Review article

DNA Barcoding in Plants and Animals: A Critical Review

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Abstract

Systematics plays the most crucial role in biodiversity conservation which is at stake due to anthropogenic activities and environmental degradations. The ever-increasing decline of classical taxonomic expertise drives the need to develop molecular marker-based tools for quick, efficient, and reliable detection of organisms, to assess their ecological impacts for deepening our understanding of systematic and evolutionary relationships between organisms which is central to biology. The pace of alpha taxonomy has quickened by its integration with an increasingly fashionable and novel concept called DNA barcoding which utilizes a short genetic marker or barcode to categorize species for enhanced biodiversity assessment. As a supplementary but not complete alternative of systematics research, DNA barcoding, however, not error-free, brings precision in identification by solving existing problems of classical taxonomy and phylogenetics, irrespective of the growth stage of organisms, particularly for known taxa rather than unknown ones. Mitochondrial gene Cytochrome C Oxidase 1 (COI) serves as a universal animal barcode but there is no such universal barcode for plants and developing a suitable one is more challenging. With the recent advancement of Next Generation Sequencing (NGS), DNA metabarcoding technology is advancing rapidly. Still, ambiguity and error prevail with the correct identification of species due to some problems. After extensive analysis of the existing DNA barcoding papers, this review discusses commonly used DNA barcodes in plants and animals, their roles, advantages, limitations to solve existing problems of conservation biology and add the author's views and recommendations.

Keywords: DNA barcoding, metabarcoding, BOLD, taxonomy, biodiversity, molecular marker.

Introduction

Species identification is the fundamental step for measuring biodiversity and developing our primary understanding of the biological world. This critical attempt is somewhat hindered by the shortage of professional knowledge of classification and sometimes due to the limitation of morphology-based identification (Chase *et al.*, 2005). The traditional morphometric taxonomic study is time-consuming as it is dependent on the growth stage of the organism. Such studies are also laborious that are dependent on pre-determined classifications and expertise (Costion *et al.*, 2011; Huang *et al.*, 2015). As taxonomic expertise is declining worldwide because of scientific reductionism (Crisci *et al.*, 2020), an integrative approach becomes highly necessary for the survival of this magnificent, crucial and quintessential branch of science.

DNA barcoding is a modern marker-based approach in molecular systematics research that aims to establish a shared community resource of DNA sequences for rapid identification, effective discrimination between taxa, and authenticated classification based on molecular data (Bandyopadhyaya *et al.*, 2013). This revolutionary technique was first proposed by Hebert *et al.* (2003) by using mitochondrial cytochrome c oxidase gene (COI) as a universal animal barcode to differentiate lepidopteran species (Hebert *et al.*, 2003). However, COI doesn't work as a universal barcode in plants due to some reasons (Kress & Erickson, 2007). Therefore, a combination of chloroplast and nuclear DNA barcodes has been recommended for plants by CBOL.

The genetic barcoding approach has been implemented in different groups of organisms including vertebrates, non-vertebrates, bacteria, bryophytes, gymnosperms, algae, fungi, angiosperms, and so on to solve their problematic taxa and improve their phylogeny. DNA barcoding serves a wider range of purposes including the safety of plant and animal-based natural products used in traditional medicine, protection of endangered species from illegal trading and poaching, revealing cryptic diversity, assessing ecological impact in the community level study, forensic analysis, phylogenetic analysis, food safety, supporting ownership or intellectual property rights (IPR), etc. (Penton *et al.*, 2004; Chen *et al.*, 2010; Nithaniyal *et al.*, 2021). The recent rapid advancements of bioinformatics and molecular biology make molecular data readily available from different databases, which improve the practical and analytical scope of DNA barcoding. With the impressive progress of high throughput next-generation sequencing (NGS) technology over the years, DNA barcoding is transforming into DNA metabarcoding to eliminate the limitations of single species sequencing by enabling multi-species sequencing simultaneously in a single PCR run to overcome the previous time-consuming specimen sorting and single species dependency. Metabarcoding enables multiplexing of hundreds of taxa from large diverse groups to study species composition and estimate population size in a complex mixed community which significantly increases surveillance measures for constructive, affordable, and dynamic management responses (Piper *et al.*, 2019; Gao *et al.*, 2019).

Although molecular barcoding can help to solve the problems of phenotypic plasticity, it never can be an alternative way of classical taxonomy as it relies heavily on some predetermined threshold value of bioinformatic analysis which is not the proper way to identify a new species (Waugh, 2007). The heavy dependence on the reference database with existing sequences is another challenge of this technique. Despite these challenges, the number of DNA barcoding studies is on the rise for its benefit to detect unknown samples quickly and efficiently (Figure 1). This review aims to shed light on the role of DNA barcoding in different sectors, commonly used barcodes, recent advancements of DNA barcoding technology in different areas, and future prospects of this technology through a problem-based discussion.

Figure 1: Publications per year since 2003 registered in PubMed searching in the title, abstract or keywords using {DNA barcod*} OR {DNA metabarcod*} (Obtained 3 September 2021)

DNA barcoding: Commonly Followed Procedures

To extract DNA, the technique begins with collecting samples, including fresh samples, mixed samples, dry samples, and processed products, as per the requirement of the experiment. Then DNA is extracted from the collected samples using one or many of the available methods such as CTAB (Doyle & Doyle, 1987), SDS method, PVP method, Phenol - Chloroform method, etc. After extracting, DNA samples are amplified using appropriate markers via PCR following

the three stages- denaturation, annealing, and extension. The PCR products are then purified and band samples are used for DNA sequencing. Different types of sequencing technologies are available, such as Sanger sequencing, next-generation sequencing, third-generation sequencing technologies, etc.

After getting sequenced DNA data of the samples, similarity search begins using bioinformatic techniques and relevant databases and tools such as NCBI, BLAST, etc. Proper data analysis at that stage is considered crucial for generating a new barcode as well as reporting a new record for an existing barcode (Yu *et al.*, 2020). If the similarity search becomes statistically authentic, the sequence is submitted to GenBank (Clark *et al.*, 2016) to get the accession number. Data from NCBI subsequently gets mined into the Barcode of Life Database (Ratnasingham & Hebert, 2007) to identify species.

There are different software used for multiple sequence alignment, molecular data analysis and the most commonly used ones are MEGA 10 (Tamura *et al.*, 2021), ABGD (Puillandre *et al.*, 2012), Taxon DNA (Meier *et al.*, 2006), Geneious vR6.1.6, MAFFT v7.017 (Kato *et al.*, 2002). Kimura 2 parameter (K2P) for genetic distance correction is used profusely to measure sequence divergences among organisms (Hebert *et al.*, 2003). Gene efficiency is tested by comparing intra- and interspecific genetic distances, focusing on the existence or lack of a barcoding gap. Species identification depends on the use of thresholds, set to differentiate between interspecific variation and intraspecific divergence. Intraspecific average genetic distances should be at least ten folds smaller than interspecific average genetic distances (Meyer & Paulay, 2005).

Figure 2: A flowchart showing generalized procedures of DNA barcoding technology.

Figure 3: A comparison between percentage of available specimens with barcodes and percentage of species with barcodes across different life forms based on records of Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007) Accessed on 3 September 2021.

Figure 4: Number of publications in PubMed till September 2021 for DNA barcoding and DNA metabarcoding across different groups of life forms.

Major Databases holding DNA Barcode Data

Continuous improvement of DNA sequencing technology has expanded the availability of DNA barcode data in public databases such as GenBank (Clark *et al.*, 2016), EMBL (Kanz *et al.*, 2005), DDBJ (Mashima *et al.*, 2017). It is unequivocal that the progress of DNA barcoding depends largely on the improvement of public databases. The

Consortium for The Barcode of Life (CBOL) was initiated in May 2004 with a view to developing global standard DNA barcodes. Later, the International Barcode of Life Consortium was founded in 2008, which took great initiative to promote biodiversity research. Under the supervision of iBOL, BARCODE 500K project (2010-15) has been completed with a generation of barcodes for 500,000 species. Recently, iBOL has initiated the BIOSCAN project (2019-2027) in collaboration with more than 1000 researchers from over 30 countries aiming to accelerate species discovery, examine species interactions, and understand species dynamics. With the progress of these projects, DNA barcodes are increasing continuously in the BOLD database (Table 1; Figure 3).

Table 1: Current state of records in the Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007)
Accessed on 3 September 2021

Group	Specimen Records	Specimens with Sequences	Specimens with Barcodes	Percentage of Specimens with Barcodes (%)	Total number of Species	Species with Barcodes	Percentage of Species with Barcodes (%)	Public BINs Availability
Animals	12425481	10029132	9307508	92.8	427824	341623	79.8	Yes
Angiosperms	440951	398242	240403	60.3	122547	67424	55.01	No
Gymnosperms	7276	6937	5516	79.5	844	785	93.00	No
Bryophytes	22307	20959	6494	30.9	3777	1808	47.9	No
Pteridophytes	12910	10661	9858	92.4	4262	4052	95.1	No
Fungi	175318	162042	142838	88.14	35145	30878	87.8	No
Algae	73241	53692	33623	62.6	9036	5141	56.9	Yes
Protists	10462	8951	5741	64.2	1224	965	78.8	Yes

DNA barcoding in Animals

DNA barcoding technique relies on three important principles such as standardization, minimalism, and scalability. Considering these principles, selecting suitable barcoding depends on selecting single or multiple standard loci for routine sequencing with the highest reliability in a very comprehensive and diverse set of samples to make data sets easily comparable to distinguish one species from another. The 5’- end of Cytochrome C Oxidase subunit 1 (COXI, COI, or COI-5P) having 600 to 1000 base pairs DNA sequences, in general, fits the purpose for interspecific variability and is considered as universal species level barcode for animals (Kress & Erickson, 2012). It is a haploid, maternally inherited, single locus with protein-coding region having high copy number per cell assuring sequence recovery from poorly preserved samples (Hebert *et al.*, 2003; Fazekas *et al.*, 2009; Hollingsworth *et al.*, 2011). COI is prioritized over other mitochondrial genes due to the reason that primers of COI are highly specific, robust, and show the highest degree of accuracy to retrieve 5’ end of target DNA (Folmer *et al.*, 1994). Since the rate of mutation in DNA shows an inverse relationship with the size of the genome, mitochondrial DNA undergoes relatively high mutation for their smaller size compared to the nuclear DNA and that makes mitochondrial COI more capable as a universal animal barcode than nuclear *rbcL*, *matK* or other nuclear barcodes (Drake *et al.*, 1998; Waugh, 2007). Intraspecific variation of COI is generally less than 10 % than interspecific variation, where deletions and insertions are rare (Blaxter, 2004).

Degree of morphological variation, poor phylogenetic info due to lack of knowledge on anatomical features, the occurrence of cryptic species drives the need for a morpho-molecular approach to bring precision in identification and solve existing problems of the animal world (Wang *et al.*, 2020). Understanding and recording cryptic biodiversity (morphologically similar species but genetically distinct) of species are critically important to achieving effective species conservation by unmasking phenotypic similarity and predicting biodiversity responses to environmental changes. Since frogs are considered as a target group for cryptic species investigation, a modern molecular systematics study of amphibian *Limnonectes Kuhl* using mtDNA data has revealed 22 distinct evolutionary lineages, 16 of which are currently subsumed under *L. kuhli* which was historically acclaimed as a single species (McLeod *et al.*, 2011). As

speciation is not always visible by morphological modifications, lack of application of molecular tools causes underestimation of species diversity (Bickford *et al.*, 2007; McLeod *et al.*, 2011).

DNA barcoding approach significantly emphasizes the need for taxonomic revision of different animal genera due to the advent of cryptic species and newly discovered species. An underestimated genus *Triplophysa* has been studied in Qinghai-Tibet Plateau, a biodiversity hotspot, to find out 24 native species with 2 cryptic species namely *T. robusta* and *T. minxianensis* by DNA barcoding of 1630 specimens (Wang *et al.*, 2020). Besides, the application of high throughput technologies for sensitive identification purposes is on the rise. Traditional trap-based surveillance strategies to control the arrival of invasive insects are less fruitful solely than when accompanied with DNA metabarcoding as the latter helps in the simultaneous, multi-species identification of mixed populations (Piper *et al.*, 2019). Insects are expected to become temperature sensitive because of their short life cycle, for example, significant poleward shifting of non-migratory butterflies in Europe, which is heavily dominated by temperatures (Parmesan *et al.*, 1999; Bale *et al.*, 2002).

Changes in the insect community are critical to understanding modifications in ecological parameters including decomposition pattern, nutrient cycling, primary productivity as well as biodiversity assessment. Combining community datasets with high throughput DNA barcoding technologies has potential aspects to minimize various logistical, financial, and systematic impediments for large-scale observation. Arctic arthropod community has been explored focusing on Arachnida, Collembola and Insecta to develop 18,096 (75%) barcodes using mitochondrial COI to be assigned to BINs, having GenBank accession number ranging from MN665381 to MN683476, of 24,198 specimens collected during study (Pentinsaari *et al.*, 2020).

In a primate study, to suggest appropriate marker for species identification, the efficiency of 12 mitochondrial protein-coding genes is tested to explore that NADH dehydrogenase subunit 5 (ND5) and cytochrome c oxidase subunit II (COII) produce the largest barcode gaps within genus and family than between species rather than COI, which is suggestive of more conservative nature of the barcodes in the species level (Jackson & Nijman, 2020). 3 species of Canidae, one of the most interesting families of Mammalia, which is threatened due to illegal poaching, poisoning, and habitat loss, have been explored by DNA barcoding approach to validate COI barcode and detect genetic divergence. The mean sequence divergence among and within species was 12.32% and 0.61% respectively indicating relatively higher genetic diversity than previously reported studies (Aksöyek *et al.*, 2017). The consistency between DNA barcoding and morphological identification for a particular species depends on the presence or absence of sibling species that are morphologically and genetically similar. Lack of conspecific barcodes, sharing the same DNA barcode may create ambiguity to identify lower operational taxonomic units (OTUs). In addition to that, misidentification and mislabeling of samples results in potential error and hesitation in later research (Pleijel *et al.*, 2008; Dixon, 2012).

In an approach to correctly identify Spanish Blackfly as a preventive measure of the outbreak in Spain, to generate important information about their species distribution, vector control strategy, disease dissemination, Ruiz-Arrondo *et al.*, (2018) used the COI gene as a potential barcode and explored 6 new records out of 22 species from 239 specimens with the average intraspecific and interspecific genetic divergence of 1.47% and 12.25%, respectively. All the sequences were submitted to the GenBank database with accession numbers ranging from MG894170 to MG894340 and five new species were registered in the BOLD database for the first time with complete DNA barcodes (Ruiz-Arrondo *et al.*, 2018). Barcodes showing high intraspecific divergence between randomly selected sibling species than morphospecies may indicate the presence of hidden diversity (Ruiz-Arrondo *et al.*, 2018). However, no uniform threshold has been reported yet for species delimitation. Maximum conspecific and minimum congeneric differences have been reported as more effective to define barcode gap than average intraspecific and interspecific sequence divergence (Meier *et al.*, 2008). Although it is a matter of dispute, distance-based technique remains as the most followed method in DNA barcoding (Reid *et al.*, 2011; Srivathsan & Meier, 2012).

Gastropods, as the most abundant group of mollusks, are different to identify morphologically for their different morphological characters at various growth stages and they form an important part of marine biodiversity with 80,000 species existing worldwide, face threats due to overexploitation as they are economically significant (Bieler, 1992; Schmidt *et al.*, 2002). Accurate species detection plays a pivotal role in the conservation of flora and fauna. To make

an efficient fisheries resource survey and natural resource management using COI gene as potential mitochondrial DNA barcode, Ran *et al.* (2020), developed a barcode reference library with 306 barcode sequences, GenBank accession number ranging from MN388943 to MN389209, obtained from 120 species containing 3 new records in China, belonging to 35 families and 7 orders, to validate the efficiency of COI gene as barcode sequence. The K2P average intraspecific and interspecific sequence divergences were 0.9% and 14.7%, respectively and a slight increase within the higher taxonomic levels of families and orders was reported but the rate of increase in higher taxa was lower due to substitutional saturation (Iyiola *et al.*, 2018; Ran *et al.*, 2020).

Ornithologists without the help of molecular tools, sometimes face challenges to provide the right identity of birds. DNA barcode reference libraries, as a useful tool, can help them to expand geographical sampling for COI sequences. A total of 26 COI sequences from bird blood samples were obtained from 18 species belonging to 10 families. A boreal migrant bird was re-identified as a different species after molecular study (Pulgarín-R *et al.*, 2021).

Overfishing of sharks is a growing problem in Brazil as the country imports most of the shark meats in the world, the majority in Asia. Feitosa *et al.*, (2018) conducted a study to trace out illegal trading of endangered shark species on the North Coast of Brazil and identified 17 species out of 427 samples examined with COI (260) and NADH2 (167). Of the species identified, 53% were listed under some extinction threatened category and it would have become 76% if near threatened categories were enlisted (Stevens *et al.*, 2000; Feitosa *et al.*, 2018).

Table 2: Some selected studies of DNA barcoding on animals with major findings.

References	Group Studied	Barcode Sequence Obtained	Total no. of Species Identified	Location	Remarks
(Penton <i>et al.</i> , 2004)	Crustacean (<i>Daphnia</i>)	43	2 (critically)	North America	Morphologically cryptic species differentiation by suggesting natural selection as the key role player of divergence.
(Francis <i>et al.</i> , 2010)	Bats	1896	157	China, Vietnam, Laos, Peninsular Malaysia, Borneo	15 species show morphologically distinct features that are either undescribed or could not be assigned on the basis of reference materials.
(Zhang, 2011)	Marine Fishes	321	121	China	Introgressive hybridization in <i>Pampus</i> was detected
(Lakra <i>et al.</i> , 2016)	Fish	72	72	India	Discriminating congeneric species without any hesitation and validating the efficiency of COI as ideal barcode

(Bilgin <i>et al.</i> , 2016)	Birds	73	73	Turkey	Indicating genetic signature of landbirds community of northeastern Turkey which is typical of northern palearctic bird communities with some unique variations.
(Bingpeng <i>et al.</i> , 2018)	Fish	284	85	Taiwan	Building up a comprehensive barcoding reference database of fishes in Taiwan to validate the efficacy of mitochondrial COI gene.
(Nneji <i>et al.</i> , 2020)	Butterfly	699	116	Nigeria	High haplotype diversity suggests population level study using COI barcodes, with 10 new records 90% species were discriminated.
(Changbunjong <i>et al.</i> , 2020)	Deer Flies	36	6	Thailand	3 new records were identified with species discrimination success of 97.22%
(Zangl <i>et al.</i> , 2020)	Reptiles and amphibians	194	34	Austria	2 new records identified, deep conspecific lineages were detected
(Habib <i>et al.</i> , 2021)	Fish, aquatic mollusks and crustaceans	185	154	Sundarbans, Bangladesh	Resolving misidentification of <i>Scylla olivacea</i> previously stated as <i>S. serrata</i>
(Ahmed <i>et al.</i> , 2021)	Fish (Elasmobranchii and Actinopterygii)	376	185	Bangladesh	Obtaining 21 species as new record from Bangladesh

Animal based Traditional Medicine and DNA barcoding

Traditional medicines derived from animal sources play a significant role in zootherapy, the availability of potential compounds for drug discovery and therapeutic practices including Traditional Chinese Medicine (TCM), Kampo medicine, Ayurvedic medicine, American folk medicine (Alves & Alves, 2011; Liu *et al.*, 2016). Numerous important medicinal animal species are threatened due to illegal hunting, poaching, and several other anthropogenic activities resulting in the increased demand for animal products to be used for medicinal purposes. To detect processed animal products rapidly for the authenticity of traditional medicines from adulterants as well as to protect threatened species, DNA barcoding can be revolutionary. (Yang *et al.*, 2018).

Table 3: Application of DNA barcoding in biomonitoring of medicinally important animal species and their status of conservation.

Medicinal Species	Class	Parts Used	Medicinal Use/Treatments in ailments	COI Accession No. (GenBank)	Conservation Status	References
<i>Saiga tatarica</i>	Mammalia	Skin and Horns	Fever, headaches, convulsion, epilepsy, agitation	KX859292	Critically endangered	(Chen <i>et al.</i> , 2015)
<i>Ceratotherium simum</i>	Mammalia	Horn	Fever, influenza, convulsion, delirium, abscess	MN124274	Near threatened	(Ewart <i>et al.</i> , 2018)
<i>Moschus berezovskii</i>	Mammalia	Musk gland	Coma, convulsion, stroke, sore	JF700175	Endangered	(Yang <i>et al.</i> , 2015)
<i>Bufo gargarizans</i>	Amphibia	Toad cake, skin	Heart disease, skin ailments, cancer	JN700880	Least concern	(Che <i>et al.</i> , 2012)
<i>Andrias davidianus</i>	Amphibia	Dried body, skin	Anemia, dysentery, chills, burns	JN700796	Critically endangered	(Che <i>et al.</i> , 2012)
<i>Eisenia fetida</i>	Annelida	Dried body	Fever, convulsion	MG737875	Not evaluated	(Römbke <i>et al.</i> , 2016)
<i>Corallium rubrum</i>	Cnidaria	Skeleton	Arthritis, ulcer	AY827536	Endangered	(Uda <i>et al.</i> , 2013)
<i>Chelonia mydas</i>	Reptilia	Shell	Insomnia, agitation, vertigo	GQ152881	Critically endangered	(Naro-Maciel <i>et al.</i> , 2010)
<i>Panthera tigris</i>	Mammalia	Bones	Pain, convulsion	MH290787	Critically endangered	(Jun <i>et al.</i> , 2011)
<i>Manta brisotris</i>	Condriichthyes	Gill rakers	Arthritis, asthma, children measles, skin sores, boils	KF899569	Endangered	(Asis <i>et al.</i> , 2016)
<i>Mobula kuhlii</i>	Condriichthyes	Gill rakers	Arthritis, asthma, children measles, skin sores, boils	KF899583	Endangered	(Zeng <i>et al.</i> , 2016)
<i>Decapterus maruadsi</i>	Actinopterygii	Muscles	Hemoptysis, dysentery	JQ738500	Least concern	(Mat-Jaafar <i>et al.</i> , 2012)
<i>Gekko gecko</i>	Reptilia	Dried body without viscera	Cough, cold, impotence, asthma, tuberculosis	HM362982	Least concern	(Gu <i>et al.</i> , 2011)
<i>Pelodiscus sinensis</i>	Reptilia	Shell	Vertigo, agitation, insomnia	JF700186	Vulnerable	(Reid <i>et al.</i> , 2011; Kundu <i>et al.</i> , 2016;)
<i>Epinephelus itajara</i>	Actinopterygii	Muscles	Alleviate hypertension, hyperglycemia	KF836462	Vulnerable	(Torres <i>et al.</i> , 2013)

Recently the tool “The Barcoding Table of Animal Species (BaTAnS)” has been developed by Matthes *et al.*, in the form of an excel sheet or spreadsheet to provide the users a collection of currently available and verified methods of DNA barcoding to facilitate the identification process of animal species (Matthes *et al.*, 2020). Furthermore, barcoding

aided with Next Generation Sequencing (NGS) is going to enhance the prospects and solve the existing problems with the rapid development of reference barcode databases for all the species in near future (Shokralla *et al.*, 2014).

DNA Barcoding in Plants

Unlike animals, molecular barcoding of plants is very challenging and it often demands the use of multiple loci, instead of a single-locus approach because the nucleotide substitution rate is lower in plant mitochondrial genome (Cho *et al.*, 1998; Mower *et al.*, 2007; Kress & Erickson, 2007; Fazekas *et al.*, 2008). Many gene loci have been experimented with to discriminate against plant species (Fazekas *et al.*, 2009) but the thumb rule is not working for plants to use single gene locus as universal barcode-like animals. To exploit the best possible result other barcodes such as trnH-psbA, rpoB, rpoCI, ITS, 23S rDNA have been experimented with to find some markers that behave well in some particular family or genus, while certain others don't that indicates the ambiguity (Janarthanan *et al.*, 2020). Based on efficacy coding genes like rbcL, rpoB, matK and non-coding genes like trnH-psbA, atpF-atpH have been shortlisted to discriminate species (Fazekas *et al.*, 2008).

One of the biggest challenges to reaching a common conclusion regarding the selection of a universal plant barcode was the paucity of comparative data enclosing all candidate markers and broad range taxonomic sampling. The Consortium for the Barcode of Life (CBOL) Plant Working Group, after testing the efficacy, proposed a two-locus-based approach using rbcL and matK as the core barcode and trnH-psbA intergenic spacer as a supplementary barcode. Instead of using a single gene locus, this multi-locus approach is taken because of the intra and interspecific variation and divergence between families and genera that shows no genetic gap in all the cases to be found (Janarthanan *et al.*, 2020). Despite lower discrimination success in plant species compared to mitochondrial COI in animals, the combination of rbcL with matK is recommended for some reasons. As both of them are plastid coding regions, it is possible to rectify sequence orientation by checking assembly errors during translation through *in-silico* analysis as well as to detect the presence of pseudogenes (Hollingsworth *et al.*, 2011).

ITS has been reported as an additional candidate plant DNA barcode by China Plant Barcode of Life (China Plant BOL Group). In a comparative study seven markers namely trnH-psbA, matK, ycf5, rbcL, rpoC1, ITS, ITS2 from medicinal plant species were tested and ITS2 was found as the best potential marker with a successful species identification rate of 92.7% (Chen *et al.*, 2010). ITS is considered as a specific reliable marker because it utilizes well-defined barcode gap to discriminate species, showing comparatively higher interspecific variation than intraspecific variation (Schoch *et al.*, 2012). However, for some environmental DNA barcoding processes, ITS creates problems due to primer mismatching. To solve that problem, a primer combination is suggested for alternative ITS regions, in parallel along with standardizing changes in melting point (Bellemain *et al.*, 2010). The presence of nuclear sequences of mitochondrial origin (NUMTs) creates difficulty in the barcoding approach. These are fragments of mitochondrial DNA that have been translocated into the nuclear genome (Williams & Knowlton, 2001) ranging from none or few in *Anopheles*, *Caenorhabditis*, and *Plasmodium* to more than 500 in human, rice, and *Arabidopsis* sp. (Vohra & Khera, 2013).

Algal Identification through DNA Barcode

DNA barcoding can play a great role to overcome insufficient algal genetic data to contribute by resolving numerous questions of algal systematics, addressing queries related to biogeography as well as establishing better cost-effective biodiversity monitoring programs emanating from UN conventions and EU directives (Bartolo *et al.*, 2020).

Red algae are economically important for carrageenan, a morpho-molecular study which using four different gene sequences namely COX1, COX2, COX2-3, and rbcL, proved the use of COX2 as a potential barcode to differentiate various commercially important carrageenophytes (Tan *et al.*, 2012). *Pyropia*, a commercially important difficult genus, has been studied up to species level using COI-5P barcode, to reveal cryptic species. The diverse sample set suggests the probability of more biodiversity in the pristine habitat which has not been explored yet (Koh & Kim, 2018). Despite comprehensive monographs, identifying algal species can be difficult as seen in the brown alga *Alaria*. With the help of COX1, rubisco operon spacer (rbcSP), and ITS, a study reported species discrimination

between closely related species of *Alaria* with the prediction of a probable collapse in the species barrier (Lane *et al.*, 2007).

Green microalgae identification is always challenging. To reveal the cryptic diversity of *Scenedesmus*, barcode-based approaches were used using *rbcL*, *tufA*, ITS, and 16S where 5 cryptic species were revealed out of 11 recovered species, suggesting a combination of 3 genes is much better to attain high species resolution than single-locus approach (Zou *et al.*, 2016).

Algae play a significant role in forensic analysis. Diatom has been identified from the internal extract of the victim using 16S, 18S, 23S, 28S, COI, *rbcL*, and ITS region through meta-barcoding aided with NGS. As most of the diatom sequence data in GenBank has been reported as mislabeled, developing standard barcodes for correctly identified voucher specimens is a great challenge (Liu *et al.*, 2020). However, a two-step approach has been recommended by CBOL Protists Working Group, referring to the use of a universal barcode at the first step and a group-specific second barcode at the second step to increase species resolution for correct identification (Pawlowski *et al.*, 2012).

Table 4: Application of DNA barcoding in different algal groups across different parts of the world.

Taxon Name	Group	Location	Accession No.(GenBank)					References
			18S/5.8S	<i>rbcL</i>	ITS1/ITS2	<i>tufA</i>	COI	
<i>Caulerpa scalpelliformis</i>	Green algae	Gujarat, India	JF932251	JF932277	JF932264	KC153492	N/A	(Kazi <i>et al.</i> , 2013)
<i>Ulva australis</i>	Green algae	Qingdao, China	N/A	KC411901	KC411881	KC411857	N/A	(Du <i>et al.</i> , 2014)
<i>Caulerpa racemosa</i>	Green algae	Gujarat, India	JF932254	JF932280	JF932267	KC153495	N/A	(Kazi <i>et al.</i> , 2013)
<i>Codium fragile</i>	Green algae	Qingdao, China	N/A	N/A	N/A	KC411835	N/A	(Du <i>et al.</i> , 2014)
<i>Monostroma articum</i>	Green algae	Qingdao, China	N/A	N/A	KC411873	KC411849	N/A	(Du <i>et al.</i> , 2014)
<i>Chara drouetii</i>	Green algae	Hildago, Mexico	N/A	HQ380444	N/A	N/A	N/A	(Hall <i>et al.</i> , 2010)
<i>Chara haitensis</i>	Green algae	Florida, USA	N/A	HQ380460	N/A	N/A	N/A	(Hall <i>et al.</i> , 2010)
<i>Chara zeylanica</i>	Green algae	Jiangsu, China	N/A	HQ380468	HQ380628	N/A	N/A	(Hall <i>et al.</i> , 2010)
<i>Caulerpa serrulata</i>	Green algae	Tamilnadu, India	JQ745683	JQ745697	JQ745711	KC153509	N/A	(Kazi <i>et al.</i> , 2013)
<i>Dictyota binghamiae</i>	Brown algae	Bamfield, Canada	N/A	N/A	N/A	N/A	FJ409139	(McDevit & Saunders, 2009)
<i>Melanosiphon intestinalis</i>	Brown algae	Black Harbour, Canada	N/A	N/A	N/A	N/A	FJ409176.1	(McDevit & Saunders, 2009)
<i>Laminaria digitata</i>	Brown algae	Churchill, Canada	N/A	N/A	N/A	N/A	FJ409151.1	(McDevit & Saunders, 2009)
<i>Macrocystis integrifolia</i>	Brown algae	Bamfield, Canada	N/A	N/A	N/A	N/A	FJ409174	(McDevit & Saunders, 2009)
<i>Eisenia arborea</i>	Brown algae	Bamfield, Canada	N/A	N/A	N/A	N/A	FJ409147	(McDevit & Saunders, 2009)
<i>Sargassum muticum</i>	Brown algae	Bamfield, Canada	N/A	N/A	N/A	N/A	FJ409215	(McDevit & Saunders, 2009)
<i>Pyropia submarina</i>	Red algae	Jeju, Korea	N/A	MG845466	N/A	N/A	MG845501	(Koh & Kim, 2018)

<i>Chondrus crispus</i>	Red algae	Peggys Cove, Canada	N/A	N/A	N/A	N/A	AY970567	(Saunders, 2005)
<i>Gracilaria pacifica</i>	Red algae	Bamfield, Canada	N/A	N/A	N/A	N/A	FJ499534	(Saunders, 2009)
<i>Navicula salinicola</i>	Diatom	Maine, USA	GQ330356	N/A	N/A	N/A	N/A	(Moniz & Kaczmariska, 2010)
<i>Skeletonema tropicum</i>	Diatom	Maine, USA	GQ330440	N/A	N/A	N/A	N/A	(Moniz & Kaczmariska, 2010)

Fungal Identification through DNA barcode

It is extremely difficult to build up a uniform, working species identification system that will work across all fungal species because fungi are extremely diverse ranging from microscopic to macroscopic life forms with a habit to colonize in every habitable niche on Earth (Xu, 2016). Proper identification of fungal species is highly significant to stop disease transmission, to monitor the spatial and temporal distribution and migration. Mitochondrial COI is not applicable for fungi as a universal barcode, though very few studies have reported its suitability for very few fungal genera for e.g. *Penicillium* sp. (Seifert *et al.*, 2007). Some fungal species don't possess mitochondria, so the use of COI has no point for those (Bullerwell & Lang, 2005; Gilmore *et al.*, 2009). Numerous fungal species are not culturable, making identification nearly impossible (Nilsson *et al.*, 2009; Begerow *et al.*, 2010).

About 50% of the described fungal species still lack any DNA sequence data in the available public databases. Mislabeling of the existing fungal DNA sequences is another issue why developing a secondary reference fungal barcode library is important (Drew, 2011).

Nuclear ribosomal ITS has been recommended as the primary fungal barcode by the International Fungal Barcode Consortium in 2012 (Schoch *et al.*, 2012) and consequently it has become a promising candidate for fungal DNA barcoding. Ease of amplification with the presence of multicopy structure in the genome, relatively shorter length of the ITS region, good species resolution power due to increased evolutionary rate, and good obtainability of universal primers have shaped ITS as the most demandable fungal barcode (White *et al.*, 1990; Vilgalys & Gonzalez, 1990; Seifert, 2009; Schoch *et al.*, 2012;). Most frequently used universal primers to amplify ITS include ITS1, ITS2, ITS3, ITS4, and ITS5 (White *et al.*, 1990) but a drawback of these primers is the amplification possibility of ITS not only from fungi but also from animals and plants (Irinzi *et al.*, 2016). To solve this problem, secondary DNA barcodes are utilized to bring precision in species delimitation where ITS does not provide sufficient efficacy. Modern fungal identification approach with the concept of meta-genomics is suggested to use a polyphasic taxonomical input combining genealogy, phenotypic autecology, and reproductive biology to identify and re-identify new and previously misidentified species, respectively (Lücking *et al.*, 2020).

Figure 6: Available species barcodes in BOLD for different groups of fungi (Accessed on 6 September 2021)

DNA Barcoding in Bryophytes

Bryophytes are well distributed all over the world and they are the second most species-rich group after angiosperms. For having small size and few easily observable morphological characters, it is often difficult to identify bryophytes up to species level and morphological plasticity in response to various environmental conditions makes them an extremely hard group to differentiate (Hassel *et al.*, 2005). As the oldest land plants on earth, they are ecologically significant which demands their rapid identification (Saddhe & Kumar, 2018). As proposed general barcode markers are challenging for bryophytes, Hassel *et al.* (2013) studied the efficiency of *atpF-atpH*, *rbcL*, *trnH-psbA*, and *ITS2* to delimit species of liverworts and explored *atpF-atpH* showing the highest accuracy (Hassel *et al.*, 2013). Liu *et al.* (2010), in a DNA barcoding approach, studied 49 moss species and 9 liverwort species and recommended *rbcL*, *rpoC1*, *rps4*, *trnH-psbA*, and *trnL-trnF* as the potential DNA barcode for mosses, while *rbcL* and *rpoC1* were claimed as the most efficient single loci (Liu *et al.*, 2010). Data deficiency is the prime cause of the establishment of a universal barcode for bryophytes. In a study on *Fissidens*, to build up a molecular phylogeny using *rbcL* and *rps4*, it was found that adaptation along with diversification in the tropical region is the prime causes behind their evolutionary build-up (Suzuki *et al.*, 2018). More study is needed worldwide to build a separate reference DNA barcode database for bryophytes to understand their phylogeography and role in the environment through effective identification.

DNA Barcoding in Pteridophytes

Pteridophytes are the seedless, vascular, nonflowering, spore-bearing plants having distinct free-living gametophyte (n) and sporophyte (2n) generations. They are evidence of vascular system evolution, connecting non-vascular cryptogams with vascular phanerogams (Malati & Rao, 2020). Therefore, proper identification of pteridophytes with a morpho-molecular approach plays a significant role in our current understanding of biology.

Authentication of medicinal pteridophytes is significant to record their status of conservation as well as to control adulteration or mixing between species which may cause serious health problems. To substantiate this, *psbA-trnH*, *rbcL*, *rpoB*, *rpoC1*, *matK* were tested on herbal pteridophytes to find that *psbA-trnH* intergenic region as the best candidate among all with the highest amplification accuracy (94.1%), sequencing success (81.3%), and species

discrimination (90.2%) rate (Ma *et al.*, 2010). ITS2 region was used exclusively to identify medicinal members of Selaginellaceae, an important family both ecologically and evolutionarily with a 100% success rate for PCR amplification and sequencing in 103 samples collected from different areas of China (Gu *et al.*, 2013). DNA barcoding technology has an immense potential to discover new pteridophytes which can be understood with the discovery of new species *Ophioglossum malviae* Patel & Reddy from Gujarat, India using chloroplast DNA regions such as trnL-F, rbcL, and psbA-trnH as barcodes that showed unambiguous lineage in the phylogenetic tree (Patel & Reddy, 2018). To reveal close similarity, *Psilotum sp.* was studied using rbcLa, trnA, trnV, matK, ITS, ycf3, and rpoB barcodes where rbcLa and trnA showed the highest species discrimination power with successful sequencing (Khan *et al.*, 2019).

A potential new ecotype of *Pteris vittata* L. has been explored in India detecting subtle changes in the size of mature sporophyte where the new ecotype was *Pteris vittata* L. ecotype nano, using rbcL as a sole locus which justifies the use of a single-locus approach for varietal identification of pteridophytes (Morajkar & Hegde, 2021).

Species complexity and polyploidization are often seen among the pteridophyte communities (Sigel, 2016). *Asplenium normale* D. Don shows species complexity in chromosome number and organization. An integrative morpho-cyto-molecular approach was taken to clarify and revise its taxonomic state using plastid marker trnL-trnF, trnG-trnR, rps4-trnS and nuclear marker gapCp, pgc, LFY to differentiate diploid and polyploid taxa distinctly (Chang *et al.*, 2018; Nitta & Ebihara, 2019). To understand the distribution of gametophyte and sporophyte, their ecological impact in climate change Nitta *et al.* (2017) studied community patterns of sporophyte and gametophyte using rbcL and trnH-psbA barcodes and showed the consistent distribution of sporophytes at high elevations than gametophytes except for some gametophytes which showed broader ranges than sporophytes (Nitta *et al.*, 2017). The most comprehensive database to date in Japan for fern and lycophytes identification was established using rbcL and trnH-psbA chloroplast barcodes (Nitta *et al.*, 2017; Nitta & Ebihara, 2019).

DNA Barcoding in Gymnosperms

Gymnosperms are seed-bearing plants predominantly woody, used commonly to enhance the aesthetic beauty of parks and gardens. Very few studies have been reported to date for their molecular authentication and as these are economically and pharmacologically significant, the more molecular study is required to reveal their true identity and properties.

Primer assessment is critical in DNA barcoding strategy, as they play a key role in the amplification process. Centering to this objective, 1 rbcL and 9 matK potential primers were assessed to evaluate their universality in 57 species of Gymnosperms belonging to 40 genera and 11 families. The study proposed 1F/724R and Gym_F1A/Gym_R1A as the best universal primer for rbcL and matK respectively (Li *et al.*, 2011).

Herbal dietary supplements of *Ginkgo biloba* L. helps to improve cognitive function via increasing blood perfusion (Kellermann & Kloft, 2011). To authenticate its herbal preparation matK mini barcode assay has been accomplished in the USA where 83.8% of samples were containing *Ginkgo biloba* L. DNA (Little & Gulick, 2014).

Sass *et al.* (2007) performed a comprehensive study in some cycads, one of the world's most threatened group of gymnosperms, using matK, ndhJ, rpoC1, rpoB, trnH-psbA, accD, ycf5, ITS to test the potentiality of these markers to discriminate species where ITS and trnH-psbA showed the highest level of accuracy (Sass *et al.*, 2007). Numerous species of Cycads were identified using rbcLa, matK as the core barcodes with the supplement of ITS and trnH-psbA from the traditional medicine market of South Africa during illegal trading from where near threatened, endangered, vulnerable, least concerned species were identified (Williamson *et al.*, 2016).

DNA Barcoding in Angiosperms

Angiosperms are a large group of flowering plants, having seeds enclosed in carpel and they account for 295,383 species belonging to 13,164 genera under 416 families (Christenhusz & Byng, 2016). Therefore, the DNA barcoding approach may play a highly promising role for their effective identification, ecological assessment, and conservation

in nature. Overcollection and habitat destruction pose a serious threat to Orchidaceae. DNA barcoding reveals a combination of *atpF-atpH*, *psbK-psbI*, *trnH-psbA* represents the best option for molecular identification of Korean orchids (Kim *et al.*, 2014). Ornamental plants are economically important for the horticultural industry and is an approach to confirm their correct identity, a combination of *rbcL* and *matK* were used in Egypt where the success rate varied at 83.4% and 40% for genus and species level, respectively which emphasizes the use of ITS or *trnH-psbA* with *rbcL+matK* for much better result (Elansary *et al.*, 2017).

Due to having no clear cut morphological differences, identification of the family Lauraceae becomes difficult, hence a molecular approach using *matK*, *rbcL*, *trnH-psbA*, ITS barcodes were taken in China for species identification, correcting the previous misidentification and reconstructing the phylogeny of the members and ITS was reported to show the highest accuracy (57.5%) to confirm species identity (Liu *et al.*, 2017).

ITS 1 is recommended to be used as a potential barcode in highly diverse genera as reported in *Passiflora* where ITS 1 becomes highly accurate than other nuclear and plastid markers (Giudicelli *et al.*, 2015). 12 medicinal plants were identified by DNA barcoding in Malaysia using ITS2, *rpoC1*, and *trnH-psbA*, where *trnH-psbA* showed the highest efficacy in the identification (Aziz *et al.*, 2015). The molecular barcode can solve cryptic lineages of many angiosperms as reported in *Dumasia*, a taxonomically problematic genus of Fabaceae family, where *matK*, *rbcL*, *trnH-psbA*, *trnL-trnF*, *psbB-psbF*, ITS were tested to solve the lineage problem. The study revealed ITS alone or ITS in combination with any chloroplast marker can be used as potential barcodes for *Dumasia* (Jiang *et al.*, 2020).

Toxic plants were identified rapidly by applying molecular tools in China using *matK*, *rbcL*, and ITS where 27 toxic plants belonging to 17 families were identified (Xie *et al.*, 2014). From the vomit samples, poisonous plants are identified through a DNA barcoding approach using *rbcL* as the primary marker and ITS2 or *trnH-psbA* as the secondary marker (Nithaniyal *et al.*, 2021). Such forensic analysis aided by DNA barcoding increases the rate of acceptability of this technique in forensic science. To test the efficacy of DNA barcodes in mangrove plants, 14 mangrove species belonging to 5 families were tested with different nuclear and plastid barcodes where a combination of *matK* and ITS2 along with *atpF-atpH* is suggested to be the best option for mangrove plants on the West Coast of India (Saddhe *et al.*, 2017).

Processed plant products for medicinal use often get adulterated due to heavy demand and insufficient supply, substitution with other products of the same genus, and contamination which cause life-threatening health implications when consumed in the form of herbal medicine. To solve that problem chemical fingerprinting technology has been developed. But due to their vulnerability to geographic populations and age of plants, chemical markers are not recommended to be used as a robust technique to solve problems of species adulteration (Gao *et al.*, 2019).

Newmaster *et al.* (2013) approached a DNA barcoding strategy to test the authenticity of 44 herbal products and reported 59% of the tested products contained barcodes of various plants not listed on the labels (Newmaster *et al.*, 2013). Srirama *et al.* (2010) reported 24% of the market samples were adulterated with 6 phenotypically similar species of *Phyllanthus* confirming species admixtures (Srirama *et al.*, 2010). Therefore, to detect the authenticity of raw herbal products, species substitution, species admixtures DNA barcoding technique may play a vital role to ensure great safety and support for the efficacy of medicinal materials (Yu *et al.*, 2021).

Table 5: Preferred / best loci for top 10 medicinal plant families recorded in the pharmacopoeia throughout the world (Yu *et al.*, 2021)

Family Name	Tested Loci	Best Loci	References
Fabaceae	ITS2, <i>matK</i> , <i>rbcLa</i> , ITS2+ <i>matK</i> , ITS2+ <i>rbcLa</i> , <i>matK+rbcLa</i> , ITS2+ <i>matK+rbcLa</i>	ITS2	(Tahir <i>et al.</i> , 2018)
Asteraceae	ITS, ITS2, <i>rbcL</i> , <i>matK</i> , <i>trnH-psbA</i>	ITS2	(Gao <i>et al.</i> , 2010)
Lamiaceae	<i>rbcL</i> , <i>matK</i> , <i>trnH-psbA</i>	<i>matK</i> , <i>trnH-psbA</i>	(Theodoridis <i>et al.</i> , 2012; Oyebanji <i>et al.</i> , 2020;)
Apiaceae	ITS, ITS2, <i>rbcL</i> , <i>matK</i> , <i>trnH-psbA</i>	ITS/ITS2+ <i>psbA-trnH</i> , ITS, ITS2	(Liu <i>et al.</i> , 2014; Parveen <i>et al.</i> , 2019)

Rosaceae	rbcL, matK, rpoC1, ITS2	ITS2	(Pang <i>et al.</i> , 2011)
Euphorbiaceae	rbcL, matK, ITS1, ITS2	ITS1, ITS2	(Pang <i>et al.</i> , 2010)
Rutaceae	psbA-trnH, matK, ycf5, rpoC1, rbcL, ITS2, ITS	ITS2	(Luo <i>et al.</i> , 2010)
Ranunculaceae	trnH-psbA, rbcL, matK, ITS1	rbcL+matK+trnH-psbA, ITS1	(Li <i>et al.</i> , 2019)
Poaceae	matK, rbcL, ITS, trnH-psbA, rps16, ndhF	ITS1, ITS2	(Yao <i>et al.</i> , 2017; Tahir <i>et al.</i> , 2018;)
Apocynaceae	ITS2, trnH-psbA, matK, rbcL, trnL-F	ITS2+trnH-psbA, matK+rbcL	(Cabelin & Alejandro, 2016; LV <i>et al.</i> , 2020)

Figure 5: DNA barcodes availability between top 10 families of medicinal plants (Yu *et al.*, 2021) in the BOLD database (Accessed on 6 September 2021).

DNA Barcoding with other Technologies

Bar-HRM Technology

High-Resolution Melting Technology or HRM has been developed in recent years to enable genotyping of plants. In this technique, the melting curve of PCR amplicons is monitored in real-time by adding specific saturated dyes as the melting curve depends on the DNA base sequences. From different shapes of the melting curve, genotype or class of different test populations is detected (Sun *et al.*, 2016; Mezzasalma *et al.*, 2017). DNA barcoding technique has been amalgamated with HRM in the name of "Bar-HRM Technology" which enables resolution accuracy to differentiate change in a single base, thus enhancing identification of medicinal plants greatly. Sequence-specific probes and sequencing are not required in this technique, thus rbcL, matK, trnH-psbA, rpoC, ITS, etc. can be employed in this technique to facilitate species identification (Yu *et al.*, 2021). The Bar-HRM method is feasible for high throughput

assay (Buddhachat *et al.*, 2015), and by applying this market samples can be identified rapidly, reliably, and accurately.

DNA Mini-barcoding

DNA mini-barcoding is useful to overcome the limitations of DNA barcoding as mini-barcodes use a smaller length of DNA usually equal to or less than 200bp which can be amplified more rapidly than regular barcodes (Meusnier *et al.*, 2008; Srirama *et al.*, 2014). As the amplicon length is shorter, the PCR success rate is higher for DNA mini-barcodes (Särkinen *et al.*, 2012). The main purpose of developing a mini-barcode is to identify individual target species of herbal plants to stop adulteration of natural herbal products rather than achieving universality for most species (Gao *et al.*, 2019). It becomes difficult to identify species present in natural herbal products using mini-barcode if the number of species is more than 10 in the herbal mixture. As DNA sequences may contain unstable mutation sites, developing a mini-barcode is critical considering the position and length to discriminate between multiple species (Hajibabaei *et al.*, 2006).

DNA Metabarcoding with NGS

Along with the development of High Throughput Technology (HTS), DNA barcoding has been transforming into a new norm called “DNA metabarcoding”. In the metabarcoding technique, universal PCR primers are used to simultaneously amplify numerous DNA barcodes to check species diversity and composition from a single environmental sample (Taberlet *et al.*, 2012). De Boer *et al.* (2017) used ITS1 and ITS2 in the DNA metabarcoding process to identify orchids and other plant species from 55 commercial products. In forensic analysis, metabarcoding holds a great promise (De Boer *et al.*, 2017). From human forensic tissue, numerous mammal species were detected with a success rate of 99.9% targeting 16S rRNA as the potential barcode (Tillmar *et al.*, 2013). DNA metabarcoding can help in the ecological analysis as significant changes in the community composition in response to elevation have been reported in carnivorous *Sarracenia purpurea* L. (Littlefair *et al.*, 2019). In a multilocus metabarcoding approach 12 barcodes were tested for universal applicability across a wide range of animal and plant taxa from a complex sample and the protocol was proved authentic after an international trial in 16 laboratories (Arulandhu *et al.*, 2017). Fish biodiversity was assessed in five different estuaries in Japan using environmental DNA (eDNA) metabarcoding approach targeting 12S rRNA (170bp) and identified 182 fish species belonging to 67 families (Ahn *et al.*, 2020). Sequencing errors during NGS may interfere with the correct identification of species. Therefore, accurate aligning along every base with the support of a comprehensive and precise reference library will decide the efficiency of DNA metabarcoding (Gao *et al.*, 2019).

Discussion and Future Prospects

DNA barcoding is incomplete without the integrity of the classical taxonomic approach to confirm species identity and it should never be replaced with the classical mainstream taxonomic study. As more DNA barcode data is generated nowadays, it's important to share equitable global access to reference databases and knowledge to ensure uniform infrastructure across all the country otherwise underdeveloped or developing countries will face difficulty to keep pace with the modern advancements. The rapid progress in NGS has been considered as a threat for DNA barcoding research in some studies as whole genome sequencing may produce better species resolution than an only barcode. But sequencing the whole genome is appropriate for studying genomic complexity, diversity, and function rather than species identification and biomonitoring (Grant *et al.*, 2021). Thus in the future, DNA barcoding will provide affordable, efficient, and high throughput solutions. International Barcode of Life Consortium (iBOL) is supervising BIOSCAN project with 180 million dollars funding, assumed to be completed in 2027. In the next phase, iBOL has planned to initiate a “Planetary Biodiversity Mission” with an estimated cost of 500 million dollars and this project will be activated in January of 2026, aiming to complete the census of all multicellular species, to establish a global biosurveillance program and to construct a library of life by preserving DNA extracts from all species.

Emphasize should be given on which specific method is applicable for which species instead of better discrimination rate between species, as whole plastid genome sequencing technology is progressing rapidly (Li *et al.*, 2015; Liu *et al.*, 2021). Rapid development and application of machine learning to check quality and disorientation of sequence data in the reference database will help to reduce errors due to mislabeling of barcodes with voucher specimens

(Krachunov *et al.*, 2019). More funding should be allocated in the herbaria for proper maintenance of voucher specimens as DNA barcodes obtained from well-curated specimens increase confidence and provide quality data stored in the reference database (Grant *et al.*, 2021). Through biomonitoring using more available barcodes, it will be possible to detect more endangered species and invasive species in the future (Kuzmina *et al.*, 2018). In the future, with the availability of exclusive bioinformatic tools, many DNA barcoding problems will be solved as biological data science is progressing rapidly.

Conclusions

DNA barcoding, significant development of molecular systematics, is a springboard to estimate diverse groups where the morphological study is painstakingly difficult and/or where the total number of species is beyond the grasp of classical taxonomists to investigate. With the advent of metagenomics and next-generation high throughput sequencing technology, DNA barcoding is progressing very fast. Integration of the taxonomic knowledge with the DNA barcoding approach is more satisfactory to bring a high level of accuracy. Beyond biodiversity monitoring, DNA barcoding knowledge can significantly reduce threats to global biodiversity by improving natural resource management, linking ecological assessments, and increasing awareness among the common mass.

Disclaimer

The author is solely responsible for the writing of this review paper.

Conflict of Interest Statement

The author has declared that there is no conflict of interest.

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