to establish a pollen album of the different *Rhizophora* species existing in the Niger Delta and possibly to infer taxonomic relationships in this taxon is the subject of this write up.

2. 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.

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

2.1 Pollen Collection and Storage

Collected pollen samples were labelled and stored in vials/sample bottles containing glacial acetic acid (GAA) for preservation prior to laboratory analysis as prescribed by [17].

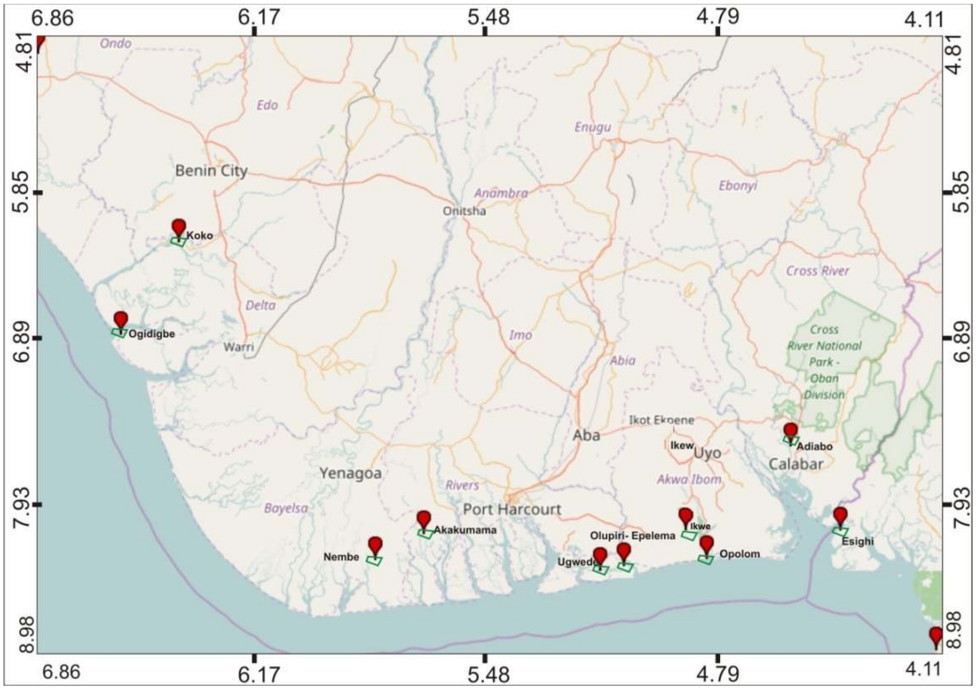

Fig. 1. Study area

Table 1. Pollen sample collection

S/N	Sample number	Location	State	Coordinates (UTM 32 in degrees, minutes and seconds) N	E	Height above mean sea level (m)	Year of collection
1	KOK 1-34	Koko	Delta	05^0 58' 33" -5^0 59' 03"	005^0 23' 119" - 005^0 24' 09"	12	2013
2	OGD 35-69	Ogidigben		05^0 23' 47" - 05^0 24' 29"	005^0 39'42" - 005^0 41' 13"	10	2013
3	OBN 70-104	Akakumama	Bayelsa	04^0 36' 29"- 04^0 37' 16"	006^0 10' 35" - 006^0 11'18"	4	2013
4	NEM 105-144	Nembe		04^0 37' 55" - 04^0 38' 29"	006^0 14' 46" - 006^0 15'28"	3	2013
5	0PM145-179	Olupiri-Epelema	Rivers	04^0 43 '35" - 04^0 44' 48"	007^0 18'49" - 007 19' 27"	13	2014
6	UGD180-214	Ugwede		04^0 40'05" - 04^0 41' 33"	007^0 22'16" - 007 23' 30"	10	2014
7	IKW215-249	Ikwe	Akwa Ibom	04^0 32' 08.6" - 04^0 33' 02.6"	007^0 54' 24.0" - 007^0 54' 56.7"	16	2015
8	UNK250-294	Opolom		04^0 32' 37" - 04^0 33' 09"	007^0 55' 5" - 007^0 55' 34"	8	2015
9	AUB 295-329	Adiabo Ukanabio	Cross River	05^0 02' 59" - 05' 02' 33"	008^0 16' 36"- 008^0 17' 07"	10	2016
10	ESG 330-364	Esighi		004^0 54' 20" - 004^0 55' 43"	008^0 26'43" - 008^0 27' 16"	5	2016

2.2 Pollen Sample Preparation

The widely accepted method of pollen analysis by [17] as adopted by [18] 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 room temperature. The centrifuged samples were decanted. The residues were washed, centrifuged, decanted and rinsed with distilled water three times. Samples were acetolysed per [17]. The acetolysed mixture [9] part acetic anhydride and 1 part sulphuric acid) was added to the samples, and water bathed at 84℃ 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.

2.3 Mounting and Photomicrography

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 as outlined in [17] were followed.

2.4 Statistical Analyses

Winks SDA version 6 and PAST software version 2 were used to calculate significant difference and cluster analysis (Principal Component Analysis and dendrogram) respectively.

3. RESULTS

The pollen characters for the 364 samples yielded five different shapes of the tricolporate pollen. These shapes shown in Figs 2-6 and Table 2.

As evident across Figs 2-6 and Table 2, the basic pollen type in the genus is Tricolporate. However four different surface patterns were observed. They are reticulate, baculate, straite regulate and germate. 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.

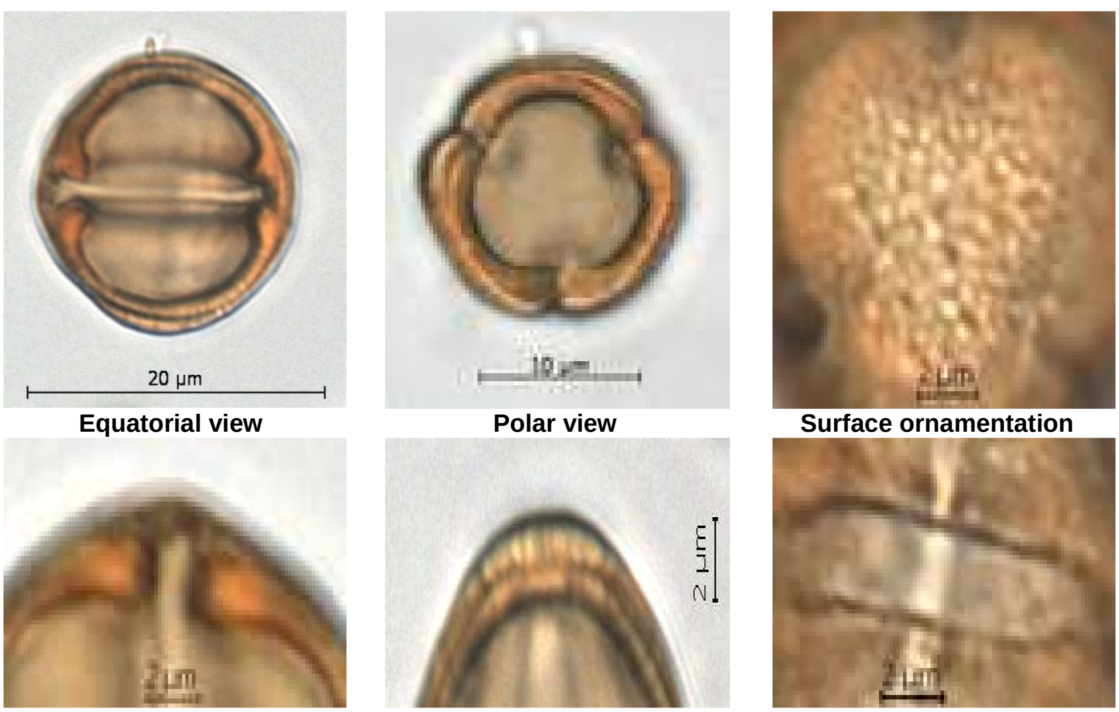

Fig. 2. Pollen characters for group 1

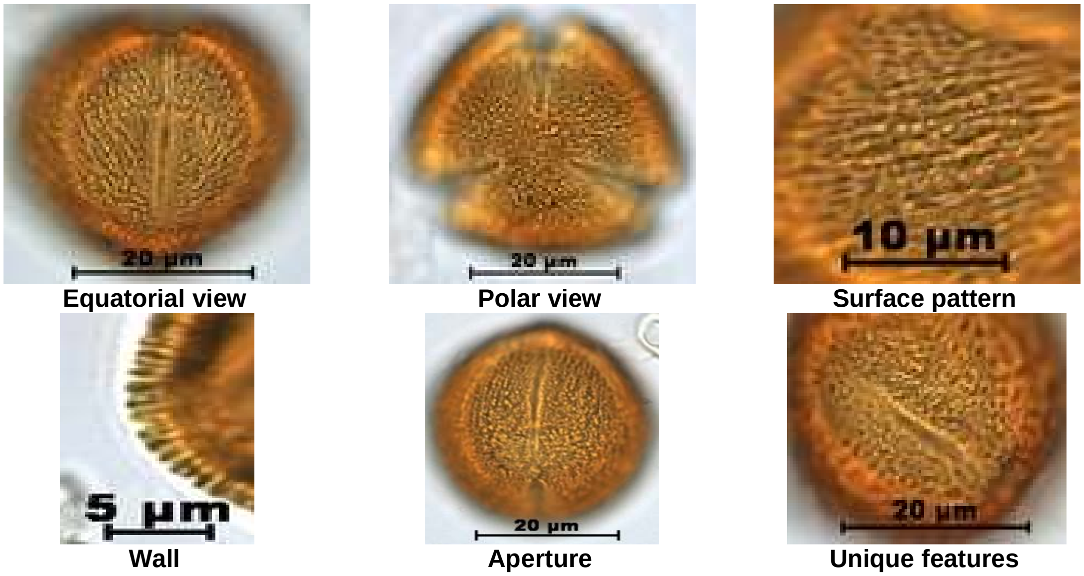

Fig. 3. Pollen characters of group 2

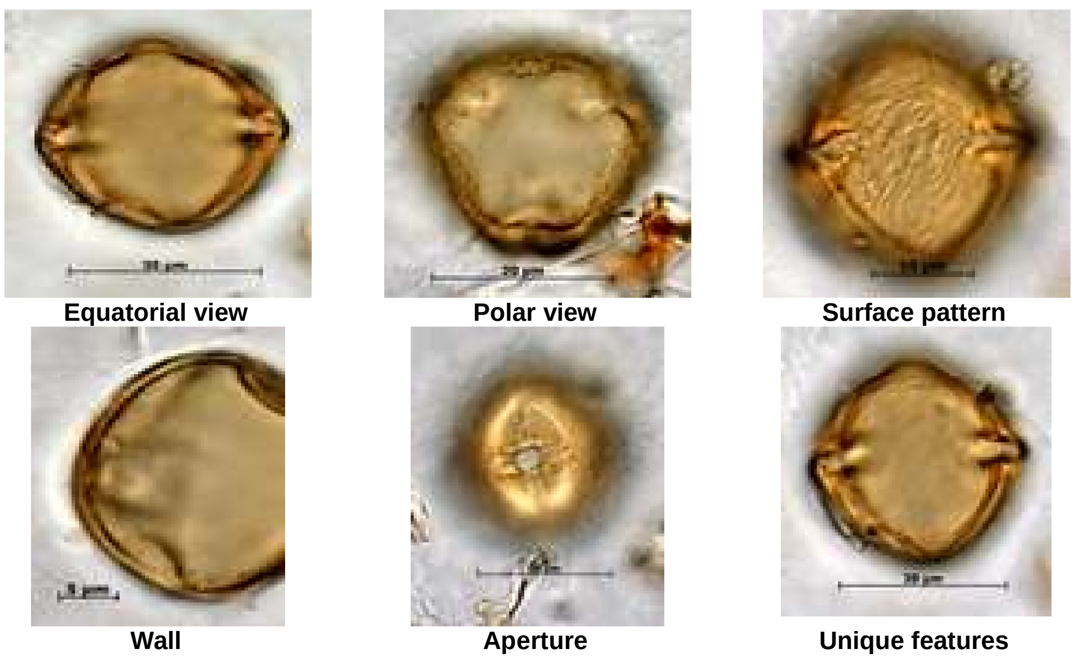

Fig. 4. Pollen characters of group 3

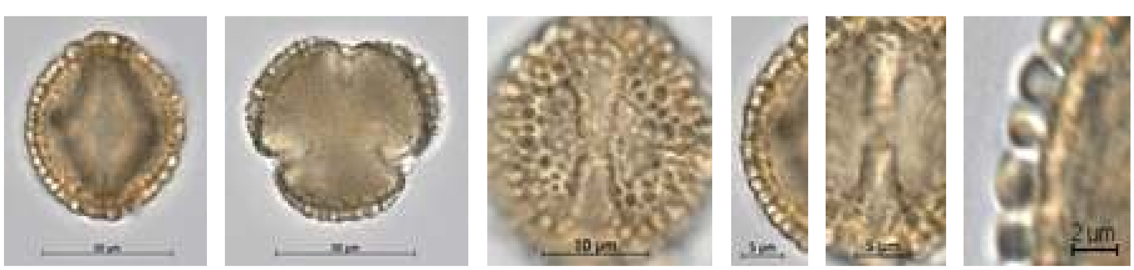

Fig. 5. Pollen characters of group 4

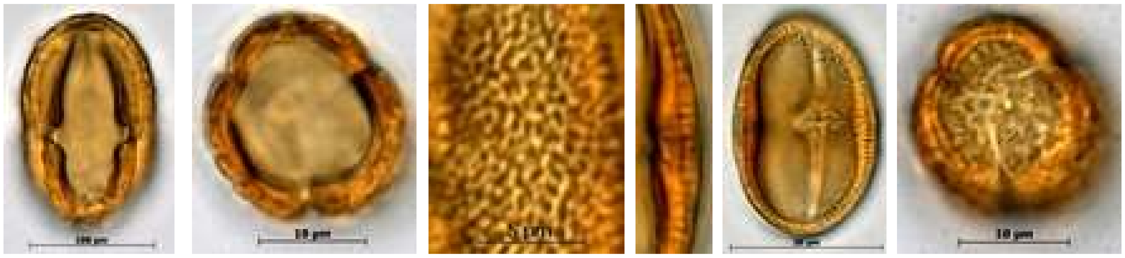

Fig. 6. Pollen characters of group 5

Table 2. Summary of pollen characters in the genus *Rhizophora*

OTU	Pollen morphology	Surface pattern	Equatorial size			Polar size			Equatorial shape	Polar shape	Grain 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

4. DISCUSSION

As could be seen in the result, the pollen dimensions showed a polar size range of 15.5 µm in pollen shapes 1 to 29.15 µm in pollen shapes 2. Similar trend was observed in the equatorial size. However, the polar to equatorial ratio (0.81) was smallest in pollen shapes 4 and largest (0.99) in pollen shapes 5. [19,20] recorded similar P/E ratio for *Rhizophora* species in Peninsular Malaysia. Result of data normalization is shown in Table 3.

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 as shown in Table 4.

Principal Component Analysis to depict the relationship of the five OTUs is shown in Fig. 7.

As shown in Table 4 and exemplified in Fig. 7, 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 is shown in Table 5.

Table 3. Data standardization for cluster analysis

Species/Pollen character	1	2	3	4	5
Polar Diameter	1.19	1.46	1.38	1.39	1.45
Equatorial Diameter	1.21	1.54	1.40	1.48	1.45
P/E	-0.02	-0.08	-0.02	-0.09	-0.01
Number of Aperture	0.48	0.48	0.48	0.48	0.48

Table 4. Similarity and distance indices among the 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

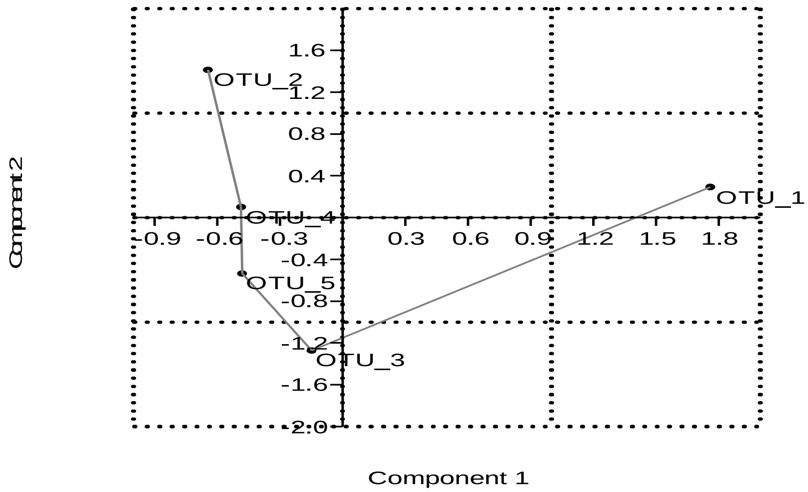

Fig. 7. Principal component analysis

Table 5. 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

Statistical analysis revealed a p<0.001 at 0.05 confidence interval. The analysis further revealed;

- Significant differences between OTUs 1 and 2, 0TUs 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. 8.

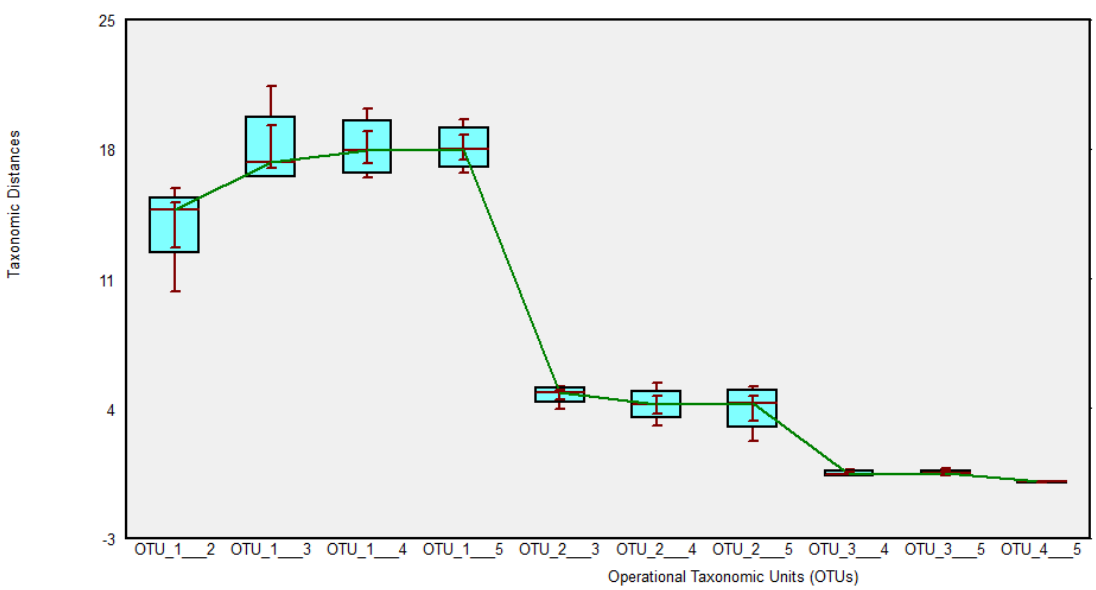

Fig. 8. Graphical illustrations of taxonomic relationships among studied OTUs

Based on this study, four 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. 9.

[21] 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*. [22] and [23] 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. [24] 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*. [25] 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. [26] 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 Fig. 10.

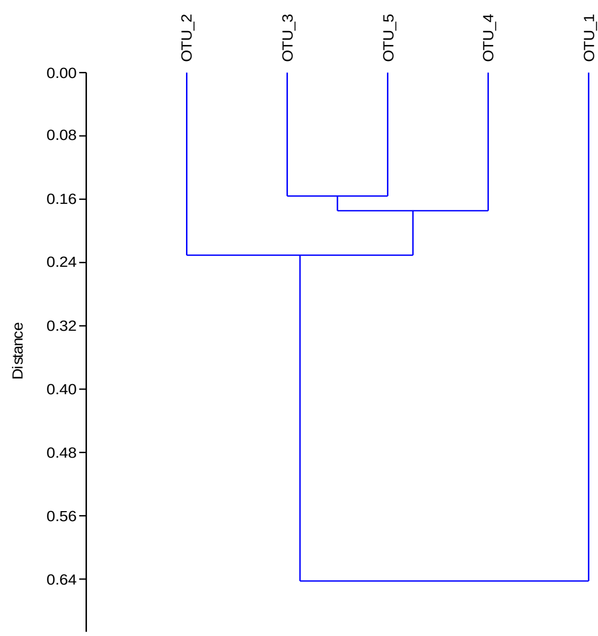

Fig. 9. Dendrogram depicting relationships among the OTUs

(A) **(B)** **(C)**

Fig. 10. *Rhizophora* in loamy soil across the study 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 [27,28].

5. CONCLUSION

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

6. RECOMMENDATION

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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