

The Use of DNA Barcoding in Identification of Genetic Diversity of Fish in Ugwu-Omu Nike River in Enugu.

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Abstract

In this study, for the first time, the use of DNA barcoding was used in identification of the genetic diversity of fish in Ugwu-omu Nike River, Enugu State, Nigeria. The fish were collected and placed in an aquarium and later transported to the Biotechnology laboratory of Godfrey Okoye University. The fish collection was followed by identification and genomic extraction using standard methods. Isolation of DNA from fish tissue was done using PROMEGA kit. Fish samples were labeled before amplification of the DNA by PCR. Samples were further analyzed using gel electrophoresis and were viewed using UV transilluminations. Fish samples were later sequenced for genetic diversity using a DNA Subway sequencer. The result shows that, gel electrophoresis had a clear DNA band on the eleven fish species collected. In the DNA subway sequences, only 9 out of the 11 fish species were identified while 2 were not identified. Fish species revealed similar and different polymorphism and genomic classification during the experiment. Therefore, it is important that continuous research be carried out in Nigeria waters for fish diversity studies.

Keywords: Biotechnology, Genetics, PCR, DNA, Biodiversity, DNA Barcodes, Taxonomy

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Introduction

DNA barcoding, a new method for the quick identification of any species based on extracting a DNA sequence from a tiny tissue sample of the organism is now being applied to taxa across the tree of life (Kress *et al.*, 2010). DNA barcoding assists in identification by expanding the ability to diagnose species by including all life history stages of an organism, helps to flag species that are potentially new to science and address fundamental ecological and evolutionary questions (Kress *et al.*, 2009). DNA barcodes consist of a standardized short sequence of DNA between 400 and 800 bp long that can be easily isolated and characterized (Savolainen *et al.*, 2005). This has become a vital new tool for taxonomists who are charged with the inventory and management of the earth's immense and changing biodiversity (Cowan *et al.*, 2006).

The process of DNA barcoding entails two basic steps: (1) building the barcode library

of known species and (2) matching or assigning the barcode sequence of the unknown sample against the barcode library for identification (Janzen, 2005). DNA analysis has been applied to the identification of pork in autoclaved meat products and to the determination of species identity in canned salmon (Filipe and Carvalho, 2012): PCR was used to amplify short fragments of DNA, which were then sequenced. For routine analysis in food control laboratories DNA sequencing has the disadvantage of being technically demanding and time consuming. Biodiversity refers to the diversity of variety of plants and animals and other living organisms in a particular area or region, for instance, the species that inhabit Enugu South is different from those in Enugu North, even though some of the same species can be found in all of those areas. Genetic diversity is essential for the continued existence and evolution of healthy fish population. Genetic difference exists among population within a species and reflect

isolation from other species to local conditions (such as water, temperature, seasonal changes in water flow, available food and presence of predators (Rose, 2008). Fish species diversity is roughly divided equally between marine (oceanic) and fresh water ecosystem. Coral reefs in the indo-pacific constitute the center of diversity for marine fishes, whereas continental freshwater fishes are most diverse in large river basin of tropical rainforest.

Fishes are the largest and most diverse class of vertebrates and fortunately among the easiest groups for which to generate DNA barcode data (Handy *et al.*, 2010). According to Weigt *et al.*, 2009, eggs, larvae and juvenile fish can be difficult to identify to species using morphology alone, however with DNA barcoding tool, it becomes easier. Therefore, the research on the use of DNA barcoding in identification of genetic diversity of fish species in Ugwu-omu Nike river of Enugu State in Nigeria was carried out.

Materials and Methods

Sample collection: The fish were collected from Ugwu-omu Nike river by the help of the fishermen and placed in a glass aquarium which was then transported to the Biotechnology laboratory of Godfrey Okoye University, Enugu State. The convenient sampling technique that was used was the random catch. The fish samples collected were identified as described by Benton, (1998). Fish samples identified include *Parachanna obscura*, *Parachanna insignis*, *Hepsetus odoe*, *Clarias gabonensis*, *Clarias jaensis*, *Channallabes apus*, *Tilapia guineensis*, *Synodontis obsesus*, *Synodontis rebeli*, *Synodontis volifer* and *Synodontis sp.*

Reagents utilized: DNA rehydration solution (100µl), 70% ethanol (600µl), Isopropanol (600µl), Nuclei lysis solution (600µl), Protein Precipitation solution (200µl) RNAse solution (3µl), 3m Sodium Acetate (150µl), TRIS/EDTA(TE) Buffer with RNAse A (250µl), dH₂O (1.5ml), Primer loading dyes for fish (rbcl aF/ rbcl aR), Ready — to — go, PCR beads in 0.2ml or 0.5ml, 2% Agarose in 1 X TBE (held @ 60°C), PBR322/BstNI Marker (20µl per gel) and SYBR Green DNA Stain (6 p.l) store on ice.

Isolation of DNA: Promega commercial kit was used. It has the advantage of working reproducibly with almost any kind of plant or animal specimen. Animal tissue was used, about —10-20 mg or 1/4 inch diameter from each of the sample. Samples were placed in a clean 1.5mL tube labelled with an identification

number. 600µl lysis solution was added to tube, and then twisted a clean plastic pestle was placed inside the centrifuge, the inner surface of 1.5mL tube to forcefully grind the tissue for 2 minute. Then incubated the tube in a water bath or heat block at 65°C for 15 minutes. 3µl of RNAse solution was added to tubes and mix by rapidly inverting tube several times. The tube was incubated in a water bath or heat block at 37°C for 15 minutes. Then stand tube at room temperature for 5 minutes. Then 200µl of protein precipitation solution was added to each tube. The tubes were vortex for 5 seconds. The tubes were placed in a balanced configuration in a micro centrifuge, with cap hinges pointing outward. Centrifuge for 4 minutes at maximum speed to pellet protein and cell debris. A clean 1.5ml tube with the sample number was labelled; Using a fresh tip to transfer 600µL of supernatant to the clean tube, being careful not to disturb the pelleted debris when transferring the supernatant. 600µL of isopropanol was added to the supernatant in tube. The tubes were placed in a balanced micro centrifuge, with cap hinges pointing outward. Centrifuge for 1 minute at maximum speed to pellet the DNA and 600µL of 70% ethanol was added. Air-dry the pellet for 10-15 minutes to evaporate remaining ethanol. 100µl of DNA rehydration solution was added to each tube, and dissolve the DNA pellet by pipetting in and out several times, then incubated the DNA at 65°C for 45-60 minutes, at 4°C and ready for amplification of DNA by PCR. This was done as described by Handy *et al.*, 2010.

Amplification of DNA by PCR: PCR tube containing Ready-To-Go PCR Bead was labeled with an identification number, micropipette of a fresh tip 23µl of the following Primer/loading dye mixes was added to each tube and allowed to dissolve for 1 minute. Then, fish samples: rbcl primers (rbcl aF / rbcl aR) using a micropipette with fresh tip 2µl of the DNA sample was then added directly into the appropriate primer/loading dye mix to ensure that no DNA remains in the tip after pipetting kept at 4°C, 45 seconds to 72°C, 45 seconds. Then samples were stored on ice until it was ready to begin thermal cycling. After thermal cycling, the amplified DNA was stored on ice or at -20 °C (Shokrallan *et al.*, 2010).

Analyzing PCR product by gel electrophoresis: The ends of the gel-casting tray was sealed with masking tape, or other method appropriate for the gel electrophoresis chamber used and insert a well-forming comb. 2% of agarose gel was poured into the tray to

a dept that covers about one-third the height of the comb teeth. The gel was allowed to solidify which took approximately 20 minutes. The gel was placed into the electrophoresis chamber and added 1x TBE buffer to cover the surface of the gel. The comb was carefully removed and then an additional 1x TBE buffer was added to fill in the wells and cover the gel, creating a smooth buffer surface. Using micropipette with a fresh tip, 5 μ of each PCR product was transferred to a fresh 1.5ml micro centrifuge 2 μ l of SYBR green DNA stain tube. 2 μ l of SYBR Green stain was added to 20 μ l of Pbr322/BstNI marker.

The gel oriented, using a micropipette with a fresh tip to load 20 μ l of PBR322/ BstNL size marker into the far left well. The remaining sample was stored on ice or at 20°C for

Discussion

The extraction of DNA from identified fish samples was successfully done using promega kit. Result from Table 1 shows that the fish species from Ugwu-omu identified had clear DNA bands. A total of 11 fish species were collected and DNA was extracted from all the samples when visualized using gel electrophoresis, out of the 11species 9 were successfully identified while were not due to the presents of stop codon. Fish Sample 25, 23 and 19 (name them) diverse from other samples in the reference library as a result of polymorphism. Fish 24 (name) and 16 (name) are closely related because, they are the same both in morphological and genomic classification. This was in line with the work of Handy *et al.*, (2010), Ahren *et al.*, (2007), Blaxter *et al.*, (2005), Calerina and Tishechkin (2006), Cox and Hebert (2001) and Smith *et*

sequencing. The gel was kept for approximately 30 minutes 130v. The gel was viewed using UV trans illumination (Lucentini *et al.*, 2006). Using the DNA Subway analysis, the identification of species and their phylogenetic relationship was carried out.

Results

The result of Table 1 showed a marked clear DNA bands on the eleven (11) fish species analyzed using gel electrophoresis. Figure 1 and 2 indicate the phylogenetic tree pattern of fish sample to those fish species at the gene bank using their maximum likelihood and nucleotide joining. Fish samples were not sequenced and recognized by the gene bank, however appeared in the form they were numbered.

al., (2005) What did these authors do?. Nevertheless, the analysis from the 11 samples shows that barcoding can work as a global species identifier for fish species. The major limitation in this study can be visualized in the aspect of primer designs which are not always stable. According to research work carried out at Cold Spring Harbor Laboratory, rbcl, matk, Dmi and universal primer were not able to differentiate citrus fruits based on their species (Brown *et al.*, 1999). With such encounter, advance research on primer design for various living organism must be initiated if the maximum goal of this research must be covered. Also, limitations include poor electricity in developing countries e.g. Nigeria where there is strong failure and fluctuations of electricity. Such negative impact will affect practical reagents due to their varied cold chain.

Table 1: Fish species from Ugwu-omu river with clear DNA bands

S/NO	NAME OF SAMPLE	GEL ELECTROPHORESIS
Fish 16	<i>Parachanna obscura</i>	Yes
Fish 11	<i>Parachana insignis</i>	Yes
Fish 18	<i>Hepsetus odoe</i>	Yes
Fish 19	<i>Clarias gabonensis</i>	Yes
Fish 20	<i>Clarias jaensis</i>	Yes
Fish 21	<i>Channallabes apus</i>	Yes
Fish 22	<i>Tilapia guineensis</i>	Yes
Fish 23	<i>Synodontis obesus</i>	Yes
Fish 24	<i>Synodontis sp</i>	Yes
Fish 25	<i>Synodontis rebeli</i>	Yes
Fish 26	<i>Synodontis volifer</i>	Yes

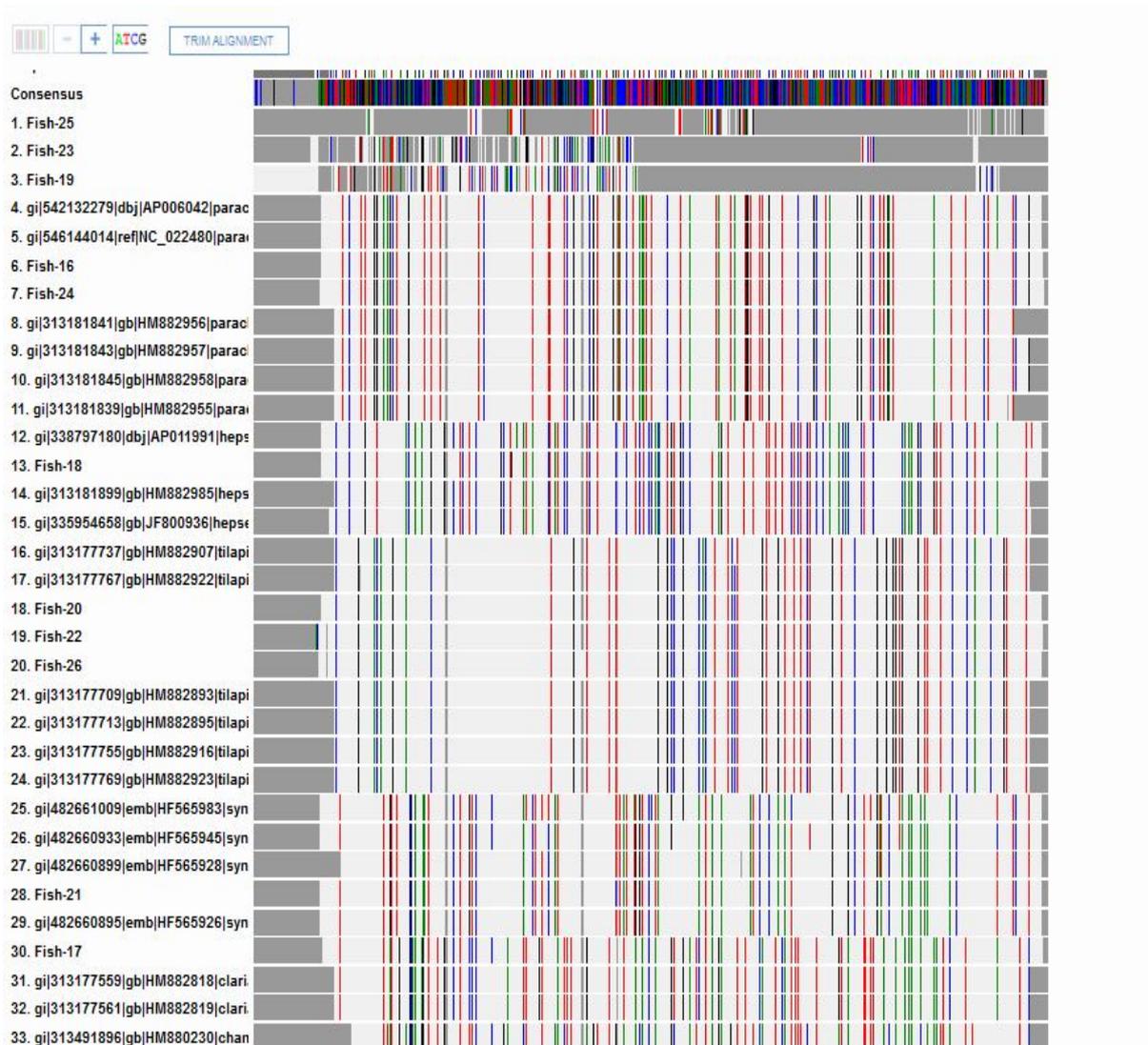


Plate 1: Fish species alignment viewer in comparison to the fish species at gene bank sequenced



Fig 1: The construction of phylogenetic tree using maximum likelihood (ML) in comparison to fish species at the gene bank

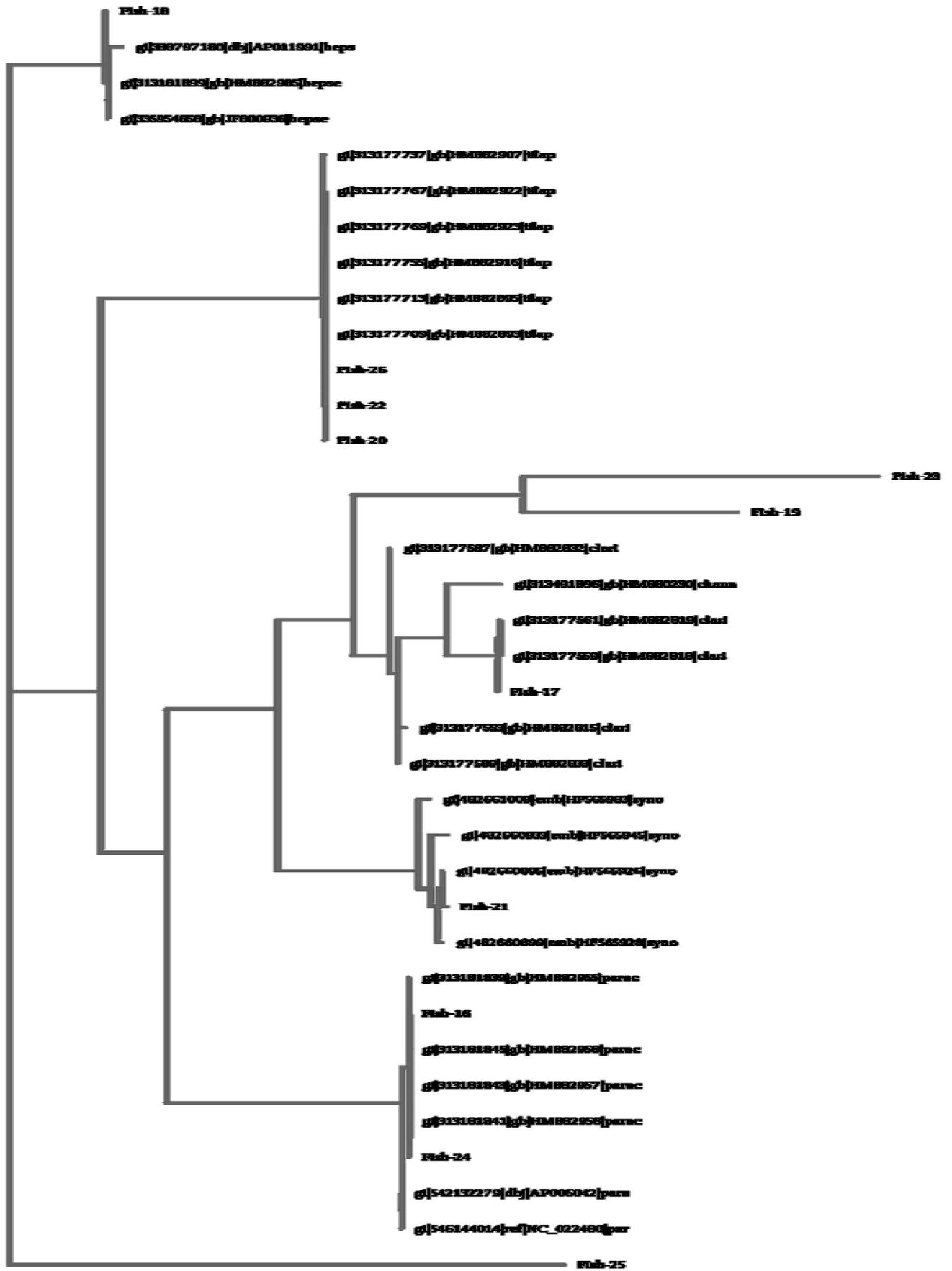


Fig 2: The construction of phylogenetic tree using nucleotide joining(NJ) in comparison to fish species at the gene bank.

Conclusion

This study has shown that DNA barcoding can be carried out in the Nigerian freshwater. Documentation of fish diversities should be encouraged in order to understand fish biodiversity and mode of diversification. Also, the DNA barcoding committee in Nigeria should be encouraged to research on fish biodiversity in order to keep track and accurate retrievable data on the various and numerous species of fish which are not discovered.

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