Global Research in Environment and Sustainability – ISSN 3033-3644
April 2024, Vol. 2, No. 4, pp. 20-34.

Genetic Diversity of Mangrove and Nypa Palm Species in the Niger Delta, Nigeria

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Abstract:
Mangroves and Nypa palm are two dominant coastal vegetation in the Niger Delta that are sources of numerous ecosystem services. We thus hypothesized that both species are identical by descent. We began the study by randomly collecting fresh leaf samples of Nypa palm in a mixed forest comprising Nypa palm and mangrove at a coastal community called Eagle Island. We put the leaves samples in zip lock bags containing silica gel crystals and transported them to the laboratory, where the DNA of the leaves was extracted. A quick-DNA plant/seed miniprep kit (Zymo-research Lab, California, USA). A total of six primer pairs were used for the amplification, namely three microsatellite markers and three universal primers. Fragments of the samples were sequenced using the Ninagen, Brilliant DyeTM Terminator Cycle Sequencing kit V3.1 BRD3-100/1000. All three samples of the nypa palm were amplified, and the BLAST prediction tool was used to identify the species. Our results show that of the three samples sequenced (NI, N2, and N3), the N1 sample was identified as Nypa fruticans with 100% identity (Accession Number is KT312925.1). In contrast, the others were the new strains identified in the study area, namely N2, identified as Astrocaryum aculeatum with 98.95% identity (Accession Number is NC 044482.1), and N3, identified as Astrocaryum aculeatum with 99.21% (Accession Number is 044482.1). The tracing of the origin of A. aculeatum reveals that it originated from Brazil in the Amazon region.

INTRODUCTION

Genetic diversity studies of nypa palm in the Niger Delta are limited, making it challenging to determine nypa palm's phylogeny and evolution (Numbere, 2021). The palms' buoyancy and floatation capability have made them undergo transboundary migration worldwide. Studies reveal that the Nypa palm was deliberately introduced from Indonesia to the Niger Delta region to prevent coastal erosion (Numbere, 2018) but became an invasive species (Numbere, 2019; Numbere, 2020; Numbere & Moudingo, 2023). Since arriving in the Delta, they have spread into areas formerly occupied by the mangroves. Thus, there is a likelihood of a genetic linkage between species in Nigeria and Indonesia, but there are few or no studies to show this linkage.

Nevertheless, previous studies have shown that genetic distribution does occur between organisms in different geographical regions (Avis, 2000; Hickerson et al., 2010) through the use of microsatellites, which are genetic markers used to construct genetic maps and trace the origin of individuals (Stefanini et al., 2023). Different genetic markers have been used to show genetic diversity between and among populations (E.g., Das and Ghose, 20023; Adhuri et al., 2006). Similarly, gene genealogies involve spatial distribution of alleles and population divergence (Zhou et al., 2023) in different regions. Similarly, the coalescent theory reveals that hierarchical gene tree branching shows organismal pedigree, and all extant lineages coalesce to a common

genealogy (Blischak et al., 2023). The ecology, structure, and function of the mangroves and nypa palms of the Niger Delta have been extensively studied (Nwobi et al., 2020; Zabbey et al., 2021; Numbere, 2021), and reveal that the mangroves of the Niger Delta originated from the Tethys Sea in the present Atlantic Ocean before the continental drift which separated the continents (Saenger, 1998; Spalding, 2010).

Nypa palm is from the family Palmae (*Arecaceae*) (Lim et al., 2022). It resembles the oil palm (*Elaeis guineensis*) tree, which grows in upland soil. Nypa palm is uniquely aquatic and grows in mudflat and intertidal regions of the marine environment (Dos Santos et al., 2015). There is no widespread use of the palms in the Niger Delta region, which is why it is regarded as a useless species. Conversely, Mangroves have numerous uses, such as producing firewood, thatch for building, scaffolding for construction, and being a haven for biodiversity. In the last 40 years, the palms have aggressively invaded and encroached into over 70% of mangrove territories. At the same time, the remaining 30% are under threat of being overrun by the palms. The invasiveness of the palms is facilitated by their harsh outer coat, which makes them withstand complex environmental conditions. In addition, human activities that pollute the marine environment have caused the proliferation of palms to the detriment of the mangroves. Oil and gas exploration, urbanization, industrialization, and coastal waste disposal facilitate Nypa palm spread. Therefore, this study aims to determine the phylogenetic relationship between mangroves and nipa palms. Our objectives are thus, (1) to identify the mangrove and nypa palm species within the study area; (2) to find out the ancestry of mangrove and nypa palm in the Niger Delta; and (3) to draw a phylogenetic tree of mangrove and nypa palm species.

MATERIALS AND METHODS

Description of the Study Area

The study was carried out on a section of a deforested and sand-filled mangrove forest at Eagle Island Niger Delta (N04°47; E6°58). The area is surrounded by chocolate-brown swampy soil bordering a river course used for boat transportation.

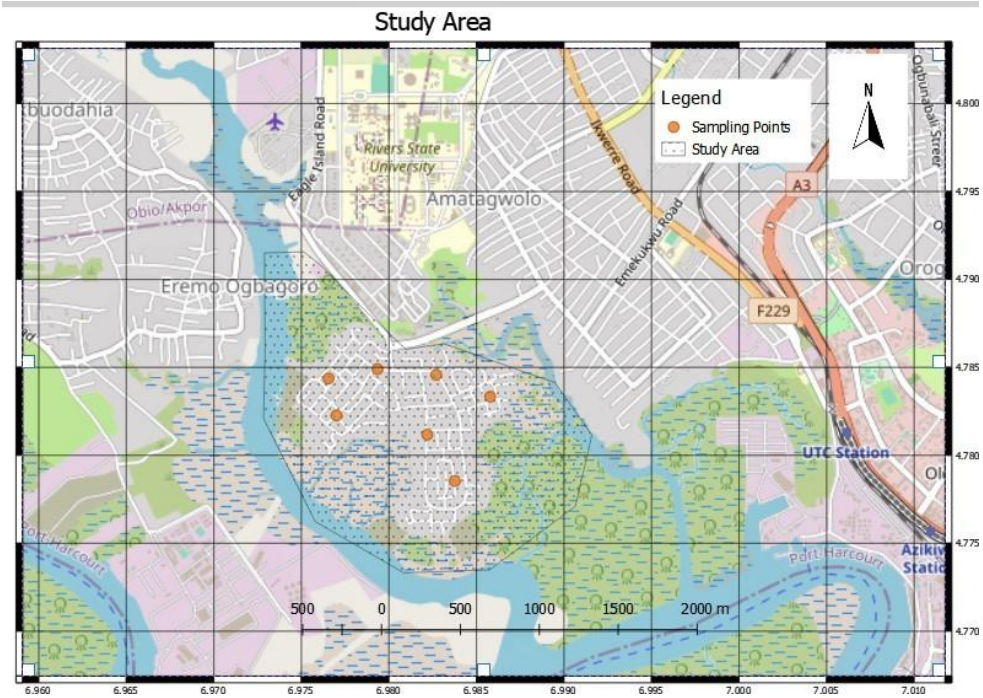

Figure 1: Map of the study area at Eagle Island, Niger Delta Nigeria

During high tide, the river flushes waterborne plastic waste, which gets trapped in the grassy sand-filled area. The sandy area has a combination of mangrove and non-mangrove species. The primary species found in the area are red (Rhizophora racemosa), black (Avicennia germinans), and white (Laguncularia racemosa) mangroves, while the non-mangrove species are dominated by grasses, e.g., Mariscus longibracteatus (Numbere, 2020; Numbere, 2018). Some Nypa palm species (Nypa fruticans) are also growing in the sand-filled area. There are also numerous fiddler crab (Uca tangeri) burrows on the sandy beach. The soil is slightly alkaline, with a pH of 7.5. The temperature of the soil is 26.1±0.01, the salinity is 1.16ppt, and the TDS is 360×10 ppm. The area has two seasons, the wet and the dry seasons. The dry season occurs from November to March, while the wet season is between March and October yearly (Numbere & Camilo, 2018).

Sample Collection

Sample Codes were used for easy identification of the study samples as follows: (Black Mangrove: B1, B2, and B3. Nypa Palm: N1, N2, and N3. Red Mangrove: R1 and R2. White Mangrove: W1 and W2). Leave samples were collected from Eagle Island in the Niger Delta in a random fashion (Figure 4B). All 20 samples were collected from mangrove (red (*Rhizophora* spp), black (*Avicennia germinants*) and white (*Laguncularia racemosa*)) and Nypa palm (*Nypa fruticans*) stands close to the river (i.e., ~1 meter from the river). The samples were put in a zip lock bag measuring 16.5 cm × 16.5cm and taken to the laboratory, where silica gels were put in the bag to maintain the integrity of the samples before being transported to the Center for Molecular Biosciences and Biotechnology of the Michael Okpara University of Agriculture in collaboration with the Department of Animal and Environmental Biology, University of Port Harcourt, Nigeria.

DNA Extraction

Deoxyribonucleic Acid (DNA) was extracted from the samples (*n* =10) using the quick-DNA plant/seed miniprep kit (Zymo-Research Laboratory, California, USA) by following the manufacturer's protocol.

Polymerase Chain Reaction (PCR)

A total of six primer pairs that included three (3) microsatellite markers (Agerm1-14F/Agerm1-14R, M47F /M47R, and Aa22F/Aa22R) and three universal primers (RBCL1/ATP, RBCL1/FLIF, and one unnamed primer pair, i.e., X and Y) were used for the amplification. However, only four (Agerm1-14F/Agerm1-14R, RBCL1/ATP, RBCL1/FLIF, and the unnamed primer) out of the six primers produced results. Three (3) primers (Agerm1-14F/Agerm1-14R, RBCL1/ATP, and RBCL1/FLIF) gave results for the six (6) samples, B1, B2, B3, N1, N2 and N3, while the remaining four samples, R1, R2, W1and W2, were amplified using the unnamed universal primer pair as they could not be amplified by the other primers listed. The primers could not amplify some samples because they (i.e., primers) could not bind to the template DNA of those samples. These primers and their sequences were selected from different literatures and then synthesized by Inqaba Biotec, West Africa (e.g., Mori et al. 2010; Craig et al., 2020). Details of the primer sequences used for this study are given in Table 1.

Table 1: Primer name and sequences used to amplify mangrove and nypa palm samples collected at Eagle Island, Niger Delta, Nigeria.

No	Primer	Sequence	Samples Amplified
1	M47F M47R	CCAATTGTGTCGTCCTTTTA AGCCTTACTTTTCCTTTGT	Nil
2	Aa22F	TCCCATTTGCATTACAGTCTG	Nil

	Aa22R	CGAGCGTGTGCTAATCTTCC	
3	Agerm1-14F Agerm1-14R	CCAATTGTGTCGTCCTTTTA AGCCTTACTTTTCCTTTGT	B2, B3, N1, N2, N3
4	RBCL1 ATP	GTAAAATCAAGTCCACCGCG ACATCKARTACKGGACCAATAA	B3, N1
5	RBCL1 FLIF	AACACCAGCTTTRAATCCAA CCACAAACAGAGACTAAAGC	B1, B2, N2, N3
6	X_Forward Y_Reverse	GTAGTCATATGCTTGTCTC GAAACCTTGTTACGACTT	W1, W2, R1, R2

PCR Amplification

The target region(s) for each of the primer set used were amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) following the calculations presented in Tables 2 and 3. The reaction mix(es) were then subjected to specified thermal cycling conditions using the Eppendorf Master cycler nexus gradient 230 (Table 3). The primers were grouped into three sets: 1 (a). X_Forward–GTAGTCATATGCTTGTCTC and (b). Y_Reverse– GAAACCTTGTTACGACTT), 2 (a). RBCL1 – AACACCAGCTTTRAATCCAA and (b) ATP - ACATCKARTACKGGACCAATAA) and 3 (a) RBCL1 – AACACCAGCTTTRAATCCAA and b. FLIF – CCACAAACAGAGACTAAAGC) for amplifications. For PCR amplification primer 2 was amplified as shown in Table 2 while primer 3 was amplified as shown in Table 3. The thermal cycling condition for sets 1 and 3 are the same apart from the difference in annealing temperature where sets 1 and 2 were annealed at 45°C and set 3 at 50°C respectively. Similarly. Microsatellite primer (Agerm 1–4) thermal cycling condition was like sets 1 and 2. Primers of set 2 was amplified as shown in Table 2, while primer 3 was amplified as shown in Table 3.

Table 2: Different primer components and volumes used for amplification of set 2.

Component	**Volumes for a 12.5µL reaction**
Template DNA	2 µL
10µM Forward Primer	0.25µL (10nM)
10µM Reverse Primer	0.25µL (10nM)
One Taq Quick Load 2X Master Mix with Standard Buffer	6.25µL
Nuclease free water	3.75 µL

Table 3: Different primer components and volumes used for amplification of set 3.

Component	**Volumes for a 12.5µL reaction**
Template DNA	4 µL
10µM Forward Primer	0.25µL (10nM)
10µM Reverse Primer	0.25µL (10nM)
One Taq Quick Load 2X Master Mix with Standard Buffer	6.25µL
Nuclease free water	1.75 µL

The thermal cycling condition for sets 1, 2, and 3 are the same apart from the different annealing temperatures where sets 1 and 2 were annealed at 45°C and set 3 was annealed at 50°C.

In the same vein, the microsatellite primer, Agerm1-14 thermal cycling condition was like sets 1 and 2. The thermal cycling conditions for the different stages of PCR and the time of the reaction is shown in Table 4. Additionally, steps 1-3 were multiplied by 35.

Table 4: Thermal cycling condition for sets 1 and 2.

Step	Stage of PCR	Temperature (°C)	Time (min: secs)
1.	Initial Denaturation	95	5:00
2.	Denaturation	95	0:30
3.	Annealing	45/50	1:00
4.	Extension	68	1:30
5.	Final Extension	68	5:00
6.	Hold	4	Hold

Gel Electrophoresis

After PCR amplification, 2µl of each PCR product was run on 1% agarose gel prepared with 1× TAE buffer and stained with Safe View Red (5µl) reagent. Electrophoresis was carried out at 100V for 40 mins. The resulting gel image was photographed using a gel documentation system (E-BOX, Vilber Lourmat, Italy, Serial number 21-0451).

Post-PCR Purification

PCR products were cleaned using an enzymatic method (ExoSAP) as follows:

1) The ExoSAP master mix was prepared by adding the following to a 0.6ml micro-centrifuge tube: (a) 50µl of 20U/µl Exonuclease I (Catalogue No. NEB M0293L); and (b) 200µl of 1U/µl of Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371).
2) The reaction mixture was prepared by mixing the following and incubating the resulting mix at 37°C for 15mins and at 80°C for 15mins: (a) Amplified PCR Product 10 µl; and (b) Exo SAP Mix (step 1) at 2.5 µl of reagent.

Sequencing

The leave sample fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions: https://www.nimagen.com/products/Sequencing/Capillary-Electrophoresis/BrilliantDye-Terminator-Cycle-Sequencing-Kit/. The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053): http://www.zymoresearch.com/downloads/dl/file/id/52/d4052i.pdf. The cleaned products were injected on the Applied Biosystems ABI 3500XL Genetic Analyser (Serial number 22309-040) with a 50cm array using POP 7: (https://www.thermofisher.com/order/catalog/product/4406016) and sequence data collected. The sequences were obtained in PDF and FASTA formats and then searched on the NCBI website.

Phylogenetic Analysis

Analyses were conducted using the Maximum Composite Likelihood model (Saitou and Nei, 1987). The differences in the composition bias among sequences were considered in evolutionary comparisons (Felsenstein, 1985). This analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (i.e., pairwise deletion option). There were a total of 1295 positions in the final dataset. Evolutionary analyses were done using MEGA11 (Tamura and Kumar, 2004).

Statistical Analysis

Pearson's product–moment correlation was done to compare whether there was any significant difference between percent genetic identity and distance from nypa palm stand. All analysis was done in *R* statistical environment 4.2.2 (R Development Core Team).

RESULTS

PCR Amplification

The PCR amplification result reveal that R1, R2, W1, and W2, were amplified using the universal primer set 1. However, they could not be amplified by the other primers earlier mentioned (Figure 2). Also, B1, B2, N2 and N3 produced amplification with the primer set 2 (RBCL1/FLIF), while B3 and N1 were amplified using primer set 3 (RBCL2/ATP). There was no attempt to amplify N1, N2, N3, B1, B2 and B3 with primer set 1 to ascertain if they could produce amplifications or not.

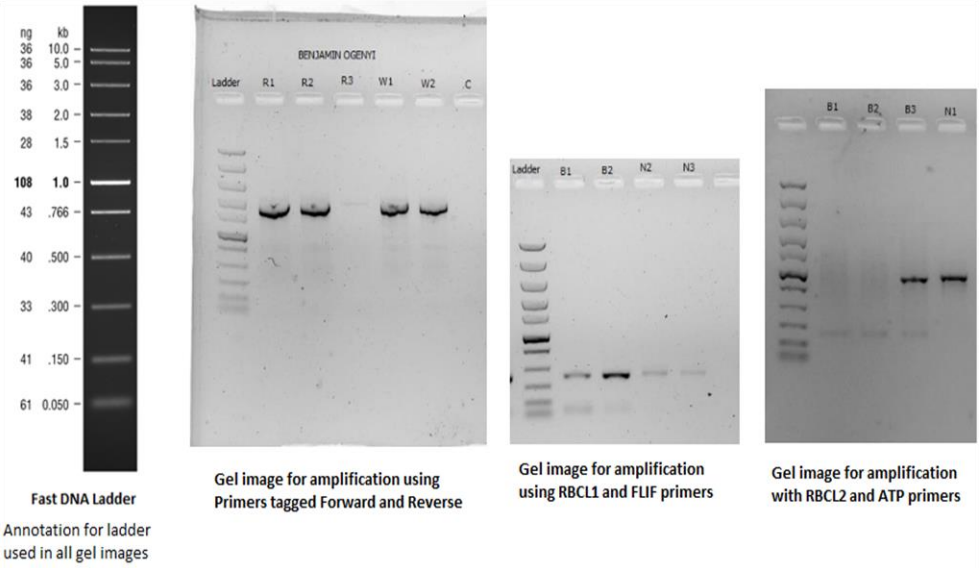

Figure 2: Gel Images obtained after PCR amplification using microsatellite primer sets 1, 2, and 3. Where N1, N2, N3 represent nypa palm samples; B1, B2, and B3 represent Black mangrove samples; R1, and R2 represent red mangrove samples; W1 and W2 represent white Mangrove samples.

The gel image reveals that B2, B3, N1, N2, and N3 were amplified as shown in Figure 3.

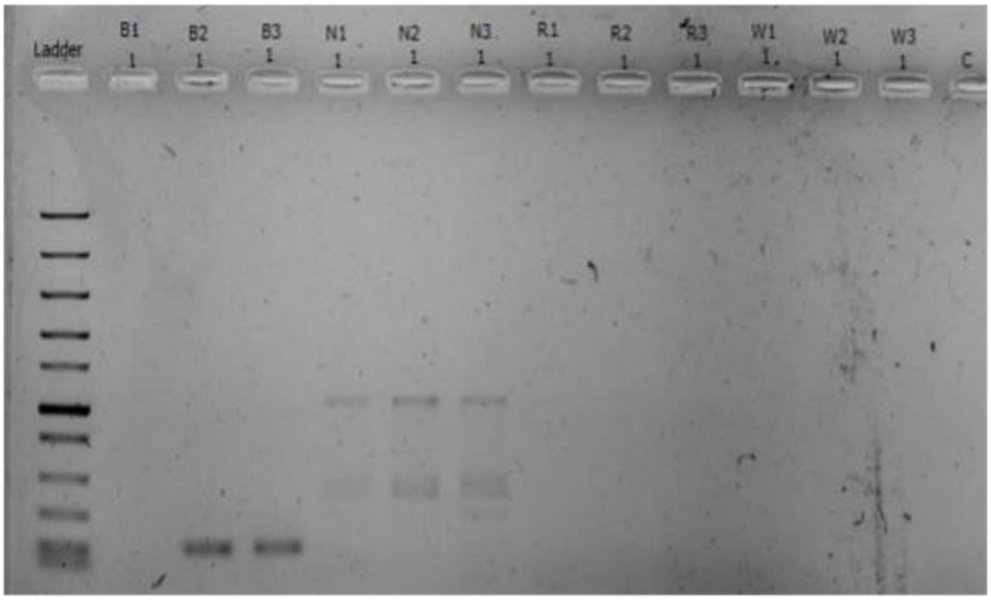

Figure 3: Gel Image amplification using primer pair Agerm1-14f and Agerm1-14r. The image reveals that B2, B3, N1, N2, and N3 were amplified.

Sequencing

The blast prediction reveals two species of black mangroves namely *A. officinalis*, and *A. marina* that has 98.42%–99.20% identity. Similarly, two species of nypa palms were identified namely: *N. fruticans* and *A. aculeatum*. that have 98.21%–100% identity. An interesting observation is the identification of a new strain of nypa palm species, *A. aculeatum*, which has not been reported in previous literatures in the region. Furthermore, only one species of red and white mangroves was identified as follows: *R. stylosa* (97%–98.47%) and *L. racemose* (92.68%–96.16%) among the leave samples collected at Eagle Island (Table 5).

Table 5: Summary of blast prediction for leave samples collected at Eagle Island, Niger Delta, Nigeria

S/N	Seq ID	Coordinates	Elevation (m)	Matched organism	% Identity	Accession number
1	B1	N04°47.318; E006°58.591	5.00	*Avicennia officinalis*	99.2	KP697352.1
2	B2	N04°47.317; E006°58.580	6.00	*Avicennia marina*	98.42	NC_047414.1
3	B3	N04°47.315; E006°58.597	6.00	*Avicennia marina*	98.68	NC_047414.1
4	N1	N04°47.274; E006°58.593	6.00	*Nypa fruticans*	100.00	KT312925.1
5	N2	N04°47.268; E006°58.593	6.00	*Astrocaryum aculeatum**	98.95	NC_044482.1
6	N3	N04°47.289; E006°58.593	6.00	*Astrocaryum aculeatum**	99.21	NC_044482.1
7	R1	N04°47.271; E006°58.505	5.00	*Rhizophora stylosa*	97.20	MZ735369.1
8	R2	N04°47.264; E006°58.501	5.00	*Rhizophora stylosa*	98.47	MZ735369.1
9	W1	N04°47.320; E006°58.599	4.00	*Laguncularia racemosa*	92.68	AY289635.1
10	W2	N04°47.322; E006°58.605	4.00	*Laguncularia racemosa*	96.16	AY289635.1

*New strain of nypa palm species identified in the study

Phylogenetic Analyses

The result of the phylogenetic analysis shows that both mangrove and nypa palm species are related and must have branched off into two clades thousands of years ago. The first clade has *Avicennia* and *Astrocaryum* species respectively, meaning both came from the same clade in the evolutionary history of mangroves in the Niger Delta (Figure 4). The second clade gave rise to white mangrove (*L. racemosa*) and red mangrove (*R. racemosa*) species.

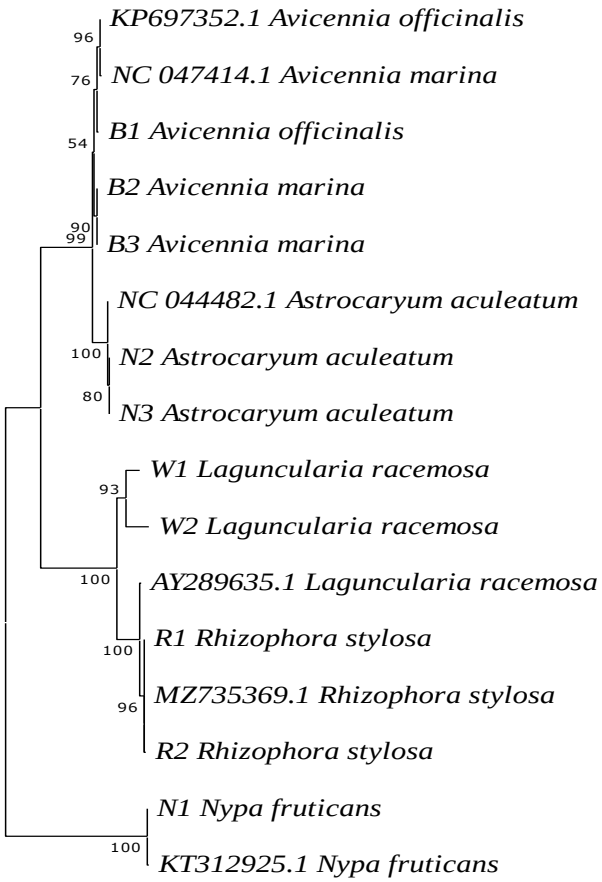

Figure 4: Original tree of mangrove and nypa palm species showing their evolutionary relationships in the Niger Delta Nigeria, Nigeria.

Based on the character formation via the bootstrapping using maximum likelihood analysis the red mangrove *R. stylosa* are related to the white mangrove, *L. racemosa*. Similarly, *N. fruticans* gave rise to the new species of palm, *A. aculeatum*, which are related to the black mangrove species i.e., *A. marina* and *A. officinalis* respectively (Figure 5).

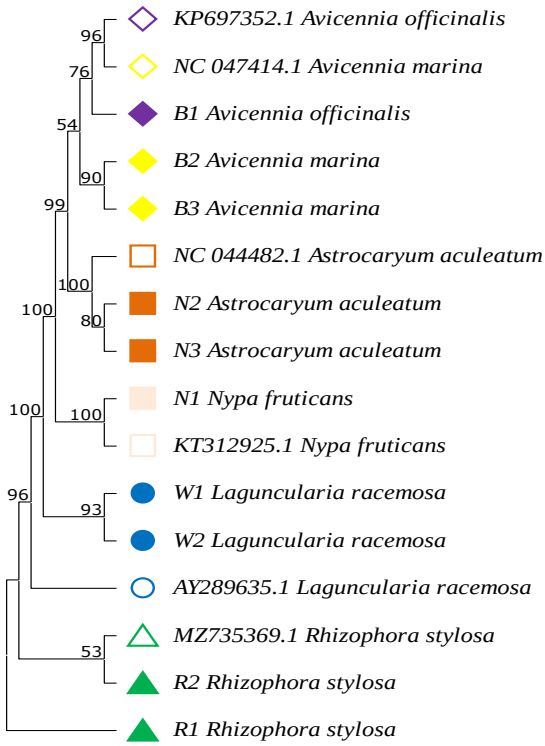

Figure 5: Evolutionary relationship of mangrove and nypa palm species using bootstrap consensus tree and consensus sequences (Appendix 1) in the Niger Delta Nigeria. The consensus tree uses the majority rule, while bootstrapping uses repeated characters at random. It shows the red mangrove (*R. stylosa*) as an originating species. Samples with the same accession numbers have the same color code.

Morphological Relationship

The boot strapping result indicates that in terms of phenotypic and ecological character traits the mangrove species (dicotyledon) are like the palms species (monocotyledon), but differ morphologically, which has made the palms to survive more in disturbed environment compared to the mangrove (Numbere, 2022).

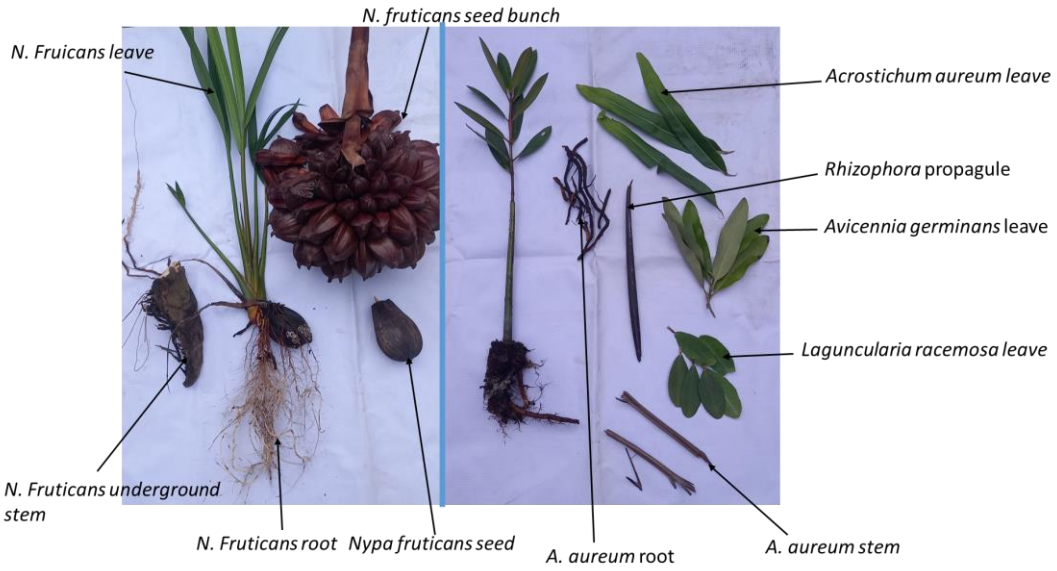

Figure 6: Morphological difference of Nypa palm (*N. fruticans*) and different mangrove species parts collected from Eagle Island, Niger Delta, Nigeria.

Figure 7: Tree stands of (A) Nypa palm and (B) mangrove at Eagle Island, Niger Delta, Nigeria Evolutionary relationships of taxa

There are a lot of differences in their anatomy and physiology like in their leave shape, seed shape, root, stem, and branch formations. For instance, the leaves of mangroves and nypa palm are not similar (Figure 6 and 7). The nypa palm species don't have stems and branches rather it has an underground stem compared to the mangroves that have above ground stems and branches.

The neighbor-joining method inferred the evolutionary history (Saitou & Nei, 1987). The percentage of replicate trees in which the mangrove and nipa palm species clustered together in the bootstrap test (1000 replicates) are shown next to each branch, as shown in Figure 4 (Felsenstein, 1987). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method (Tamura & Kumar, 2004; Tamura & Kumar, 2002). This analysis involved 16 nucleotide sequences. There were a total of 1295 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

Correlation Between Percent Genetic Identity and Distance from Nypa Palm Stand

There was little or no correlation between the percent genetic identity and distance from the Nypa palm stand (t = -0.34729, df = 8, p-value = 0.7373; cor = -0.1218688, Figure 8). However, the negative correlation sign indicates a tendency for the percent identity to drop with increasing distance from the nypa palm stand.

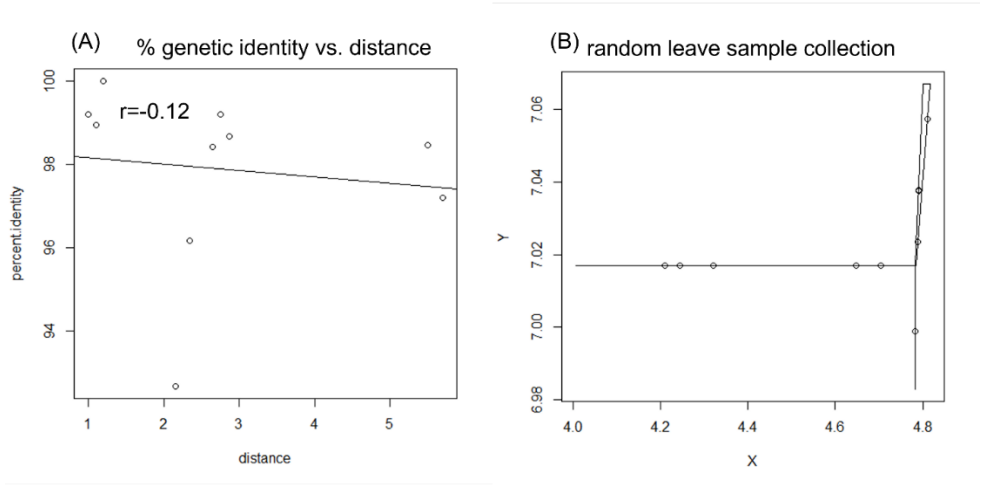

Figure 8: Graph of (A) Correlation of percent genetic identity and distance from Nypa palm and mangrove stands and (B) random leave collection design at Eagle Island, Niger Delta, Nigeria.

DISCUSSION

The nested clade phylogenetic analysis was done because it is a cladistic approach to identify sub-sets of associated species within geographic locations. Thus, the phylogenetic tree was done to show the relationship between nypa palm and mangrove species. The result shows that mangrove, a dicot, and Nypa palm, a monocot, originated from the same ancestor but branched off millions of years ago (E.g., Peng et al. 2014). Supporting this view is that mangrove and nypa palm species are of similar ecotypes, which are locally adapted populations but differ phenotypically and genetically. The palms and the mangroves survive in the same semi-aquatic environment (Figure 7). However, in the Niger Delta region, different selection pressures must have resulted in the divergence of the palms species (e.g., hybridization) into more than one species (i.e., *N. fruticans* and A. *aculeatum*), like in the mangroves that have over five different species. Mangrove and Nypa palm are ecologically similar but genetically and morphologically dissimilar (Figure 6) because, over the years, they have evolved to form new character traits with new identities. The results reveal that ten samples belong to six species of mangrove plants with different percentage identities (Table 5). For the black mangrove, two samples belong to the species *Avicennia marina* but with different percentage identities, while the remaining one belongs to the species *Avicennia officinalis.* Similarly, two samples of the nypa palm belong to the species *Astrocaryum aculeatum* but with different percentage identities, while the remaining one belongs to the species *Nypa fruticans.* Furthermore, two samples of the red mangrove belong to the same species, *Rhizophora stylosa*, though with different percentage identities. Finally, two samples of the white mangrove belong to the same species, *Laguncularia racemose*, but with different percentage identities.

Our results show that of the three samples sequenced (NI, N2, and N3), the N1 sample was identified as *Nypa fruticans* with 100% identity (Accession Number is KT312925.1). In contrast, the others were the new strains identified in the study, namely N2, identified as *Astrocaryum aculeatum* with 98.95% identity (Accession Number is NC 044482.1), and N3, identified as *Astrocaryum aculeatum* with 99.21% (Accession Number is 044482.1). Furthermore, the evolutionary relationship shows that *Nypa fruticans* was among the first generation (Clade 1) that gave rise to the sister species of *Astrocaryum aculeatum* several years ago in branch two of the fourth generation (Clade 4). Similarly, in the first branch of the fourth generation, there is another

sister species of the nypa palms: the two black mangrove species, namely *Avicennia officinalis* and *Avicennia marina*. Mangrove and Nypa palms have highly contrasting morphological differences but are halophilic and semi-aquatic. The situation in the Niger Delta and neighboring African countries shows that the palms are invasive species and spread and displace large populations of mangrove forests, which defy their shared ancestry and calls for more future research to determine their genetic makeup and phylogeography.

CONCLUSION

Our study shows that *N. fruticans* and *A. aculeatum* are identical and indistinguishable in the appearance of their leaves, seeds, branches, and growth but are genetically different. Morphologically, they also look alike and grow in the same environment. In contrast, the palms are morphologically different from the mangroves. Therefore, our result revealed that the palms arrived in the Niger Delta centuries ago and began branching out because of cross-fertilization and hybridization of their gametes, which made their genes vary, leading to the formation of a new species. Also, since the Niger Delta is linked to the Atlantic Ocean, near the equator, the *A. aculeatum* might have migrated from Brazil in Latin America to the coast of the Niger Delta. Lastly, this study has shown that the Nypa palm is not monospecific as previously thought but has a sister species called *A aculeatum* in the Niger Delta region.

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APPENDIX 1

Consensus Sequences

>N1_ *Nypa fruticans*

TGGAAACCACAGGACTAGAAGGAGTAGGATTGATTCTCATAATTATAATAAAGTGAAATATGTCGAAATTTTTTTTG
GAATAGTGCCATGTCAAATCGAAAATAAATGTCCGATAGCAAGTTGATCGGTTAATTCAATAAGAAAAGAAAAAAA
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TTTCAAGTTCAGTCAATTTTTTTTTTTCAAAATGTCAAGTAGATGAAATCGTGAGTAAGTGTGTTTCATTTCTCTATCA
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CAAAGGTTTCATTCACGCCTAATCCATATCGAGTAGACCTTGTCGTTGTGAGAATTCTTAATTCATGAGTTGTAGGGA
GGGGACC

>N2_ *Astrocaryum aculeatum*

CCACAAACAGAGACTAAAGCAAGTGTTGGATTYAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGA
CTACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAGGAAGCAG
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TACAAAGGACGATGCTACCACATCGAAACCGTTGTCGGGGAGGAAAATCAATATATTGCTTATGTAGCTTATCCTTT
AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAAGCTGGTG

>N3_ *Astrocaryum aculeatum*

CCACAAACAGAGACTAAAGCAAGTGTTGGATTYAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGA
CTACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAGGAAGCAG
GGGCAGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACTGATGGGCTTACCAGTCTTGATCGT
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AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAAGCTGGTG

>W1_ *Laguncularia racemosa*

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ACTACTCTGGATAAATTAGAGTGCTTAAAGCAATCCTACGCTCTGGATACTTGCGGAAGGGATGCATTTATTGGATT
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>W2_ *Laguncularia racemosa*

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>R1_ *Rhizophora stylosa*
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>R2_ *Rhizophora stylosa*
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>B1_ *Avicennia officinalis*
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>B2_ *Avicennia marina*
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