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CURRENT DEVELOPMENTS IN THE APPLICATION OF DNA BARCODING TO SOLVING BIODIVERSITY CONSERVATION PROBLEMS IN DEVELOPING COUNTRIES

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ABSTRACT

A DNA barcode is a genetic signature that occurs naturally within the genome of every living species. One of the gene regions commonly used for all animal groups is a 648 base pair region in the mitochondrial cytochrome oxidase 1 gene (CO1), it has been effectively used in identifying birds, flies, butterflies, fishes and many other animal groups due primarily to the high polymorphisms among species. However, CO1 is not effective in identifying and distinguishing plants because it changes too slowly in plants. Currently, two gene regions in the chloroplast, **MatK** and **rbcl** are employed in bar-coding land plants. In 2003, Paul Herbert and research group published a paper entitled "Biological Identification through DNA barcodes, which created awareness among scientists (taxonomists in particular) on the usefulness of DNA Barcode as an effective technique for identification of species. In the one decade of research after this publication, DNA barcode has evolved rapidly into a tool that can be employed for solving many environmental, agricultural, health and conservation problems around the globe. It also has applications in disease and pest control, market fraud detection and protection of endangered species. Some developing countries like Nigeria are known for their rich biodiversity, but technology is very low in area of conservation and management of these biological resources. This paper reviews the current developments in the application of DNA Barcoding to solving biodiversity conservation problems and its adoption in developing countries.

Key words: *DNA Barcode, mitochondrial cytochrome oxidase 1 gene (CO1), MatK and rbcl*

1.0 INTRODUCTION

Developing countries are described according to their Gross National Income (GNI) per capita per year. Countries with GNI of US$11, 905 and less are defined as developing (World Bank, 2013). There are 145 countries in this category and they include Algeria, Bangladesh, Haiti, Morocco and Nigeria etc. These countries have lower standard of living, undeveloped industrial base and low Human Development Index (HDI) when compared to developed countries (Sullivan and Sheffrin, 2003). The strength of most of the developed countries lies not in the richness of their biodiversity but in the application of science-based research and development to conservation, utilization and management of their biodiversity (Onyia *et. al.*, 2009).

According to DFID *et. al.* (2002), poor people tend to be most dependent upon the environment and the direct use of natural resources and therefore most affected when their access to biodiversity is limited or denied (Isoun, *et. al.*, 2009). A country's biodiversity is a valuable and irreplaceable resource because its extinction is forever. Unfortunately, developing countries in the tropics, which are repositories of agro-bioresources, have remained the areas of low productivity and high population density. Many of them in the Sub-Saharan Africa fall within the "hunger belt" of the world due to poor scientific and technological development of their natural resources.Knowledge about biodiversity and the ability to identify organisms (taxonomical knowledge) that comes with it are global public goods.

Taxonomy is the science of classifying living things according to shared features. Biological classification is a system of binomial nomenclature that assigns each organism a genus and species name. Species identification and conservation are becoming more important with the current issues in global climate change, habitat destruction and genetic erosion. According to Scientists, the yearly rate of extinction has increased from about one species per million to 100 -1,000 species per million. Conventional taxonomists are therefore facing the challenge of cataloging the huge biological diversity before it disappears.

Nigeria is known for its rich biodiversity but she is loosing some of its flora and fauna not only to extinction but to unguided and illegal trading of endangered species, climate change and commercial development/ urbanization. For example, elephant tusk being used as ornaments, some wild animal skin used for decorations in homes and leather accessories. Some are processed into powder to prevent easy detection, and some plants have their genes sequenced and taken out of indigenous countries just to be processed and brought back at a very high cost for the people to buy as drugs. According to CBOL, (2011), Sampling, identifying, and studying biological specimens are among the first steps toward protecting and benefiting from biodiversity. In line with the United Nation's Millennium Development Goals (MDG), DNA barcoding could assist in achieving some of these goals by controlling agricultural pests to reduce poverty and hunger (MDG1), identifying disease vectors to combat disease (MDG6) and environmental sustainability (MDG7).

DNA barcoding is a tool that is aimed at providing an efficient process for species-level identifications and thereby contributing effectively to taxonomic and biodiversity research. DNA barcoding was first discovered in the scientific community in 2003 when a research group led by Paul Herbert at the University of Guelph made a publication on a paper

titled "Biological identifications through DNA barcodes". He described it as a system proposed for species to have same features where we can use a machine to read the DNA of any organism like in the case of a supermarket scanner where the cashier does not have to read the label but just scan the black stripes of the Universal Product Code (UPC) as shown in fig. 1. It is a tool used in the rapid identification of species based on the extraction of a DNA sequence from a small tissue sample of any organism.

Fig1: Zebra and Barcode (BBC 2009)

For the past several years, DNA sequences have been used to build evolutionary history of animals and plants. However, the DNA regions that they use are chosen to enhance the resolution for their specific experimental purposes.

2.0 GENE REGIONS FOR DNA BARCODING

A hypervariable region (HVR) is a particular spot within nuclear DNA in which base pairs of nucleotides repeat. Changes in hypervariable regions are highly polymorphic. A 648 base pair region (hypervariable region) in the mitochondrial cytochrome c oxidase 1 gene (CO1) is the gene region that is used as the standard barcode for almost all animals (Herbert et al. 2003). This region is of potential importance for facilitating inventories of biodiversity and performing species' identification. CO1 is effective in identifying birds, flies, butterflies, fish and many other animal groups. CO1 is short enough to be sequenced quickly and cheaply, and long enough to identify variations among species. CO1 is **not** effective in identifying plants because it evolves too slowly.

In the publication of Peter Hollingsworth titled "choosing and using a plant DNA Barcode" in 2011, "establishing a standardized DNA barcoding region in plants has been more challenging". As a result, two regions of DNA have been chosen to form the plant barcode- portions of the genes rbcL and matK in chloroplast gene. However, it is not clear whether this combination provides the resolution required to identify most plants to the species as additional regions may be necessary for certain taxa of plants (CBOL Working Group 2009).

RBCL is an acronym for Ribulose-1, 5-bisphosphate carboxylase oxygenase, abbreviated as RuBisCO. It is an enzyme involved in the first step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants to energy-rich molecules such as glucose. It catalyzes the carboxylation of ribulose-1, 5-bisphosphate and probably the most abundant protein on earth. MATK is an acronym for Megakaryocyte-associated tyrosine-protein kinase, an enzyme that in humans is encoded by the *MATK* gene.

2.1 The barcode production pipeline

According to Barcode of Life (2010), there are four factors of barcoding namely:

- The specimens: these are biological materials stored in repositories such as the field, National Parks, Botanical Gardens, zoological Gardens / Zoos, seed banks, National Herbaria and Gene Banks
- The Laboratory analysis: it is done in molecular biology labs where DNA is extracted from the specimen of any organism in line with laboratory protocols, DNA amplification is done with PCR machine, The PCR product sequencing – the sequence is represented by 4 nucleic acids namely – Cytosine, Adenine, Thymine, Guanine. The data are then kept in database for subsequent analysis.
- The Database: it is a reference library where unknown specimens could be assigned to known species. The two main barcode databases are the International Nucleotide Sequence Database Collaborative (GENBANK) and the Barcode of Life Database (BOLD).
- Data analysis: specimens are identified by finding the closest matching reference record in the database.

2.2 Adoption of DNA Barcoding

Many developed countries adopted DNA Barcoding technique as a molecular tool for species identification shortly after the Paul Herbert's publication in 2003.Subsequently, groups and outreach programs emerged such as: International Barcode of Life (iBOL),Consortium for Barcode of Life (CBOL) etc. Projects such as Encyclopedia of all butterflies / all pollinators project, Fish BOL, Fungi / Tree BOL etc were organized.

2.2.1 Trends in Developed World

DNA Barcoding is making news around the world. As a research tool for taxonomists, it assists in identification by increasing the ability to diagnose species by including all life history stages of an organism. As a biological tool, DNA barcoding is being used to tackle fundamental ecological and evolutionary questions. DNA Barcoding developed by University of Guelph researchers in Canada has proven up to 88 per cent efficient in authenticating natural health products (Sciencedaily 2012). This study has helped in regulating the health product industry. In Brazil, DNA Barcoding has helped in convicting illegal traffickers/ loggers behind the Amazon's environment crime wave (The Scotsman, 2012).

In recent times, some violins were recovered which the police believe is from an endangered tree that gave Brazil its name. DNA Barcoding is a tool used by Agricultural Research Service scientists to control and monitor insects that create threats to crops as diverse as potatoes, wheat and barley (USDA, 2012).

DNA barcoding played a vital part in the investigation of the bird strike involving US Airways Flight 1549 in 2009. Biodiversity Institute of Ontario analysed samples retrieved from the air craft and the DNA barcodes corresponded to *Branta Canadensis*, the Canada goose (Steinke, 2014)

2.2.1.1 Other Applications of DNABarcoding

- •Re-discovering lost species: some species of fauna and flora that were rarely seen were rediscovered through barcoding. Example is the predatory water beetle *Graphoderus bilineatus* that was thought to have gone into extinction for 26years.
- •Cataloguing hidden diversity: groups of plant and animals with very similar features have been found to be of distinct species through barcoding
- •Discovering diagnostic differences: DNA barcoding has helped taxonomists to discern species that have been misclassified into other groups.
- •For safeguarding public health
- •Used in developing standardized methods for bioassessment in environmental monitoring
- •Used in untangling names when morphologic features of the defining original are lost
- •Used in finding facts from fragments
- •Protecting consumers from mislabeling

2.2.2 Trends in Developing World

In the research work of Contreras Gutiérrez *et. al.,* (2014) titled "DNA barcoding for the Identification of Sand Fly Species (Diptera, Psychodidae, Phlebotominae) in Columbia, Sand flies are of medicinal importance and differ in ecology, geographic distribution and pathogen transmission in Columbia. The presence of sand fly species is important for predicting risks and observing the increase of diseases which sand flies can transmit. However, some species' important morphological similarity, especially among females, may cause difficulties during identification process. Species identification is intricate because it requires a considerable degree of skill and taxonomic expertise. DNA barcoding has turned out to be an increasingly useful and promising tool for projecting Sand fly diversity and for ensuring the rapid and precise identification of species thereby enhancing control measures.

According to Mankga *et. al*., (2013) research work "Efficacy of the core DNA barcodes in identifying processed and poorly conserved plant materials commonly used in South African traditional medicine", DNA barcoding was used to identify commonly used medicinal plants in South Africa. South Africa is a country with rich tropical and temperate flora, of about 24, 000 species that account for more than 10% of the world's vascular plants (Germishuizen and Meyer 2003). Approximately 3,000 of this unique diversity are used as medicines, with a greater number exported to other countries (Van Wyk *et. al*., 1997). Given the increasing pressure on medicinal plants, there is need for increasing commitment towards proficient controls that can help preserve medicinal plant diversity in South Africa. DNA barcoding which is a reliable tool for identification is being used to reach this objective.

3.0 ADOPTION OF DNA BARCODING IN NIGERIA

CBOL had an outreach visit to Nigeria in 2005 to sensitize the government on the use of DNA barcoding for rapid species identification for the purpose of protecting her biodiversity and benefit sharing in case of product development by third party. A number of Research Institutes / Universities in the country became members of CBOL from 2006 and a few joined have the iBOL (international Barcode of Life). Nigeria in collaboration with CBOL organized the first Regional workshop on DNA barcoding of national biodiversity for West and Central Africa in October 2008. This opened the door for the adoption of DNA barcoding as a veritable tool for biodiversity conservation and training.

The first national DNA barcoding project involving several Federal Ministries, Departments, Agencies, and some Universities is on-going. The project's goal is to use DNA barcode as evidence in court for prosecution of violators of Convention on International Trade in Endangered Species (CITES). This project, referred to as Barcode of Wild Life Project (BWP), is being sponsored by Google Impact Award and coordinated by CBOL, worldwide.

4.0 CONCLUSIONS

DNA Barcoding is as important in developed countries as it is in developing nations, like Nigeria for preventing the extinction of endangered species and poaching of our useful biodiversity such as medicinal plants and parts of wild mammals (e.g. elephant tusks) which are taken elsewhere, developed into useful products and exported back for trade at huge costs. With DNA barcoding, processed items can be identified and traced back to place of origin, thereby checking illegal trade and providing opportunity for benefit sharing among nations.

Grants such as the Google Impact Award and awareness on the application of DNA barcoding would help developing countries with low technology in biodiversity conservation and management.

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