FISH SPECIES IDENTIFICATION AND BIODIVERSIFICATION IN ENUGU METROPOLIS RIVER BY DNA BACODING

PRESENTED BY

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INTRODUCTION

➤ The identification and classification of species provides a nomenclatural backbone and a key prerequisite for numerous biological issues.

➤ Among which are the need to maintain biodiversity and ensure biosecurity, protect species and avoid pandemics.

➤ The process and procedure to achieving such goal involves the use of barcoding to identify and describe species (Frezal et al., 2008).

➤ Fish is a proteinous animal which plays a vital role in protection and prevention of human diseases (McManus, 2011).

INTRODUCTION CONT.

➢ DNA barcoding which uses the 50 region of the mitochondrial Cytochrome C oxidase subunit (COI) as the target gene is an efficient method of standardized species level identification for biodiversity assessment and conservation.

➢ It involves the analysis to identify its family and species they belong to by using their genomic content.

➢ The current popularity of DNA barcoding relates to its potential power coupled with its intuitively pleasing simplicity

➢ This is based on the premise of using standardized short region of DNA as a universal tool for identification of organisms.
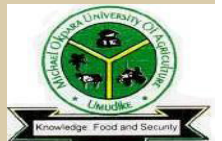
OBJECTIVES

➢ The aim of this research is solely to establish a large-scale reference sequence database against which unknown samples that are cryptic in nature can be barcoded.

➢ And queried for identification where sequences are found that are divergent from others in the database, the corresponding specimens are flagged up as potential new species warranting further investigation (Herbert *et al*, 2003).

➢ To identify unknown species or assess whether species should be combined or separated (Newmaster *et al., 2009*).

MATERIALS AND METHODS

➢ Nike Lake is the source of this research samples, it rests on the outskirt of Enugu City, Eastern Nigeria.

➢ Nike Lake is located in an area called "Nike" within the Enugu Metropolis.

➢ This research was carried out with 18 fish specimens, 5 genera and 10 different species out of the known 285 fish species in all Nigerian freshwater systems as described by Raji *et al*, 2008.

MATERIALS AND METHODS CONT.

➢ The samples collected were morphologically identified in-situ by visual inspection and taxonomically classified with standard guides.

➢ The samples were kept in aquarium alive until the date of extraction.

➢ The DNA extraction involved: 10-20mg from the fish fin clip was obtained using the universal promega kit.

Materials and methods cont.

➢ Amplification of DNA by PCR was done using the primer that contains both forward and reverse primer.

- 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3' (forward primer - VF2_t1)
- 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' (forward primer - FishF2_t1)
- 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3' (reverse primer - FishR2_t1)
- 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3' (reverse primer - FR1d_t).

➢ Analysis of **PCR product by gel electrophoresis was done were the** UV Tran illuminator imaging system was used to place the e-gel to take a photograph of the DNA bands and record if there is DNA in your extracted DNA samples.

➢ DNA sequencing was carried out using Applied Bio-system (ABI) Prism 310 Genetic Analyzer. The laser will record these colour transmission and sends the result to the computer for analysis.

RESULTS

SAMPLE NUMBER	GENUS	SPECIE	IGBO NAME	NICHE	SCALES
1	*Chrysichthys*	*nigrodigitatus*	okpọ	Under grass	No
2	*Parachinna*	*obscura*	Evi	Mud	Yes
3	*Parachinna*	*obscura*	Evi	Mud	Yes
4	*Ctenopoma*	*acutirostre*	Ikiko mmanu	Open water body	Yes
5	*Tilapia*	*sp.*	Ikpopo	Open water body	Yes
6	*Ctenopoma*	*acutirostre*	Ikiko mmanu	Open water body	Yes
7	*Clarias*	*sp.*	Alila	Mud	No
8	*Clarias*	*sp.*	Alila	Mud	No
9	*Clarias*	*sp.*	Alila	Mud	No
10	*Clarias*	*sp.*	Alila	Mud	No
11	*Clarias*	*sp.*	Alila	Mud	No
12	*Clarias*	*sp.*	Alila	Mud	No

RESULT CONT.

NUMBER OF FISH	CONTAMINATED	NO DNA EXTRACTED	NO DNA SEQUENCE	DNA SEQUENCE	POLYMORPHISM	NO POLYMORPHISM
18	2	3	2	12	8	4

RESULT CONT.
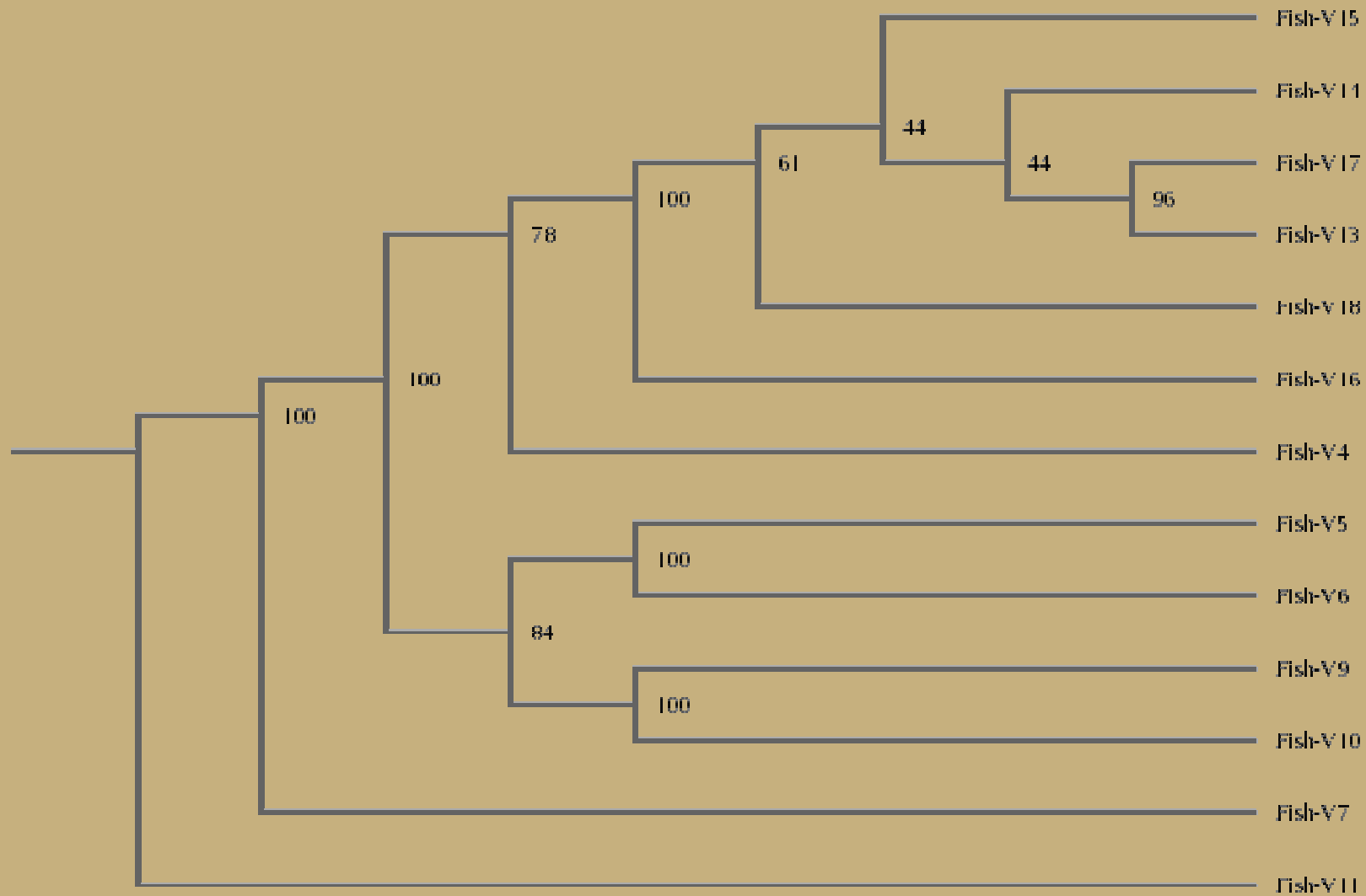
Results cont.
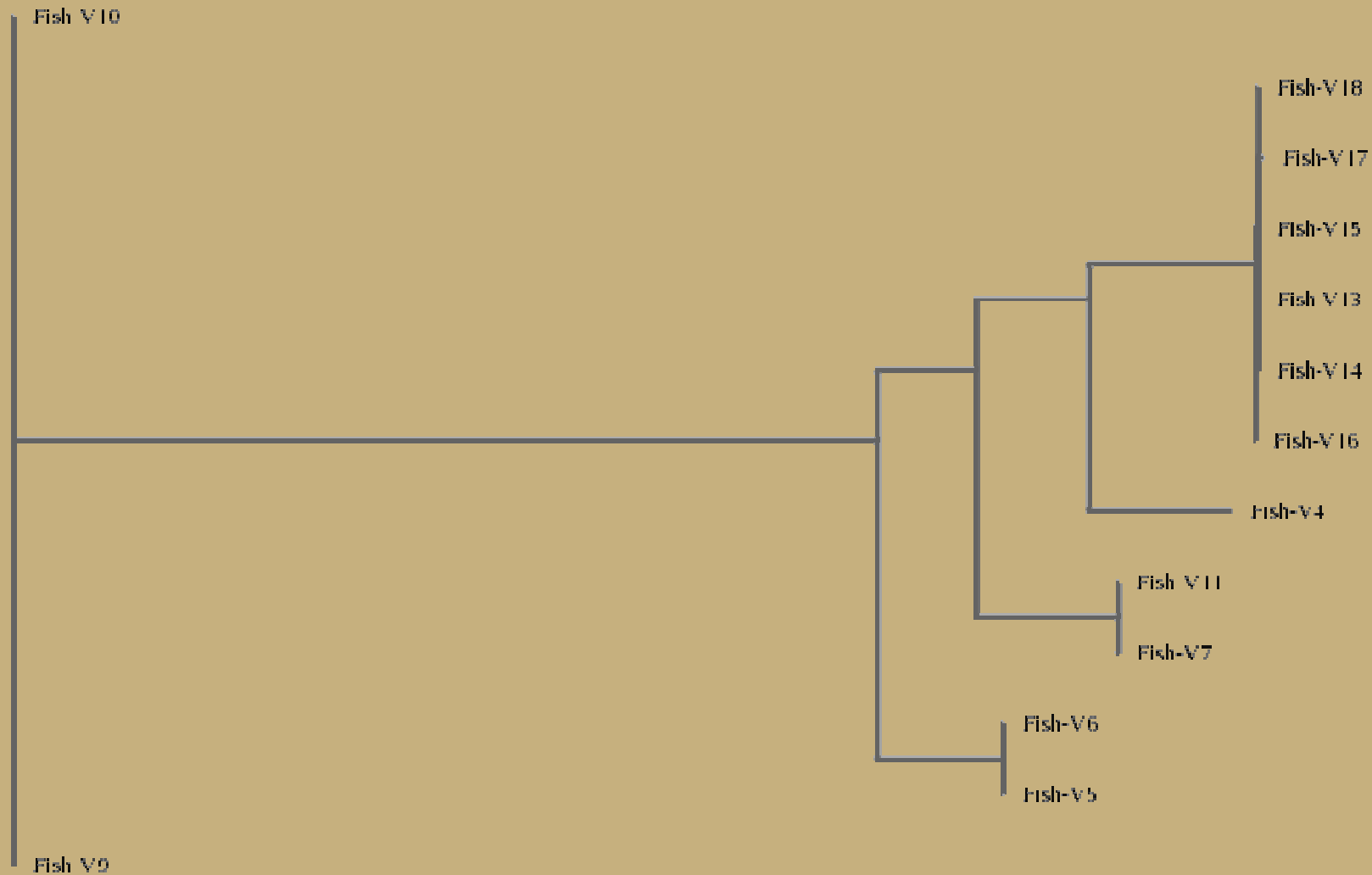
Discussion

- The extraction of the DNA was successfully done using promega kit. Out of 18 samples, 11 samples were good, 2 samples were contaminated with pseudomonas and also presence of stop codon.

- No DNA was extracted from sample (1, 2, 3) and no DNA sequence from sample 5 and 9 (S-8 AND S- 12).

- The contamination was solely analyzed to be as a result of intestinal flora.

- 15 fish samples were analyzed using their genomic make-up, 4 out of 15 samples (*Parachinna obscura* - 2) (*Clarias sp* - 2) did not show statistical significant evidence of spatial genetic differentiation in their nucleotides despite the enormous geographical distance separating populations. This is in relation to findings from Wong *et al* (2011).

Conclusion

- The 11 practical samples and the samples from BLAST tool were the same in their varied genus and species but they were existence of polymorphism in their nucleotide bases.

- Nevertheless, the analysis from the 13 samples shows that barcoding can work as a global species identifier for fish species.

- Although there is a complete morphological and genomic relationship between the sample species and that from gene bank, there was a partial difference in their genomic nucleotide base pairs when muscled.

THANKS FOR LISTENING