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Review

DNA barcoding of Ghanaian fish species: Status and prospects

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Ghana's waters are biologically diverse with different fish species due to different ecological habitats and niches harboring several cryptic fish species. However, the fish production has reduced over the past decade; hence an urgent conservation and management strategies are required to save Ghanaian fishery such as accurate identification of species to formulate species-specific conservation and management strategies. Molecular method of fish identification, DNA barcoding, has proven its efficacy in species identification for both freshwater and marine species. In recent years, DNA barcoding has been accepted as a bio-identification system for living organisms globally. This system is fast and produces accurate species identification by using a short DNA sequence marker from a standard region of the DNA sequence of an organism's genome to identify it as belonging to a particular individual or species based on Cytochrome C Oxidase type I (COI/ Cox 1) gene instead of the whole genome. Unfortunately, unlike the developed countries, this molecular method of fish identification is new in Ghana. This review article aims to examine the issues regarding fish identification and the need for DNA barcoding as a tool for taxonomic identification, grouping, and naming of fish species in Ghana. Also, this review takes a look at the current status and future direction of DNA barcoding fisheries in Ghana. In addition, the benefits of DNA Barcoding in fishery management and conservation are discussed.

Key words: DNA barcoding, Ghana, marine species, current status, management, conservation.

INTRODUCTION

Aquatic organisms are the largest and most diverse class of invertebrate and vertebrate species. Recently, the estimated species exceeds 30,000 (Eschmeyer et al., 2010) with an annual description of about 300 new

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species (Weigt et al., 2012). Hence, taxonomic ambiguity exists for several fish genera and identification (Lakra et al., 2016). The basic research that helps characterize their taxonomic diversity is important for the conservation of these taxa and for improvement of their management and conservation strategies (Wang et al., 2010). Fortunately, they are among the easiest groups of organisms for which to generate DNA barcode data (Weigt et al., 2012).

DNA-based approaches (DNA Barcoding) for taxon diagnosis exploiting DNA sequence diversity among species can be used to identify fishes and resolve taxonomic ambiguity including the identification of new species (Hebert et al., 2003). DNA barcoding is a taxonomic method that uses a short genetic marker from a standard region of the DNA sequence of an organism's genome to identify it as belonging to a particular individual or species (Hebert et al., 2003). This short fragment of mitochondrial (650 base pairs) cytochrome c oxidase subunit I (COI) is used to identify the teleost that has been previously described morphologically (Barros-Garcia et al., 2016). The short fragment (DNA sequence) is made of four nucleotide bases A (Adenine), T (Thymine), C (Cytosine) and G (Guanine) (Kaur, 2015). Apart from facilitating species identification, other benefits of DNA barcoding include highlighting cases of range expansion for known species, flagging previously overlooked species and enabling identifications where traditional methods cannot be applied (Ward et al., 2009).

Previously, Polymerase Chain Reaction (PCR) and hybridization methods such as Random Amplification of Polymorphic DNA (RAPD), probe hybridization, gene-specific primers and PCR- Restriction Fragment Length Polymorphism (RFLP) were used for species identification (Teletchea, 2009). However, these methods of identification lacked a database for reference and universal primer (Vartak et al., 2014). Hebert et al., (2003) proposed that a single gene sequence would be sufficient to differentiate all, or at least the vast majority of animal species, and proposed the use of the mitochondrial DNA gene cytochrome oxidase subunit I (cox1/ COI) as a global bio-identification system for animals. This method has proven its efficacy in fish species identification, both freshwater and marine species. However, due to the lack of reference sequences, there is still a long way to go before we could identify every matured fish, larva, egg or organ by their barcode sequences (Wang et al., 2012).

Ghana's waters, both marine and freshwater serve as habitat for diverse fish species (Dankwa et al., 1999; http://www.fishbase.org). However, the management of these species is challenged by inadequate information on the specific species available which subsequently affects management policies (Ministry of Fisheries and Aquaculture Development (MoFAD), 2015). Traditional method of fish identification based on the morphology is the technique applied in fish identification in Ghana (Dankwa et al., 1999). This review aimed to provide an overview and potentials of Ghana's fishery sector and to access the current and future state of DNA barcoding as a fish identification tool for Ghanaian fish species with a secondary aim to establish a DNA barcode reference library for utilization in biodiversity assessment and conservation for the entire country.

GHANAIAN FISHERY SECTOR

Ghana is a country located in West Africa on the GPS coordinates of latitude: 7° 57' 9.97" N Longitude: -1° 01' 50.56" W (https://latitude.to/map/gh/ghana). The southern part of the country is bordered with the Gulf of Guinea and Atlantic Ocean. The Volta Lake, an artificial lake, is also located in the heart of the country (van Zwieten and Kolding, 2011). These waters, as well as other minor water bodies within the country, serve as sources for capture fisheries.

Based on observation, almost all Ghanaians diets include fish as a source of proteins and micronutrients. Additionally, the fishery sector plays a substantial role in contributing significantly to national economic development objectives related to employment, livelihood support, poverty reduction, food security, foreign exchange earnings and resource sustainability development of Ghana (MoFAD, 2015). The capture fishery industry is based on resources from the freshwater (inland), marine and estuaries (coastal lagoons) sources. According to the MoFAD (2017) report, fish production was about 87.2% for capture fisheries (marine: 71.1% and in-land: 16.1%) and 12.8% for aquaculture. Marine fishery contributes significantly to Ghana's economy, accounting for about 4.5% of the Gross Domestic Product (GDP), 12% of the agriculture GDP and 10% of the workforce (Chauvin et al., 2012). Aquaculture, on the other hand, is still growing as compared to other countries. The capture fisheries sector in Ghana is limited by a number of factors such as seasonal fluctuations of fish abundance; implying that income from fishing is also unstable, poor landing sites, post-harvest losses, poor equipment base and a lack of refrigeration facilities (Gordon et al., 2013), and climate change (Mohammed and Uraguchi, 2013). Other principal concerns are fish stocks overexploitation, leading to the decline of harvests and inadequate information about fish identification, fish biology leading to the formulation of poor conservation and management strategies (Zemlak et al., 2009). Moreover, there is no detailed knowledge about the abundance, diversity, and distribution of fish species in wild Ghanaian waters.

Ghana's waters are biologically diverse environment with different ecological habitats and niches which harbor several different fish species. For marine ecosystem, species are broadly classified in four classes based on their bathymetric distribution: small pelagic-*Sardinella aurita, Engraulis encrasicolus, Ethmalosa frimbiata*; large pelagic species-*Katsuwonus pelamis, Auxis thazard, Thunnus obesus*; coastal demersal species-*Dantex* species*, Pagellus bellottii, Pagrus ehrenbergi*; and deep-water demersal species-*Sepia officinalis, Epinephelus* species (Ago and Ofori-Adu, 2005). The main commercial species targeted in Ghanaian waters are Clupeid (Sardinellas, Scombridae - chub - mackerels and Engraulidae (anchovies). The large pelagic species represent the Thunidae whereas the demersal species are: Sparidae, Lutjanidae, Mullidae, Pomadasydae, Serranidae, Polynidae and Penaeidae (Mensah et al., 2003).

Within the in-land sector, Volta Lake is the major source of freshwater fish. Other sources are Black Volta River, White Volta River, Lake Bosomtwe, Barekese Reservoir (MoFAD, 2015) and minor water bodies. Common species among the landings are various Tilapia species and *Clarias* species among others. In a study conducted by Dankwa et al. (1999), the Volta Lake hosts about 121 fish species. Common freshwater species landed from the lake are various species of *Tilapia, Chrysichtys, Synodontis, Mormyrids, Heterotis, Clarias, Bagrus, Brychinus, Barbus, Alestes, Labeo, Parailia, Schilbe, Sarotherodon, Distichodus,* and *Citharinus* species and the Nile perch (*Lates niloticus*) (Dankwa et al., 1999). According to FishBase (http://www.fishbase.org), about 491 marine species and 212 freshwater species have been discovered and identified within Ghana waters as at December 2018. Majority of these species are native while few others migrated from neighboring countries.

Irrespective of the many benefits derived from the Ghanaian capture fishery, anthropogenic activities pose a serious threat to its sustainability. This is evidenced by pollution of water bodies, reclamation wetlands due to urbanization, the reduction in catches over the years and the extinction of some species as reported by the local fishers (MoFAD, 2015). Also, taxonomic knowledge is still incomplete and scattered in the scientific literature (Hubert et al., 2015). Therefore, development of new tools such as molecular genetics (DNA barcoding) for species identification is urgently needed to improve the sustainability of the exploitation of the ichthyofauna within Ghana's waters.

SPECIES RECOGNITION, IDENTIFICATION, AND FAMILY GROUPING

Essential for the sustainable development of fish biodiversity for Ghanaians is to generate accurate knowledge of the identity, evolution, and the geographic distribution (Berkes et al., 2000) of Ghana's fish species. Unfortunately, such important information is often only partially or not available for taxonomists and other stakeholders. Ghana's waters are endowed with several species including cryptic species. The documentation and description of these species have significant implications for fishery resource management and conservation. Moreover, cryptic species require special attention in conservation planning, especially for endangered species complex. Because the species that are considered as endangered might be composed of multiple species that are even fewer than previously supposed and different species might require different conservational strategies (Bickford et al., 2007).

DNA barcoding is the system that can provide accurate, fast and automatable species identification by using short and standardized gene regions from a fish species' whole genome (Hebert and Gregory, 2005) using GenBank as a reference.

ISSUES REGARDING FISH IDENTIFICATION IN GHANA AND THE NEED FOR DNA BARCODING

The traditional method of fish identification is based on external morphological features comprising body shape, the pattern of colours, scale size and count, number and relative position of fins, type of fin rays, gill, otoliths and geography (Granadeiro and Silva, 2000). This method of identification has proven not to be effective as it is unable to differentiate a fish species during its egg or larval stages (Zemlak et al., 2009). Even when the undamaged adult sample is available, the morphological characters alone are not enough to identify it sometimes resulting in taxonomic doubt (Lakra et al., 2016). In the event of phenotypic plasticity among fish, morphological based identification may provide a less accurate result (Pavan-Kumar et al., 2016). Moreover, the traditional method does not consider the genetic composition of an organism for the variability of the characters used for species recognition and identification. As a result, species misidentification, an erroneous grouping of different taxa and faulty synonymous taxon names occur. This may lead to paying less attention to species which need immediate conservation strategies. Also, the existing cryptic species are overlooked (Zemlak et al., 2009; Hubert et al., 2012). Furthermore, it results in giving less priority to species which are needed to be conserved and also overlooks morphologically cryptic taxa that are common in many fishes species (Hubert et al., 2008). These associated challenges with the traditional technique of fish identification have resulted in the invention of genetic-based species identification method called DNA barcoding (Mitochondrial DNA).

Grouping of fishes is sustainable in the same species group if their records are not based on inaccurate sub-infra species-level identifications and validation (Halford and Marko, 2004). In a case of large-scale fishery surveys, this issue becomes complicated, many taxonomic experts may be required to identify specimens from a collection (Ward et al., 2009). In such a situation, it is expensive to assemble and deploy such teams and to distribute specimens for identification. Moreover, it is time-consuming. Also, accessing the historical literature and assessing the validity of species with a controversial taxonomic history are challenging tasks (Ward et al., 2009), even for well-trained taxonomists. For a novice faced with an assemblage of unknown specimens, identification can be challenging and even be close to an impossible task unlike the use of the molecular method. This taxonomic issue generally hinders the assessment, conservation, and management of Ghanaian fish biodiversity.

Again, new species are landed onshore occasionally which require to be identified for scientific study and resource management. This indicates that the application of DNA barcoding is highly required in fisheries sectors for proper species authentication (Rasmussen and Morrissey, 2008).

BENEFITS OF DNA BARCODING IN FISHERY MANAGEMENT AND CONSERVATION

DNA barcoding assists in formulating conservation policies by rapidly assessing the biodiversity at low cost, and this information help prioritize conservation areas or evaluate the success of conservation actions (Krishnamurthy and Francis, 2012). Prioritization of different ecosystems for conservation depends on information of species diversity, its richness and value. Phylogenetic diversity (PD) is an indicator that measures taxonomic divergence between species and an index of phylogenetic diversity can appraise conservation strategies by ignoring tedious species counts and using evolutionary lineages (phylogenies) to boost predictions about biodiversity patterns (Mitchell et al., 2008). Barcoding plays an essential role in terms of phylogenetic (Hajibabaei et al., 2007). Faith and Baker (2010) showed a potential role of DNA barcoding in PD assessments for biodiversity conservation strategies.

CURRENT STATUS AND FUTURE DIRECTION OF DNA BARCODING IN GHANAIAN FISHERIES

With the advent of polymerase chain reaction (PCR) and the reduced cost of DNA barcoding, DNA sequencing has become ubiquitous (Margulies et al., 2005) especially in developed countries. DNA barcoding has gained considerable validation as a key tool for accurate species discovery and identification (Bhattacharya et al., 2015). Moreover, the emergence of different bioinformatics software tools has increased the taxonomic coverage of nucleic acids sequences confined in DNA sequence libraries, also known as GenBank (Bucklin et al., 1999).

Several scientific studies have demonstrated its effectiveness in identifying both marine and freshwater fishes (Eischeid et al., 2016). However, DNA barcoding is still in its new in the third world. Currently, in Ghana, this molecular method of fish identification is in fragments. This can be attributed to the slow pace development in the area of scientific research as a developing country. Again, the lack of resources to carry out such project is another factor hindering molecular method of fish identification. Therefore, Ghanaian waters are fertile grounds for the introduction of DNA barcoding for fish identification and subsequently formulate informed conservation and management strategies to save the deteriorating Ghanaian capture fishery.

As important as DNA barcoding is, there is a need for researchers to consider conducting studies on some of the important fish species in Ghana. For instance, there have been speculations regarding the introduction of tilapia into Ghana believed to have originated from Asia. It will be prudent to conduct studies and characterize the tilapia species in Ghana and generate DNA barcodes for easy identification and comparisons.

CONCLUSION

DNA barcoding has been accepted as a bio-identification system for living organisms globally. It is an essential tool to vouch for species identification and discovery of new 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 (Hubert et al., 2008). This system is fast and produces accurate species identification by using standardized short DNA sequence. It is the simplest way to identify an unknown specimen by comparing its COI sequence generated with the Barcode of Life Database (BOLD) identification engine or data from GenBank (Ward et al., 2009).

Based on successes of studies conducted on DNA barcoding as fish identification, it is the hope to save Ghana’s fishery. It has the efficacy to be adopted as a new tool to identify fish to make scientific conservation and management strategies to protect Ghanaian fishery.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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