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Short Introduction of Dna Barcoding

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ABSTRACT

DNA barcoding is a system for fast and accurate species identification. It's creates ecological system more accessible by using short DNA sequence instead of whole genome and is used for eukaryotes and prokaryotes. The short DNA sequence is generated from standard region of genome known as marker. This marker is different for various species like CO1 cytochrome c oxidase 1 for animals, matK and rbcL for plants and Internal Transcribed Spacer (ITS) for fungus. It has many uses in various fields such as agriculture, sustaining natural resources, protecting endangered species, water quality, preserving natural resources, identification of medicinal plants.

Key words: matK, ITS, rbcL, taxonomy mitochondrial gene, PCR primers

INTRODUCTION

DNA barcoding is a taxonomic method in which a short genetic marker to identify DNA to which organisms or a particular species it belongs. It helps to identify an unknown sample in terms of a preexisting classification. Barcodes are used in an effort to identify whether unknown species in sample should be combined or separated. The most commonly used barcode region in animal is a segment of mitochondrial gene cytochrome oxidase I (COI) that approximately contains 600 base pairs. Applications include identifying plant leaves due to absences of flowers or fruit, helps to identifying larvae stages of insects, which may not have significant characters than adults and are less well known , identifying the nutrition level of an animals , and identifying products of herbal supplements , wood ,or skin and other animal parts[1].

specific locus should be standardized in DNA barcoding , present in most of the texa of interest and sequenceable without specific PCR primers , short time to be easily sequenced with current technology , and provide a large variation between species yet a relatively less variation within a species. A several loci set as standardized regions were selected by the respective committees. For

animal and many other eukaryotes, the mitochondrial COI gene for plant, the concatenation of the rbcL and matK are chloroplast genes [2]. These genes are providing poor resolution for land plants, and the regions to be assessed that could complement rbcL and matK. In fungi, the internal transcribed spacer region. It can be applied in algae, animal and also fungi, perhaps to a lesser degree due to a lower incidence of hybridization compared to higher plants [3].

Population genetic studies can be done by these techniques and have large numbers of specimens at their disposal when the DNA quality is a lesser concern, and high-quality DNA samples gives more accurate in barcoding techniques would depend.

To improve species concepts, It's to be develop a more sophisticated approach to barcoding, which would ideally include sequences from multiple independent markers, a multi-locus barcode, and specific inference tools that could be used to be limits and identify genetic 'gaps', and also improve the information base depend on cruder plastid and mitochondrial DNA barcodes [4].

Molecular analyze data is says that several barcodes DNA regions are suitable in plants. It has four significant limitations in identification of species. First, both phenotypic plasticity and genetic variability in the characters employed for species recognition can leads to incorrect identifications. Second, the morphologically cryptic taxa, which are common in many groups [5]. Third, since morphological keys are often effective only for a particular life stage or gender, many individuals' species cannot be identified. Finally, the modern interactive versions represent a major advance; the use of keys often demands in high level of expertise that misdiagnoses are common [6].

Figure1: DNA based Identification system

Maturase K gene (matK)

The chloroplast maturase K gene (matK) is, with the exception of some ferns, situated

within an intron of the trnK gene. The gene is approximately 1535 basepair long in monocots and is the only chloroplast-encoded group II intron maturase. Universal primers situated in the trnK gene are used to amplify the entire gene region for phylogenetic studies in orders or families, but are sometimes effectively used on genus or species level, i.e. in the genus Paeonia (Paeoniaceae)[7]. Only 600-800basepair regions of the matK gene are utilized for DNA-barcoding purposes. The matK gene evolves fast (three times faster than rbcL and atpB) and some studies suggest it can effectively discriminate between species in the angiosperm [8].

***Figure* 2: The matK chloroplast coding region based on the schematic drawing of Wakasugi*et al.* (1998), Matsumoto *et al.* (1998), Shaw *et al.* (2005) and Barthet&Hilu (2007)**

Internal Transcribed Spacer (ITS)

Refers to the insert DNA to be found between the small-subunit and large-subunit of ribosomal RNA (rRNA) genes in the chromosome or the corresponding transcribed region in the polycistronic rRNA precursor transcript. In bacteria and archaea, ITS issituated between the 16S and 23S rRNA genes [9]. Then again, there are two ITS's in eukaryotes; ITS1 is located between 18S and 5.8S rRNA genes, while ITS2 is between 5.8S and 26S (in plants, or 28S in animals and fungi) rRNA genes. ITS1 relates to the ITS which in bacteria and archaea, while ITS2 created as an insertion that disturbed the familial 23S rRNA gene. In Bacteria and Archaea, theITStake place in one to several copies, as do the neighboring 16S and 23S genes. When there are multiple copies, these do not occur in line to one another. Fairly, they occur in discrete locations in the circular chromosome[10].

In eukaryotes, genes encrypting ribosomal RNA and inserts occur in cycle repeats that are thousands of copies long, each separated by regions of non-transcribed DNA

termed *intergenic spacer* (IGS) or *non-transcribed spacer* (NTS).

Each eukaryotic ribosomal cluster contains the 5' external transcribed sequence (5' ETS), the 18S rRNA gene, the ITS1, the 5.8S rRNAgene, the ITS2, the 26S or 28S rRNA gene, and finally the 3' ETS[11].

During rRNA maturation, ETS and ITS pieces are eliminated as non-functional by-products of this maturation, they are rapidly degraded.[12]

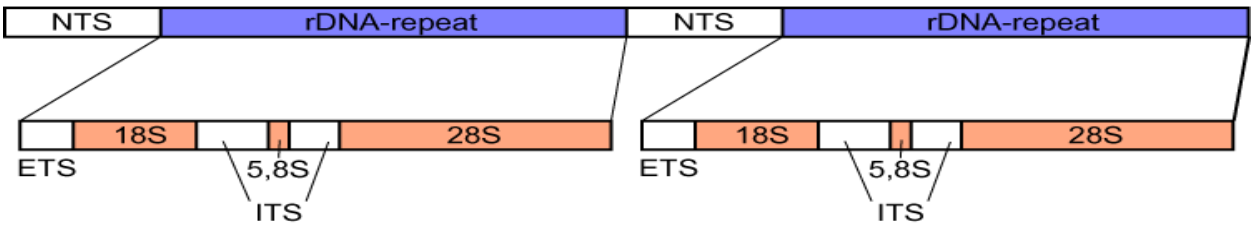

Figure: Organization of the eukaryotic nuclear ribosomal DNA tandem repeats.

Sequence evaluation of the ITS region is widely used in taxonomy and molecular phylogeny because of several favorable properties:

- It is routinely amplified thanks to its small size associated to the availability of highly conserved flanking sequences ;
- It is stress-free to discoverlevel from small quantities of DNA due to the high copy number of the rRNA clusters ;
- It undergoes rapid concerted evolution via unequal crossing-over and gene conversion. This endorses intra-genomic homogeneity of the repeat units, even if high-throughput sequencing showed the occurrence of frequent variations within plant species.
- It has a high degree of variation even between closely related species. This can be explained by the relatively low evolutionary pressure acting on such non-coding spacer sequences [13],[14].

RBCL

The chloroplast gene *rbc*L, which codes for the larger unit of Ribulose-1, 5-bisphosphate carboxylase (RuBisCO). Which broadly used as an appropriate locus for analysis of phylogenetic in plant taxonomy[15].Ribulose-1,5-bisphosphate carboxylase/oxygenase, commonly known by the abbreviations RuBisCO, RuBPCase,

or RuBPco, is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants and other photosynthetic organisms to energy-rich molecules such as glucose. In chemical terms, it catalyzes the carboxylation ofribulose-1, 5-bisphosphate (also known as *RuBP*). It is probably the most abundant enzyme on Earth [16][17].

Cytochrome c oxidase I

Cytochrome c oxidase I (COX1) also known as mitochondrially encoded cytochrome c oxidase I (MT-CO1) is a protein that in humans is encoded by the MT-CO1 gene.[18]

In other eukaryotes, the gene is called COX1, CO1, or COICytochrome c oxidase I is the main subunit of the cytochrome c oxidase complex It is a gene that is often used as a DNA barcode to identify animal species[19].

MT-CO1 gene sequence is suitable for this role because its mutation rate is often fast enough to distinguish closely related species and also because its sequence is conserved among conspecifics. Contrary to the primary objection raised by skeptics that MT-CO1 sequence differences are too small to be detected between closely related species, more than 2% sequence divergence is typically detected between such organisms, suggesting that the barcode is effective. In most if not all seed plants, however, the rate of evolution of cox1 is very slow[20].

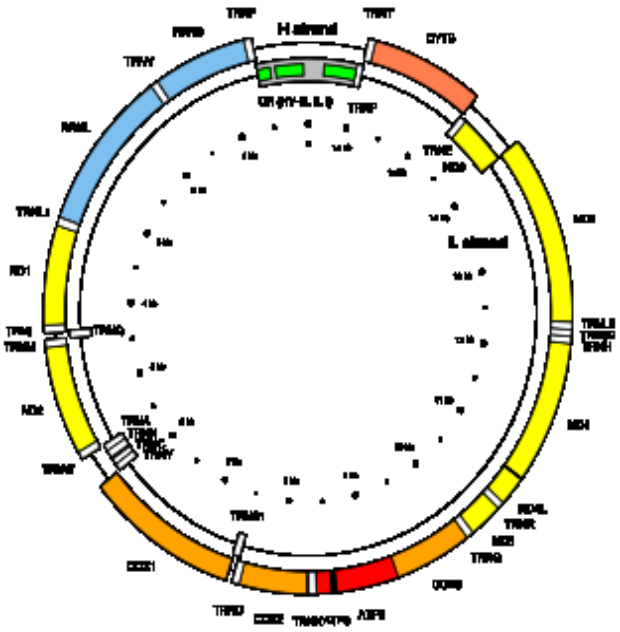

Figure: Location of the MT-CO1 gene in the human mitochondrial genome. MT-CO1 is one of the three cytochrome c oxidase subunit mitochondrial genes (orange boxes).

REVIEW OF LITERATURE

Hebert *et al.,* (2003) implies the employment of sequences of DNA for the diagnosis of a species. By their research they emphasize the use of mitochondrial gene cytochrome c oxidase I in the global identification system, to understand the diversity of life and also to study

molecular evolution. They created three COI profiles, for seven phyla of animals, for eight for largest order of insects and three for two hundred closely allied species of lepidopterans to provide an overview of COI diversity. They demonstrated that difference in COI sequences were sufficient to assign organisms to their taxonomic categories providing resolutions that cannot be obtained through morphological analysis. Joly *et al.,* (2014) considered the several reviews on DNA barcoding and presents the potential uses of DNA barcoding in eco-informatics, community ecology, invasive species, macroevolution, trait evolution, food webs, trophic interactions and spatial ecology. They suggested that DNA barcoding would also lead us to understand interactions between species and the flow of energy in a food web. Hebert *et al.,* (2004) tested the effectiveness of barcoding using the COI gene in 260 bird species of North America for the purpose of identification and discrimination. The large COI sequence variation concluded that the variation within closely related species were higher than variation the within species.

Ward *et al.,* (2005) barcoded 207 species of Australian Marine fishes and were able to differentiate one from the other by cox I barcoding. Their results showed some phylogenetic relation that species invariably clustered within genera and genera clustered within families. Lowenstein *et al*., (2010) has used DNA barcoding in the identification of commercial fishes (tuna) classified under the genus *Thunnus*and addressed the issue of public health by estimating the level of mercury in this specific species and also banned the trade of certain critically endangered species that were in the menu in certain sushi restaurants of New York. Nwani*et al.*, (2011) made an application of DNA barcoding in order to obtain detailed facts on the distribution of fresh-water fish species in Nigeria. They also established a reference library for the use of information in biodiversity evaluation and conservation giving the remark that DNA barcoding could also help in food research and market analysis. Triantafyllidis*et al.*, (2011) analyzed the diversity of fish species in four Greek lakes using DNA barcoding with an objective to conserve and manage the threatened species. They examined 37 species and found deep divergences among the specimens collected from the four lakes. De Carvalho*et al.,* (2011) studied the diversity of COI sequences among 100 species of fish in the Sao Francisco River basin to evaluate the efficacies of barcoding in the differentiation of species. They found deep

intra-specific divergences within nine species and discovered several new species and genera**.** Luiz *et al*., (2011) proved the efficiency of DNA barcoding in the detection of hidden biodiversity in the Upper Parana Basin of Brazil. Their study showed that the fish *Piabinaargentea*represented a minimum of five new species of fish suggesting that geographic isolation possibly enabled the formation of new species.

Ward *et al*., (2008) studied 191 species of echinoderms that included five classes and concluded the barcode sequence; COI that comprises of 657bp is an effective, accurate and useful method for the diagnosis of all the five classes of Echinodermata.

Radulovici*et al.*, (2009) used DNA barcoding for species identification of metazoans including crustaceans in the Estuary and Gulf of St Lawrence .They concluded that genetic distances between species were 25 times higher than within species.

Their research also led to the identification of invasive *Amphipods* in St. Lawrence estuary marking the importance of this system of COI gene identification.

Hogg *et al.*, (2007) evaluated the sequence diversity in the mitochondrial cytochrome-c oxidase 1 gene as a tool for resolving differences among species of Arctic springtails. Foottie*t al*., (2008) using DNA barcoding were able to discriminate nearly 300 species of aphids (*Hemiptera: Aphidae*) and concluded that 96% were well differentiated though sequence variation was low.

The complex life cycles of the aphids and their parthenogenic mode of reproduction did not prove to be a barrier. Sheffield *et al*., (2009) used DNA barcoding which is a reliable and rapid means of species-level identification for ecological studies of bee communities. Their work led to the identification of two undescribed genetically unique species of bee. Utsugi*et al*., (2011) with respect to the extreme diversity of insects and their ecological, epidemiological and agricultural importance imply that this technique of DNA barcoding has attracted the attention of many taxonomists, agriculturists, conservation biologists and ecologists. Ander *et al.,* (2012) sequenced and studied the COI gene of 237 specimens of biting midges (*Culicoides*), who were insect vectors of veterinary diseases and by their study found that there was deep intraspecific

divergence among the 237 specimens and were able to differentiate 95% of the species.

Ng'endo*et al.,* (2013) sequenced the mitochondrial DNA Cytochrome oxidase subunit 1, COI gene from 47 ants of the genus *Pheidole*. Their work resulted in significant findings where most sequences clustered into well differentiated groups and the sequences in a cluster were quite distinct. Smith *et al.,* (2013) used DNA barcoding and studied the identification of microgastrine wasps that are parasitoids of caterpillars, the host-parasitoid biology and also ecology. Because of their use as biological control agents the study resulted in biological control programmes, description of new cryptic species and taxa. Smith *et al*., (2014) performed barcoding on a group of arthropod, the ants of Madagascar, which exhibited hyper diversity.

These high divergences furthermore paved the way for detailed genetic, morphological and behavioral studies. They barcoded 280 specimens belonging from 28 genera and derived vast diversity patterns across the locality of Madagascar. Kumar *et al.,* (2007) barcoded DNA sequences of mitochondrial cytochrome oxidase gene and were able to identify 62 species out of 63 specimens. Wiemers*et al*., (2007) did a study on blue butterflies belonging to the family *Lycaenidae*and found that inter specific variation exceeds intraspecific variation and found the absence of a barcode gap. They were able to identify several cryptic species that which did not differ phenotypically from the others. They gave the impression that minimum distances between species are critical and not average distances. Their success rate was 58%.Hajibabaei *et al.,* (2006) were able to effectively discriminate three families of tropical lepidopterans using the mitochondrial cytochrome c. Their result showed barcoding of the COI gene helped to distinguish 97.9% of the specimens.

Kronestedt*et al.,* (2010) proposed a new genus *Draposa*for the former Genus *Pardosa*that consisted of eight species of wolf spiders in the Indo-Malayan region.

Barrett *et al*., (2005) worked on DNA based identification and established the potential of COI as a rapid and accurate identification tool for biodiversity survey of spiders. Astrin*et al*., (2006) worked on the identification of species using species identification methods COI and 16 sRNA. Binford*et al.,* (2008) studied that Phylogenetic relationships of *Loxosceles*and *Sicarius*spiders are consistent with western GondwananVicarience using the help of

molecular dating analyses of 28S, COI, 16S and NADHI sequences.

They presented data which made evident that *Loxosceles*and *Sicarius*spiders were very old and have diversified since the separation of African and South American continents. Robinson *et al.,* (2009) has applied the knowledge of DNA barcoding for the identification of 19 species- rich genera of spiders in order to find out the existence of a barcode gap. From their study, they deduced that values of divergences were quite variable among genera. Their study also revealed maximum intraspecific divergence and suggested the collaboration of molecular and morphological identification system for global identification of spiders.

Peterson *et al.,* (2007) worked on the identification of Mexican Tarantulas by investigating the mitochondrial DNA sequence from the cytochrome - c oxidase subunit 1 gene. Identification of these species were important for International Wildlife Law enforcement and conservation, researches were done accurately without the death of other species.

Regagnon*et al.*, (2010) tested the potential of cox 1 gene in the discrimination of Helminthes (Platy helminthes: Digenea) that were *Digenean*parasites of amphibians with an objective discovering new species and accurate discrimination. They described nearly five new species of frog lung flukes out of 13 species and explained that apart from morphological diagnosis, DNA barcoding of species further enhanced the identification of intraspecific specimens.

Gu*et al.,* (2011) used DNA barcoding for the identification of the crude drug gecko, *Gekko gecko* which is valued as a traditional Chinese medicine. As a result, the population of this crude drug gecko was declining and DNA barcoding was done to identify the adulterants used instead of the crude drug gecko. Nagy *et al.*, (2013) did the first large scale reptile barcoding assessment of entire reptile fauna of the fourth largest island in the world, the Biodiversity Hotspots of Madagascar.

They used newly designed reptile specific primers and identified nearly 40 new species of snakes, skinks, chameleons and geckos. Meganathan*et al.*, (2013) utilized the knowledge of DNA barcoding for the identification of threatened crocodile species in India due to illegal hunting. They adopted DNA barcoding of the barcode region that consisted of 750bp in crocodiles in their conservation strategy. Vences*et al.*, (2014) used DNA

barcoding to understand the genetic variation in two widespread skinks from Madagascar, *Trachylepiselegans*and *Trachylepisgravenhorstii.* Although both the species were well-differentiated morphologically except for some slight differences DNA barcoding proved to be a promising tool for identification.

Clare s*et al*., (2007) sequenced the cytochrome c oxidase subunit gene 1 in 87 species of bats from Guyana for the sole purpose of studying diversity. 81% of the species showed low intraspecific variation and their sequences showed clear divergences. Six species of bats showed deep intraspecific divergences. Muller *et al.,* (2013) utilized the technique of barcoding in relation to human health issues so that it could aid in the identification and correct elimination of rodents classified under the subfamily *Sigmodontidae*. This was done in order to reduce the risk of transmission of the hanta virus through these rodent species as they are the reservoirs of these viruses.

APPLICATIONS

DNA barcoding has many applications in various fields like preserving natural resources, protecting endangered species, controlling agriculture pests, identifying disease vectors, monitoring water quality, authentication of natural health products and identification of medicinal plants.

Controlling Agricultural Pest: DNA barcoding can help in identifying pests in any stage of life making easier to control them saving farmers from cost of billion dollars from pest damage. The global tephritid barcoding initiative contributes to management of fruit flies by providing tools to identify and stop fruit flies at border.

Identifying Disease Vectors: DNA barcoding allows non ecologists to identify the vector species that can cause serious infectious diseases to animals and humans, to understand these diseases and cure them. A global mosquito barcoding initiative in building a reference barcode library that can help public health officials to control these diseases causing vector species more effectively and with very less use of insecticides.

Sustaining Natural Resources: Using DNA barcoding, natural resource managers can monitor illegal trade of products made of natural resources like hardwood trees. Fishbol is reference barcode library for

hardwood trees to improve management and conservation of natural resources.

Protecting Endangered Species: Primate Population is reduced in Africa by 90% because of bush meat hunting. DNA barcoding can be used by law enforcement to bush meat in local markets which is obtained from bush meat.

Monitoring Water Quality: Drinking water is a process resource for living being. By studying organism living in lakes, rivers and streams, their health can be measured or determined. DNA barcoding is used to create a library of these species that can be difficult to identify. Barcoding can be used by environmental agencies to improve determination of quality and to create better policies which can ensure safe supply of drinking water.

Routine Authentication of Natural Health Products: Authenticity of natural health products is an important legal, economic, health and conservation issue. Natural health products are often considered as safe because of their natural origin.

Identifying of plant leaves even if flowers or fruit are not available.

Identification of medical plants [21].

CONCLUSION

DNA barcoding is a system for fast and accurate species identification which will make ecological system more accessible. It has many applications in various fields like controlling agricultural pests, sustaining natural resources, protecting endangered species, monitoring water quality, preserving natural resources, protecting endangered species and identification of medicinal plants.

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