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Molecular identification of *Lentinus squarrosulus* and *Marasmius palmivorus* harvested from the stump of mangrove trees in the Niger Delta, Nigeria

Elijah I. Ohimain[1*], Harry A. Gijo[2] and Timipanipiri T. Wood[3]

[1]Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. E-mail: elijahohimain@ndu.edu.ng
[2]Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. E-mail: ayebapreye.gijo@ndu.edu.ng
[3]Fungi Biotechnology, National Biotechnology Research and Development Agency, Abuja, Nigeria. E-mail: timipriwood@gmail.com

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Abstract

Some macrofungi within the basidiomycetes are known to play major role in the sustenance of mangrove ecosystem, however, there are limited molecular reports on the characterization of these fungi found in the estuarine areas of Africa. In this study, the Universal Internally Transcribed Spacer (ITS 1 and 4) primers were used to identify and characterize two distinct fruiting bodies that were harvested from two mangrove tree species in Nun River, Central Niger Delta. Molecular results of this study identified the edible *Lentinus squarrosulus* as the species fruiting on the trunk of *Rhizophora racemosa* while the pathogenic *Marasmius palmivorus* was fruiting on a fallen branch and pneumatophores of *Avicennia africana*. Gel images of amplicons of these fungi reveal similarity at 500 bp while phylogenetic assessment using neighbor-joining at 500 bootstrap value showed that the two species were of different common ancestry. The study therefore suggest that these mushroom species might be tolerant to the high salinity in coastal mangrove areas. They might be playing roles as decomposers and nutrient recyclers in the estuarine ecosystem.

Key words: *Avicennia africana, Coastal biodiversity, Mangrove fungi, Molecular ecology, Rhizophora racemosa, Saprophytes, White rot*

1. Introduction

Mangrove vegetation are important components of tropical coastlines. They perform several vital ecosystem services such as the storage and purification of water including overland runoff before they enter the sea (Ohimain *et al*., 2024). They immobilize heavy metals and radionuclides in unavailable forms in their sediments (Wu *et al*., 2019). Mangroves sequester carbon, control coastal erosion and provide physical traction against sea surges (Allard *et al*., 2020), all of which are important in climate change resilience, adaptation and mitigation (Mai *et al*., 2021). Mangrove also provides provisioning services such as provision of water, timber, medicinal plants, fuel wood and fisheries (Ohimain *et al*., 2024). They are important breeding grounds for shell and fin

* *Corresponding author: Elijah I. Ohimain, Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. E-mail: elijahohimain@ndu.edu.ng*

fishes. For instance, the Niger Delta wetlands, which contained the largest mangrove stands in Africa, is the breeding and spawning ground for coastal and marine fishes coming from the Atlantic Ocean (Ohimain *et al.*, 2024). However, the Niger Delta region is more known for its vast oil and gas resources. Exploitation of the natural resources of the Niger Delta, particularly fisheries and oil and gas, has put the region under environmental stress (Turner and Ohimain, 2024).

It has been recently realized that much of the ecosystem services performed by mangroves are closely linked to their microbiome. For instance, fixing of heavy metals in mangrove sediments are facilitated by sulphate reducing bacteria, which produced hydrogen sulphide that precipitates heavy metals in unavailable forms in mangrove sediments (Wu *et al.*, 2019). A host of heterotrophs and saprotrophs are abundant in mangrove ecosystem, which perform the ecological role of mangal leaf litter and xenobiotics degradation resulting in the biogeochemical cycling of nutrients (Lin *et al.*, 2019; Baker *et al.*, 2021). Hence, these microbes enhance the productivity and facilitate the survival and resilience of mangrove vegetation species (Haldar and Nazareth 2018; Mai *et al.*, 2021). Mangrove ecosystems are areas of high microbial diversity containing archaea, bacteria and fungi (Zhang *et al.*, 2020). But majority of the microbiome study in mangrove ecosystems has focused largely on bacteria, with few on archaea and rare on fungi and the mushroom are even worse.

Lin *et al*. (2023) used qPCR analysis of fungal DNA to characterize the fungal communities in sediments and reported 115 isolates that were classified into 23 genera, which are mostly saprophytes that are involved in the decomposition of leaf litter/organic matter and nutrient cycling. In the Niger Delta mangrove area, some studies on the microbiome of mangrove ecosystem that have been done using molecular methods focused on bacteria (Chikere *et al.*, 2019; Iturbe-Espinoza *et al.*, 2022; Ohimain *et al.*, 2024). Ohimain *et al*. (2024) studied the bacterial guides associated with the sediments of the major mangrove trees in the Niger Delta including *Avicennia africana*, *Laguncularia racemosa, Rhizophora racemosa*, *Rhizophora mangle,* and *Nypa fruticans*. Hence, in this study, we used molecular method based on the amplification of the ITS region of the fruiting bodies of two mushroom species that were harvested from mangrove tree trunk of *Rhizophora racemosa* and fallen branches/pneumatophores of *Avicennia africana* in the Niger Delta.

2. Materials and methods

Field sampling: The fruiting bodies of suspected *Lentinus* sp. (Figure 1) was observed in March 2024 and were sampled from the trunk of *Rhizophora* racemosa in the lower reaches of River Nun, Central Niger Delta along with another unknown species that was sampled from the fallen branch and pneumatophores of *Avecenia africana* (Figure 2) in November 2024. The fruiting bodies were harvested at Ereweibie Creek, which is a tributary to the River Nun Estuary around Akassa Clan, Brass LGA, Bayelsa state, Nigeria. The samples were preserved and taken to the Microbiology Laboratory of Niger Delta University for DNA extraction.

Figure 1: ***Lentinus squarrosulus*** **fruiting on the trunks of** ***Rhizophora racemosa***

3. Molecular identification

3.1. DNA extraction

To extract DNA from the fresh fruit bodies, mini prep extraction kit from ZR fungi DNA were used to run the samples. For cell lysis, the fruit bodies were first crushed and tissue suspended in a 200 ul isotonic buffer and then placed on the lysis tubes of ZR Bashing bead. The volume of lysis solution introduced into the tube was

Figure 2: ***Marasmius palmivorus*** **fruiting on the pneumatophores and branches of** ***Avicennia africana***

750 ul. For the removal of proteins and other cell debris, the tubes were kept in a bead container that is fitted with a small tube of 2 ml and then centrifuged at top speed for 5 mins. Again, the bashing bead lysis tube were centrifuged at 10,000 xg for 1 min. Of the supernatant collected, 400 ul were transferred to the orange top spin filter (Zymo-Spin IV) and then placed on a collection tube and centrifuged at 7000 xg for a minute. For efficient

binding and recovery of DNA from enzymatic reactions and impure DNA samples, 1.2 ml of fungal DNA binding offer were put into the filtrate in the collection tube. This brings the final volume to 1.6 ml. Furthermore, 800 ul was transferred to a Zymo-spin column in a receiving tube and centrifuged for a minute at 10,000 xg. The filtrate was disposed while the remaining volume were put back in the same Zymo-spin and ran. To remove any impurities, 200 ul of the pre-wash buffer was introduced to the spin column in another collection tube, then centrifuge for a minute at 10,000 xg. Next, was the addition of 500 ul wash buffer and then centrifuged again for a minute.

Finally, the column (Zymo-spin IIC) was transferred to a 1.5 ul centrifuge tube with 100 ul of DNA elution buffer. This was later added to the column matrix and centrifuged at 10,000 xg for 30 seconds to elute the DNA. The pure DNA was then preserved at -20 °C until they are ready for use.

3.2. Quantification of DNA

The quantification of DNA was performed by measuring the absorbance at a wavelength of 260 nm using a Nanodrop 1000 spectrophotometer to ascertain the purity of the DNA. DNA BAND imaging: This was done by the use of Agarose Gel electrophoresis to observe the genomic DNA bands while spectrophotometry.

3.3. DNA amplification

This was done using the step-wise protocol established by White *et al*. (1990). The ITS1: 5'-CTTGGTCATTTAGAGGAAGTAA -3' and ITS4 : 5'-TCCTCCGCTTATTGATATGC-3' primers were used to amplify the ITS region of the mushrooms on ABI 9700 Applied Biosystems thermal cycler for 35 cycles at a final volume of 30 ul. The PCR cocktail was; master mix (Taq polymerase, DNTPs, MgCl), primers at a concentration of 2 M, and the extracted DNA. The PCR calibrations were; initial denaturation: 5 mins at 95 °C , denaturation: 30 secs at 95 °C, annealing: 30 secs at 53 °C, extension: 30 secs at 72 °C running 35 cycles, and 5 mins at 72 °C for final extension. The product was visualized using a blue light transilluminator after being resolved on a 1% agarose gel at 120 V for 15 minutes.

3.4. Sequencing

Sequencing was carried out at Inqaba Biotechnological, Pretoria South Africa. The BigDye Terminator kit was used to perform the sequencing on a Thermo-Fisher Scientific 3510 ABI sequencer. The composition for the final volume of 10 ul were; 0.25 µL of BigDye terminator, 2.25 µL of 5x sequencing buffer, 10 µM PCR primer, and 4 ng PCR template DNA per 100 bp. The conditions for sequencing were 32 cycles setting for 10 secs at 96 °C, 5secs at 55 °C, and 4mins at 60 °C.

3.5. Phylogenetic analysis

The Trace edit bioinformatics algorithm was used to edit the sequences that were obtained from the study after they were converted to fasta files. BLASTN was used to upload query sequences from the National Center for Biotechnology Information (NCBI) database. Clustal X was used to align these sequences. The Neighbor-Joining method in MEGA 6.0 was used to infer the evolutionary history in accordance with the process outlined by Saitou and Nei (1987). It was believed that the evolutionary history of the taxa under study was represented by the bootstrap consensus tree derived from 500 replicates (Felsenstein, 1985). The Jukes-Cantor method (Jukes and Cantor, 1969) was used to calculate the evolutionary distances.

4. Results and discussion

Figure 3 presented the gel visualization of the amplicons visible at 500 bp. The obtained query sequence from the mushroom samples matched the reference sequence in the megablast search at different similarity indexes. The referenced sequences of the mushrooms showed a 100% similarity to query sequences revealing *Lentinus squarrosulus* and *Marasmius palmivorus* (Figure 4). The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement revealing close-relatedness between *Lentinus squarrosulus* and *Marasmius palmivorus* from different ancestral lineage (Polyphyletic). However, this study has further shown that ITS bar-coding region is a good universal marker for delimiting macrofungi species of different taxa. Furthermore, the bootstrap value of the Neighbor-Joining method revealed that *Marasmius palmivorus* were of different taxonomic lineage and appears earliest species compared to *Lentinus squarrosulus*

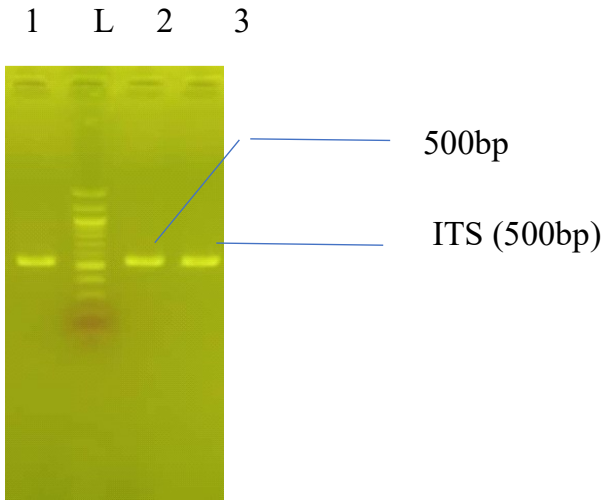

Figure 3: Agarose gel electrophoresis showing the amplified ITS fragment. Lane 1-3 represent the ITS bands at 500 bp while lane L represent the 100bp DNA ladder

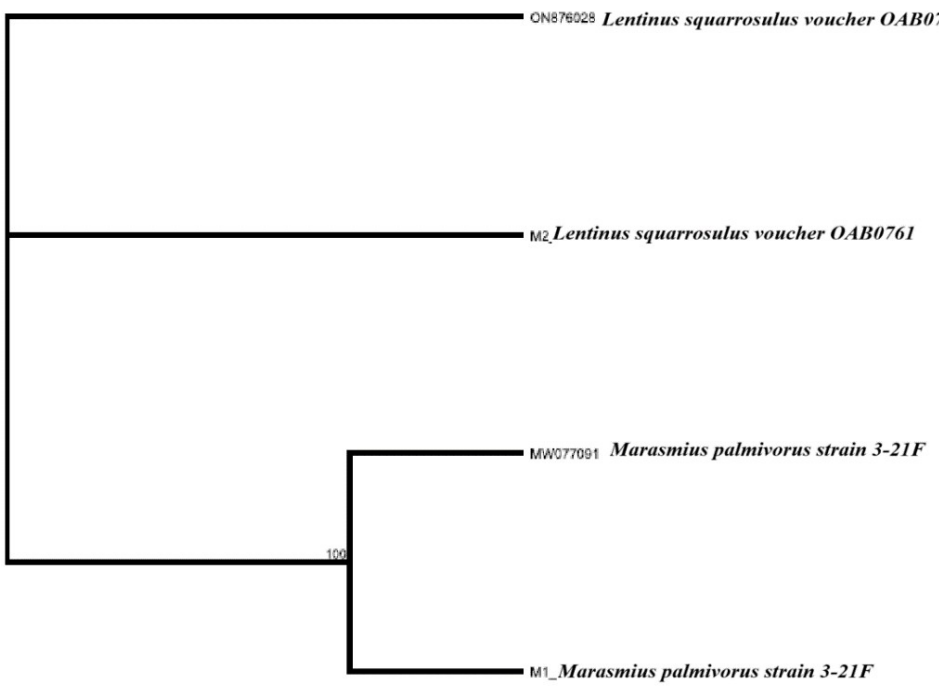

Figure 4: Phylogenetic tree showing the evolutionary distance between the mushroom isolates

even though they both share some genetic similarities coming from the same class of basidiomycetes (Figure 4). This work aligns with recent findings by Abiodun *et al*. (2022) observed that ITS sequences obtained *Lentinus squarrosulus* compared to sequences in the database from NCBI-BLAST displaying significant sequences comparable to the query sequences used to identify the mushroom with a high percentage similarity.

The fruit body of *Lentinus squarrosulus* have been reported especially in Africa and Asia to be an alternative protein source for locals (Isikhuemhen *et al*., 2010). Recently, this species is attracting attention specifically in herbal medicine, food and biotechnology sectors due to its high temperature tolerance, fast growth and expression of diverse enzymes (Isikhuemhen *et al*., 2010 and 2012). Recent studies have shown the safety and efficacy of extracts from *Lentinus squarrosulus*. The mushroom is widely used in Gabon for food and medicinal purposes and was demonstrated to be rich in bioactive substances particularly polyphenols, alkaloids and coumarins, and exhibited antioxidant activity (Ndong *et al*., 2021). Abiodun *et al*. (2022) demonstrated that the

methanol/chloroform extract of the fruiting bodies of the mushroom is rich in phytochemicals particularly flavonoids and polyphenols, and exhibited substantial á-glucosidase and pancreatic lipase inhibitory activities, which contributed to their antidiabetic and anti-obesity properties in vitro. Prateep *et al.* (2017) extracted peptides from the mushroom that exhibited antiapoptotic activity against human lung cancer cells. Ugbogu *et al.* (2019) used GC-MS analysis to demonstrate the presence of diverse bioactive substances from the mushroom including fumaric acid, 1-tetradecene, monochloride, 9-eicosene, phytol, 3-trifluoroacetoxypentadecane 6-ethyloct-3-yl ester, and octahydropyrrolo[1,2-a]pyrazine. They also show that the aqueous extract of the mushroom is nontoxic and exhibited antimicrobial, antioxidant and antinocieptive activities.

Adenipekun and Isikhuemhen (2008) demonstrated the bioremediation of used engine oil polluted soil by *Lentinus squarrosulus* that was isolated from Nigeria, while Tripathi *et al.* (2011) established that the fungus is able to degrade and completely mineralize 2-, 3- and 4-nitrophenols. Their ability to degrade wood biomass, petroleum and other phenolic hydrocarbons is due to the expression of lignocellulosic enzymes especially laccases, peroxidases, CMCase, xylanase (Tripathi *et al*., 2011; Isikhuemhen *et al*., 2012). The fungus being a hydrocarbon degrader and an indigenous species in Nigeria, is therefore not surprising that it is fruiting in the wild in the oil rich mangrove ecological zones of the Niger Delta region. To the best of our knowledge, the ability of this mushroom to grow on mangrove trees and its tolerance to saline environment has not been previously reported. This fungus is often found fruiting in the wild in freshwater areas during the wet season, which ranged from May to October in Nigeria (Isikhuemhen *et al*., 2010). However, during this field work, we observed them fruiting among red mangrove trees (*Rhizophora racemosa)* in the month of March and November 2024. The presence of wood degrading enzymes such as laccase, cellulase and hemicellulase are thought to confers on the mushroom the capacity to interact with a wide host of lignocellulosic substrates including the bark of mangrove trees (Isikhuemhen *et al*., 2012; Anike *et al*., 2015). The observation of *Lentinus squarrosolus* in mangrove ecosystem is therefore noteworthy. Tropical coastal areas experience moderate to high salinities and tidal fluctuations, which support a mix of mangrove species, including red (*Rhizophora racemosa*), black (*Laguncularia racemosa*), and white (*Avicennia germinans*) mangroves (Ohimain *et al*., 2024). Other factors that may have contributed to succession of these fungi in mangrove ecosystems are spore dispersals by wind, physicochemical parameters such as temperature, humidity, luminosity, hydrogen ion concentration and quality of host substrates.

On the other hand, *Marasmius palmivorus* is a pathogenic fungus that causes bunch rot, a disease that affects mostly the palmae family particularly oil palm and coconut and has also been reported in other plants such as rubber trees, pineapple, and banana located in tropical rainforest zones (Pham *et al*., 2020; Maizatul-Suriza *et al*., 2021). The disease of this fungus was previously reported among oil palm in few countries/ regions such as Malaysia, Indonesia, India and West Africa (Gorea *et al*., 2023). Pham *et al*. (2020) first reported *Marasmius palmivorus* triggering stem rot disease leading to the death of indigenous Formosa palm, *Arenga engleri* in Taiwan. Sridhar *et al.* (2022) first reported white root rot disease caused by *M. palmivorus* in *Arachis hypogaea* L. (groundnut) in India. Gorea *et al*. (2023) had stated that the cause of the infection by this fungus can be due to poor sanitation, inadequate pollination, wet seasons with high humidity, damaged or aborted bunches due to pests, and acid sulphate soils. Long wet season with high humidity and acid sulphate soils are predominant features in the Niger Delta mangrove ecosystem. Our study is reporting *Marasmius palmivorus* for the first-time as a potential cause of degradation and decay in mangrove trees, suggesting a possible expansion in the host range of the organism. In a related study carried out by Maizatul-Suriza *et al.* (2021), molecular identification of the *Marasmius palmivorus* showed amplicon size observed to be 700 bp whereas the amplicon assessment for this study is around 500 bp. Members of the genus *Marasmius* belong to the phylum Basidiomycota, which are mushroom-producing fungi, in the order Agaricales (Gorea *et al*., 2023). Species in the genus *Marasmius* are generally regarded as cosmopolitan fungi perhaps because they are found in both tropical and temperate climates, however, there are no sufficient information to confirm the presence of *Marasmius palmivorus* in mangrove regions. This fungus is predominantly a saprotroph in the ecosystems playing important ecological role in the decomposition of litters in the forest floor and nutrient cycling, which is important in carbon sequestration (Gorea *et al*., 2023). Among the marasmioids, few members like *Marasmius palmivorus* also exhibits pathogenic behaviour (Maizatul-Suriza *et al*., 2021). The pathogenicity of this fungus was unequivocally established in oil palm (Maizatul-Suriza *et al*., 2021), groundnut (Sridhar *et al*., 2022) and Formosa palm (Pham *et al*., 2020) following the Koch's principle.

In our study, *M. palmivorus* was found fruiting on a fallen branch upon the pneumatophores of *Avicennia africana*. *Avicennia africana* are typically located in relatively higher salinity zones in the mangrove ecosystems. The fungus therefore seems to have ability to withstand saline environment, because beyond the water, *Avicennia* are known to store and excrete high concentration of salts from their tissues especially their leaves (Waisel *et al.*, 1986; Griffiths *et al.*, 2008; Naidoo, 2025; Shearman *et al.*, 2025). It therefore follows that this mushroom could be tolerant to the high salt concentration in mangrove tissues. Studies on coastal and marine fungi in the Atlantic coast of West Africa are rare. Aleem (1980) studied the distribution of marine fungi in the Atlantic coast of neighbouring Sierra Leone and identified 27 species, which comprised of 14 Ascomycetes, 3 Phycomycetes, and 10 Deuteromycetes, of which about 50% of them occurred in mangrove forests under marine influence. The climate of Sierra Leone, is quite similar to the conditions in the Niger Delta, which is characterized by two main seasons, a short dry season from December-April and long wet season from May-November.

The two species detected, *Lentinus squarrosulus* and *Marasmius palmivorus* were not found by Aleem (1980) in Sierra Leone. Jonathan *et al.* (2025), detected *Ganoderma* species in the coastal mangrove ecosystem of Nigeria, fruiting on the trunk of mango tree (a non-mangrove plant) in Badagry, southwest and on a hard wood in Patani, in the Niger Delta. However, Hyde (1989) working in Brunei found that marine and coastal fungi vary with habitat type, landscape and pollution status. They reported the commonest fungi species in healthy mangrove habitats was *Halocyphina villosa* whereas in oil-polluted mangroves, they were *Cirrenalia pygmea* and *Lulworthia grandispora.* Devadatha *et al.* (2021) reviewed the global occurrence and distribution of mangrove fungi and reported 58 Basidiomycota, 773 Ascomycota, one Blastocladiomycota, 13 Mucoromycota and five Chytridiomycota. They however recorded only 109 fungal taxa in the Atlantic Ocean. But they also did not report the presence of *Lentinus squarrosulus* and *Marasmius palmivorus*. Our study has shown the presence of these mushrooms in the mangrove ecosystem of the Niger Delta in saprophytic relationship with mangrove species. However, further research is necessary to ascertain if *Marasmius palmivorus* is pathogenic to *Avicennia africana* or other mangrove plants.

5. Conclusion

This study revealed the molecular identification of two mushroom species harvested from the two foremost mangrove trees in the Niger Delta. Results of the study identified the *edible Lentinus squarrosulus* as the species fruiting on *Rhizophora racemosa* while the parasitic *Marasmius palmivorus* was fruiting on *Avecinnia africana*. To the best of our knowledge, this is the first time these mushrooms were reported fruiting on these mangrove species in the Niger Delta and perhaps elsewhere.

Availability of data and material

The data are deposited in the GenBank with accession number(s) SUB15009893 M1 PQ878813 for the nucleotide sequence(s).

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Authors' contributions

EIO: Conceptualisation, project administration, supervision, visualisation, writing–original draft and final version.

HAG: sample collection, reviewed draft manuscript and approved final version.

TTW: Result validation, visualisation, reviewed draft manuscript and approved final version.

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