Biodiversity and Phylogenetic Study of Mushrooms from Southeast Nigeria Using DNA Barcoding Methods

Ede, E.M; Dania-Lawrence, F; Ozoma, P.O; Eze, P.C; Akingbade, E. O; Ike-Okafor, N.C; Maduekobo, M.C; Emmanuel, F.I; Emmanuel, L.A; Enuka, S.O; Umeanaeto, E.F; Nwokeke, N.T; Emenogu, S.C; Okoshi, J.C; Fidelis, C.P; Ibegbulem, U.D; Agbasi, K.C; Aroh, E.C; Okoli, M.C; Anochili, E.N; Okereh, C.F; Nworgu, S.M; Manasseh, V; Offordile, E.G; Stephen-Eka, D; Chizam, K; Onyia, A.N; Ezeonu, J.C; Ugoji, E.C; Udeh, E.O; Ogbonna, C; Ogukah, F.E; Beetseh, F; Ibeagha, C.S; Ezu, E.M; Omegu, B.I; Amadi, A.A; Wanogho, C., Irunegbo, D; Anya, J; Ozioko, N**; Mgbodile, F**; Ude, C**; Aniakor, U**; Okoli, C**; Daniels, M; Ekeogbede, E**; Okoro, M.O**; Olisaka, F**; Ude, G.N**

*(*300L Students, Department of Biological Sciences, Faculty of Natural & Applied Sciences, Godfrey Okoye University, Enugu State, Nigeria)*

*(**Staff from Godfrey Okoye University and DNA Learning Center Nigeria)*

Abstract

Over the past two decades, DNA barcoding has gained popularity in the field of molecular biology as a tool for solving the issue faced by traditional taxonomical methods. Here, we explored the use of this tool to identify various mushroom species found in the diverse ecology of south east Nigeria (Enugu and Ebonyi states).Thus, having an efficient identification system could significantly enhance our ability to make the most use of them for both nutritional and therapeutic values as well as monitor the spatial and temporal patterns of fungal distributions and migrations. By using DNA barcodes from the nuclear ribosomal internal transcribed spacer (ITS) of the rRNA gene, with fungal specific ITS primers (ITS 1 AND 4), ITS barcodes were generated for 71 representative fungal samples, the generated barcodes were sequenced and the result of BLAST identified to 64 samples to species levels with *Lentinus squarrosulus* and *Neonothopanus hygrophanus* as the most abundant species.

Background

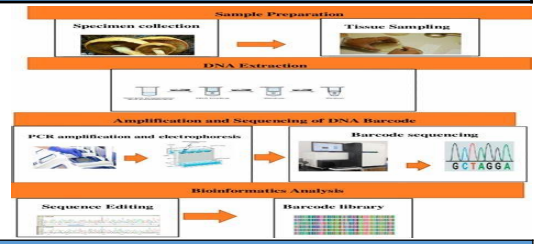
Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogenous and large enough to be seen with naked eye and to be picked by hand. They are widely distributed in the temperate and tropical regions in countries such as Nigeria where they have found importance for use in medicinal and food (Chang Shu-Ting and Philop, 2004). Mushroom are a type of fungi which ubiquitous in both natural and human-made environments. They play important roles in the health of plants, animals, and humans, and in broad ecosystem functions. Adequate knowledge of mushroom diversity and distribution are imperative for successful conservation, management and optimum exploitation of the ecosystem for innumerable benefits to mankind (Nwordu et al. 2013). Mushrooms recognized as visible fungus with characteristic carpophores that symbolize the reproductive stage of several Ascomycetes and Basidiomycetes life cycles. With a variety of nutritional, therapeutic, and ecological benefits, mushrooms constitute a significant bioresource. DNA barcoding is a technique that uses a short DNA sequence to identify species. In the case of fungi, DNA barcoding typically uses the ITS region of the rRNA gene as the barcode region.

Impact and Future Direction

- This investigation serves as a teaching plot and pilot study for undergraduate research where the students gained basic molecular biology skills such as sample collection and documentation, pipetting techniques, gel electrophoresis, DNA extraction and much more.
- There will be a better study involving more students as well as the survey of more sites in the different LGAs in Enugu State to ensure a better representation of the samples size required to publish accurate data on this subject matter.
- Other undergraduate students in the future will use this data as their reference data to compare the nature of species collected and also process for seasonal variations that occur with the change in seasons and time.
- This project will form the background to introduce CURE into the undergraduate curriculum in Nigeria beginning from Godfrey Okoye University, Enugu.

Materials and Methods

The materials used were gloves and ziplock bags during the sample collection. Then we extracted the Mushroom DNA using micropipettes, silica resin, microcentifuge tubes, centrifuges and a water bath. Then PCR was performed using the thermocycler,. Gel electrophoresis was done then the DNA was sequenced and sent back to us through DNA subway. The last step was bioinformatics.



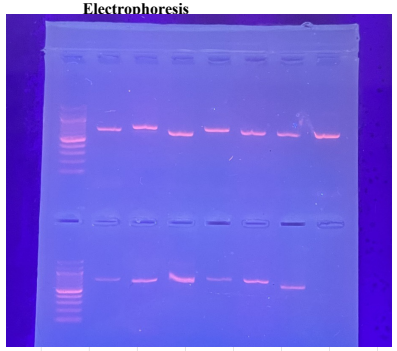
Results

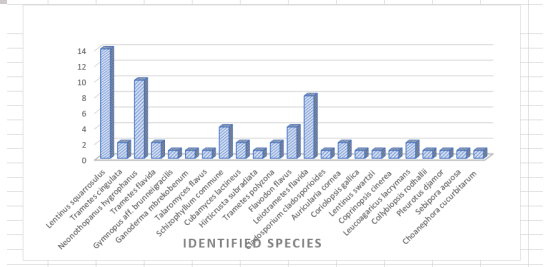
A total of 102 samples were collected

46	85-ITS	Trametes polyzona	MH131681.1	1
47	87-ITS	Lentinus squarrosulus	OM780271.1	0
48	88-ITS	Lentinus squarrosulus	MN856299.1	0
49	89-ITS	Leucoagaricus lacrymans	OP985115.1	12
50	90-ITS	Lentinus squarrosulus	MN856300.1	1
51	91-ITS	Lentinus squarrosulus	MN856300.1	1
52	92-ITS	Lentinus squarrosulus	MN856300.1	1
53	93-ITS	Auricularia cornea	MH213359.1	0
54	95-ITS	Leucoagaricus lacrymans	OP985115.1	12
55	96-ITS	Leucoagaricus lacrymans	OP985115.1	12
56	97-ITS	Neonothopanus hygrophanus	MK931357.1	1
57	98-ITS	Collybiopsis rodhalliii	MF100982.1	0
58	99-ITS	Schizophyllum commune	MT460518.1	0
59	100	Pleurotus djamor	KT273359.1	2
60	101 ITS	Sebipora aquosa	KJ854615.1	2
61	46 ITS	Choanephora cucurbitarum	MT586126.1	1
62	68-ITS	Trametes cingulata	MK736979.1	17
63	94-ITS	Lentinus squarrosulus	MN856300.1	0
64	102-ITS	Neonothopanus hygrophanus	MK931357.1	28

ITS barcodes were generated for 71 representative fungal samples, the generated barcodes were sequenced and the result of BLAST identified to 64 samples to species levels with *Lentinus squarrosulus* and *Neonothopanus hygrophanus* as the most abundant species.

Polymerase Chain Reaction (PCR) with ITS primers (1F and 4R) and Gel Electrophoresis





There are four major Clades - A: comprising majorly of genus Leiotrametes, Clade B: Lentinus, Clade C: Neonothopanus and Clad D: Choanephora and Rust Fungus

CONCLUSIONS

Out of 71 samples, 64 were identified to specie level. These species are in 4 major Clades: Clade A: Leiotrametes Complex, Clade B: Lentinus Complexes, Clade C: Neonthopanus Complexes, Clade D: Choanephora and rust fungus. The most abundant species were Lentinus Squarrosulus and Neonthopanus hygrophanus. Leucoagaricus lacrymans (samples 89, 95 and 96), Lentinus squarrosulus (Sample 33), Coriolopsis galica (Sample 65) and Neonthopanus hygrophanus (Sample 102) ,had significant mismatches. With reference data on the NCBI reference database. This result conflicts with related research conducted in Cross River and Southwest states of Nigeria , where the main genera found were *Agaricus and Aleuria*. on Hence, further bioinformatics studies should be done to understand the nature of these dissimilarities.

Acknowledgements and References

We want to appreciate the Carnegie African Diaspora Fellowship Program administrators for the funding & support that enabled Professor George Ude to engage with Godfrey Okoye University and their students for 9 months. We also thank the Vice Chancellor of Godfrey Okoye University, Prof. Christian Anieke for the financial investments and the moral support that has given the sciences a platform to thrive in GOU, Nigeria. We acknowledge the efforts and contributions of the Executive Director, DNALC New York, Dr. Dave Micklos who consistently sends supplies and reagent that support the work done in CURE. Finally, we appreciate the Staff (Dean of NASES, HOD of Biological Sciences, Senior lecturers etc.) and students of GOU for your immensurable contributions to this project.