Extended Abstract
Volume 3:2, 2015

Journal of Biodiversity & Endangered Species

Open Access

Biodiversity - 2015: Fish species identification and biodiversification in Enugu metropolis river by DNA barcoding

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Abstract

The inland fisheries in tropical Africa face threats both by stress from climate change and by overexploitation. Species are becoming extinct and populations decline at an alarming but poorly understood rate. Many species may face extinction before they can be identified or described. This presents a problem for conservation planning and prioritization, because those species that have not been identified obviously cannot be protected effectively. Caddy and Garibaldi reported that only 65.09% of worldwide fishery captures reported to the FAO for the year 1996 was identified at species level, ranging from about 90% in temperate areas to less than 40% in tropical regions. Surveys into the accuracies of species identifications have not been reported, but a significant percentage of identifications may still be erroneous. The limitations inherent in morphology-based identification systems and the limited pool of taxonomists paved the way for the introduction of new molecular diagnostic tools for effective species identification. Hitherto, a wide variety of protein-based and DNA-based methods have been evaluated for the molecular identification of fish species in Africa. These studies, however, are not comparable for the purposes of species identification because they lack standardization (e.g. different regions of the mitochondrial genome such as cytochrome *b* and 16S rDNA were used). Hebert et al. (2003) proposed a single gene sequence to discriminate the vast majority of animal species, using a 650-bp fragment of the 5′ end of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene as a global bioidentification sequence for animals. This technology (DNA barcoding) relies on the observation that the 'barcode' sequence divergence within species is typically much lower than the divergence exhibited between species, making it an effective marker for species identification and discovery. The startling efficiency of the method may arise from selective sweeps and the intricacies of mito-nuclear coadaptation, raising the profile of bioenergetics as a possible speciation mechanism.

DNA barcoding has since gained global support as a rapid, accurate, cost-effective, and broadly applicable tool for species identification, particularly with respect to fishes as coordinated by the fish barcode of life campaign. Barcoding has also been adopted by the census of marine life project, a growing global conglomerate of 50 countries engaged in a 10-year initiative to assess and explain the diversity, distribution, and abundance of life in the ocean. Although there has been criticism of both the philosophical and the practical underpinnings of DNA barcoding, its successful application for both species identification and discovery has been demonstrated in many studies, involving many taxonomic groups, for example birds, fish, fish parasites, bats, spiders, crustaceans, nematodes,

earthworms, mosquitoes, and diverse arrays of Lepidoptera. In addition, DNA barcoding strategies are now being applied for other groups of organisms including plants, macroalgae, fungi, protists, and bacteria. Furthermore, DNA barcoding has gained wide application in forensic analysis to investigate cases of illegal poaching, separation of species, gut content analysis in ecological studies, food product analysis and market substitution, and Asian medicine trade regulation. DNA barcoding has also been employed to validate the identity of biomaterial collections and cell lines. A sufficient accumulation of DNA barcodes can also help conservation managers to identify interim priority areas for conservation efforts in the absence of species data. Currently, DNA barcode reference library records are available for more than 1 million sequences representing more than 94,000 species on the barcode of life data systems, an informatics workbench aiding the acquisition, storage, analysis, and publication of DNA barcode records. Nearly, 10% of these records comprise marine and freshwater fish species.

The COI divergence and species identification success based on DNA barcodes have been previously assessed for many freshwater fish species, for example in Canada, Mexico and Guatemala, and Brazil. Since, to date, there is no detailed knowledge about the diversity and distribution of freshwater fish species in Nigeria, the aim of this study was to determine whether DNA barcoding can be used as an effective tool to perform unambiguous species identification of freshwater fishes in this region, with a view toward establishing a DNA barcode reference library for utilization in biodiversity assessment and conservation for the entire country.

Fish is a proteinous animal which plays a vital role in the protection and prevention of human diseases. DNA barcoding which uses the 50 region of the mitochondrial cytochrome c oxidase subunit as the target gene was implored as an efficient tool in the identification of fish species in the Enugu Metropolis River (Nike Lake and Abakpa River). 10-20mg fish tissue sample of 18 species were extracted for DNA using Promega kit. The polymerase chain reaction (PCR) was used to amplify short sequences of mitochondrial DNA, which were denatured and analysed by polyacrylamide gel electrophoresis (native PAGE), for detection of single strand conformation. Polymorphism species specific muscle alignment patterns of DNA bands were obtained for Chrischthys sp, Parachinna sp, Ctenopoma sp, Tilapia sp and for a number of Clarias species. Out of the 18 fish species, only 15 fish samples were analysed using their genomic make-up, 4 out of the 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. The morphological studies on this fish species have shown that these lines of evidence are taxonomically important and also partial differences in genomic nucleotide base pairs when noticed. This difference is the polymorphism, which is the key to flagging new specie in a particular genus can be attributed to environmental changes and diversity.

This work is partly presented at 4th International Conference on Biodiversity June 15-17, 2015 Las Vegas, USA