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)](image)

**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 (Katoh 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).

![Flowchart showing generalized procedures of DNA barcoding technology.](image)

**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 Kuhlii* using mtDNA data has revealed 22 distinct evolutionary lineages, 16 of which are currently subsumed under *L. kuhlii* 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 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 congenic 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 et al., 2004) | Crustacean (Daphnia) | 43                        | 2 (critically)                  | North America              | Morphologically cryptic species differentiation by suggesting natural selection as the key role player of divergence. |
| (Francis et al., 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 Pampus was detected                    |
| (Lakra et al., 2016) | Fish              | 72                        | 72                             | India                      | Discriminating congeneric species without any hesitation and validating the efficiency of COI as ideal barcode     |
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               |
|----------------------|-------------|---------------------|---------------------------------------------------------------------------|-----------------------------|--------------------------|--------------------------|
| Saiga tatarica       | Mammalia    | Skin and Horns      | Fever, headaches, convulsion, epilepsy, agitation                          | KX859292                    | Critically endangered    | (Chen et al., 2015)      |
| Ceratotherium simum  | Mammalia    | Horn                | Fever, influenza, convulsion, delirium, abscess                           | MN124274                    | Near threatened          | (Ewart et al., 2018)     |
| Moschus berezovskii  | Mammalia    | Musk gland          | Coma, convulsion, stroke, sore                                            | JF700175                    | Endangered               | (Yang et al., 2015)      |
| Bufo gargarizans     | Amphibia    | Toad cake, skin     | Heart disease, skin ailments, cancer                                     | JN700880                    | Least concern            | (Che et al., 2012)       |
| Andrias davidianus   | Amphibia    | Dried body, skin    | Anemia, dysentery, chills, burns                                         | JN700796                    | Critically endangered    | (Che et al., 2012)       |
| Eisenia fetida       | Annelida    | Dried body          | Fever, convulsion                                                        | MG737875                    | Not evaluated            | (Römbke et al., 2016)    |
| Corallium rubrum     | Cnidaria    | Skeleton            | Arthritis, ulcer                                                         | AY827536                    | Endangered               | (Uda et al., 2013)       |
| Chelonia mydas       | Reptilia    | Shell               | Insomnia, agitation, vertigo                                              | GQ152881                    | Critically endangered    | (Naro-Maciel et al., 2010)|
| Panthera tigris      | Mammalia    | Bones               | Pain, convulsion                                                         | MH290787                    | Critically endangered    | (Jun et al., 2011)       |
| Manta brisotris      | Condrichthyes| Gill rakers         | Arthritis, asthma, children measles, skin sores, boils                  | KF899569                    | Endangered               | (Asis et al., 2016)      |
| Mobula kuhlii        | Condrichthyes| Gill rakers         | Arthritis, asthma, children measles, skin sores, boils                  | KF899583                    | Endangered               | (Zeng et al., 2016)      |
| Decapterus maruadsi  | Actinopterygii| Muscles             | Hemoptysis, dysentery                                                   | JQ738500                    | Least concern            | (Mat-Jaafar et al., 2012)|
| Gekko gecko          | Reptilia    | Dried body without viscera | Cough, cold, impotence, asthma, tuberculosis                      | HM362982                    | Least concern            | (Gu et al., 2011)        |
| Pelodiscus sinensis  | Reptilia    | Shell               | Vertigo, agitation, insomnia                                            | JF700186                    | Vulnerable               | (Reid et al., 2011; Kundu et al., 2016;) |
| Epinephelus itajara  | Actinopterygii| Muscles             | Alleviate hypertension, hyperglycemia                                     | KF836462                    | Vulnerable               | (Torres et al., 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, rpoCl, 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          |
|-----------------------|------------|----------------|------------------------|---------------------|
| *Caulerpa scalpelliformis* | Green algae | Gujarat, India | JF932251 / JF932277 / JF932264 / KC153492 / N/A | (Kazi et al., 2013) |
| *Ulva australis*      | Green algae | Qingdao, China | N/A / KC411901 / KC411881 / KC411857 / N/A | (Du et al., 2014)   |
| *Caulerpa racemosa*   | Green algae | Gujarat, India | JF932254 / JF932280 / JF932267 / KC153495 / N/A | (Kazi et al., 2013) |
| *Codium fragile*      | Green algae | Qingdao, China | N/A / N/A / N/A / KC411835 / N/A | (Du et al., 2014)   |
| *Monostroma articum*  | Green algae | Qingdao, China | N/A / N/A / KC411873 / KC411849 / N/A | (Du et al., 2014)   |
| *Chara drouetii*      | Green algae | Hildago, Mexico | N/A / HQ380444 / N/A / N/A / N/A | (Hall et al., 2010) |
| *Chara haitensis*     | Green algae | Florida, USA   | N/A / HQ380460 / N/A / N/A / N/A | (Hall et al., 2010) |
| *Chara zeylanica*     | Green algae | Jiangsu, China | N/A / HQ380468 / HQ380628 / N/A / N/A | (Hall et al., 2010) |
| *Caulerpa serrulata*  | Green algae | Tamlilnad u, India | JQ745683 / JQ745697 / JQ745711 / KC153509 / N/A | (Kazi et al., 2013) |
| *Dictyota binghamiae* | Brown algae | Bamfield, Canada | N/A / N/A / N/A / N/A / FJ409139 | (McDevit & Saunders, 2009) |
| *Melanosiphon intestinalis* | Brown algae | Black Harbour, Canada | N/A / N/A / N/A / N/A / FJ409176.1 | (McDevit & Saunders, 2009) |
| *Laminaria digitata*  | Brown algae | Churchill, Canada | N/A / N/A / N/A / N/A / FJ409151.1 | (McDevit & Saunders, 2009) |
| *Macrocytis integrifolia* | Brown algae | Bamfield, Canada | N/A / N/A / N/A / N/A / FJ409174 | (McDevit & Saunders, 2009) |
| *Eisenia arborea*     | Brown algae | Bamfield, Canada | N/A / N/A / N/A / N/A / FJ409147 | (McDevit & Saunders, 2009) |
| *Sargassum muticum*   | Brown algae | Bamfield, Canada | N/A / N/A / N/A / N/A / FJ409215 | (McDevit & Saunders, 2009) |
| *Pyropia submarina*   | Red algae   | Jeju, Korea     | N/A / MG845466 / N/A / N/A / MG845501 | (Koh & Kim, 2018)   |
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 multicycopy 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 (Iriyí *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).

| **Species**       | **Phylum** | **Collection** | **Genbank** | **Accession No.** | **Year** |
|-------------------|------------|----------------|-------------|-------------------|----------|
| *Chondrus crispus*| Red algae  | Peggys Cove, Canada | N/A         | N/A               | N/A      | AY970567 (Saunders, 2005) |
| *Gracilaria pacifica* | Red algae | Bamfield, Canada | N/A         | N/A               | N/A      | FJ499534 (Saunders, 2009) |
| *Navicula salinicola* | Diatom    | Maine, USA      | GQ330356    | N/A               | N/A      | (Moniz & Kaczmarska, 2010) |
| *Skeletonema tropicum* | Diatom    | Maine, USA      | GQ330440    | N/A               | N/A      | (Moniz & Kaczmarska, 2010) |
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 malvaiPatel & 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 viittata L. has been explored in India detecting subtle changes in the size of mature sporophyte where the new ecotype was Pteris viittata 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-cytomolecular approach was taken to clarify and revise its taxonomic state using plastid marker trnL-trnF, trnG-trnR, rps4-trnS and nuclear marker gapCp, pgIC, 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 (Giudicielli et al., 2015). 12 medicinal plants were identified by DNA barcoding 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, matK, rbcLa, ITS2+matK, ITS2+rbcLa, matK+rbcLa, ITS2+matK+rbcLa | ITS2 | (Tahir et al., 2018) |
| Asteraceae  | ITS, ITS2, rbcL, matK, trnH-psbA | ITS2 | (Gao et al., 2010) |
| Lamiaceae   | rbcL, matK, trnH-psbA | matK, trnH-psbA | (Theodoridis et al., 2012; Oyebanji et al., 2020;) |
| Apiaceae    | ITS, ITS2, rbcL, matK, trnH-psbA | ITS/ITS2+psbA-trnH, ITS, ITS2 | (Liu et al., 2014; Parveen et al., 2019) |
| Family               | Barcode Markers                      | Accession          | Reference                  |
|---------------------|--------------------------------------|--------------------|-----------------------------|
| Rosaceae            | rbcL, matK, rpoC1, ITS2              | ITS2               | Pang et al., 2011           |
| Euphorbiaceae       | rbcL, matK, ITS1, ITS2              | ITS1, ITS2         | Pang et al., 2010           |
| Rutaceae            | psbA-trnH, matK, ycf5, rpoC1, rbcL, ITS2, ITS   | ITS2            | Luo et al., 2010            |
| Ranunculaceae       | trnH-psbA, rbcL, matK, ITS1         | rbcL+matK+trnH-psbA, ITS1 | Li et al., 2019             |
| Poaceae             | matK, rbcL, ITS, trnH-psbA, rps16, ndhF | ITS1, ITS2       | Yao et al., 2017; Tahir et al., 2018 |
| Apocynaceae         | ITS2, trnH-psbA, matK, rbcL, trnL-F | ITS2+trnH-psbA, matK+rbcL | Cabelin & Alejandro, 2016; LV et al., 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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References

1. Ahmed, M. S., Datta, S. K., Saha, T., & Hossain, Z. (2021). Molecular characterization of marine and coastal fishes of Bangladesh through DNA barcodes. Ecology and Evolution, 11(9), 3696–3709. https://doi.org/10.1002/ece3.7355
2. Ahn, H., Kume, M., Terashima, Y., Ye, F., Kameyama, S., Miya, M., Yamashita, Y., & Kasai, A. (2020). Evaluation of fish biodiversity in estuaries using environmental DNA metabarcoding. PLoS ONE, 15(10 October), 1–15. https://doi.org/10.1371/journal.pone.0231127
3. Aksöyek, E., İbiş, O., Özcan, S., Moradi, M., & Tez, C. (2017). DNA barcoding of three species (Canis aureus, Canis lupus and Vulpes vulpes) of Canidae. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis, 28(5), 747–755. https://doi.org/10.1080/24701394.2016.1180512
4. Alves, R. R. N., & Alves, H. N. (2011). The faunal drugstore: Animal-based remedies used in traditional medicines in Latin America. Journal of Ethnobiology and Ethnomedicine, 7(9), 1–43. https://doi.org/10.1186/1746-4269-7-9
5. Arulandhu, A. J., Staats, M., Hagelaar, R., Voorhuijzen, M. M., Prins, T. W., Scholtens, L., Costessi, A., Duijssings, D., Rechenmann, F., Gaspar, F. B., Barreto Crespo, M. T., Holst-Jensen, A., Birck, M., Burns, M., Haynes, E., Hochegger, R., Klingl, A., Lundberg, L., Natale, C., … Kok, E. (2017). Development and validation of a multi-locus DNA metabarcoding method to identify endangered species in complex samples. GigaScience, 6(10), 1–18. https://doi.org/10.1093/gigascience/gix080
6. Asis, A. M. J. M., Lacsamana, J. K. M., & Santos, M. D. (2016). Illegal trade of regulated and protected aquatic species in the Philippines detected by DNA barcoding. Mitochondrial DNA, 27(1), 659–666.
7. Aziz, N. A. A., Ahmad, M. I., & Naim, D. M. (2015). Molecular identification of medicinal plants used by traditional healers in Malaysia. Genetics and Molecular Research, 14(4), 1593-15947. https://doi.org/10.4238/2015.December.7.5
8. Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symminoudis, I., Watt, A. D., & Whittaker, J. B. (2002). Herbivory in global climate change research: Direct effects of rising temperatures on insect herbivores. Global Change Biology, 8(1), 1–16. https://doi.org/10.1046/j.1365-2486.2002.00451.x
9. Bandyopadhyaya, S., Vankayalapati, R., Rajanna, L., & Kulkarni, S. (2013). DNA barcoding and its applications - A critical review. Cnims J. Res. and Dev., 1(1), 77–81. https://www.researchgate.net/publication/285322603
10. Bartolo, A. G., Zammit, G., Peters, A. F., & Kupfer, F. C. (2020). The current state of DNA barcoding of macroalgae in the Mediterranean Sea: Presently lacking but urgently required. Botanica Marina, 63(3), 253–272. https://doi.org/10.1515/bot-2019-0041
11. Begerow, D., Nilsson, H., Unterseher, M., & Maier, W. (2010). Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Applied Microbiology and Biotechnology, 87(1), 99–108. https://doi.org/10.1007/s00253-010-2585-4
12. Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: An in silico approach reveals potential PCR biases. BMC Microbiology, 10(189), 1–9. https://doi.org/10.1186/1471-2180-10-189
13. Bickford, D., Lohman, D. J., Sudhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, N. (2007). Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution, 22(3), 148–155. https://doi.org/10.1016/j.tree.2006.11.004
14. Bieler, R. (1992). Gastropod phylogeny and systematics. Annual Review of Ecology and Systematics, 23(1), 311–338. https://doi.org/10.1146/annurev.es.23.110192.001523
15. Bilgin, R., Ebeoglu, N., Inak, S., Kirpik, M. A., Horns, J. J., & Sekercioglu, C. H. (2016). DNA barcoding of birds at a migratory hotspot in eastern Turkey highlights continental phylogeographic relationships. PLoS ONE, 11(6), 1–17. https://doi.org/10.1371/journal.pone.0154454
16. Bingpeng, X., Heshan, L., Zhihan, Z., Chunguang, W., Yanguo, W., & Jianjun, W. (2018). DNA Barcoding for Identification of Fish Species in the Taiwan Strait. PLoS ONE, 13(6), 1–13. https://doi.org/10.1371/journal.pone.0198109
17. Blaxter, M. L. (2004). The promise of a DNA taxonomy. Philosophical Transactions of the Royal Society B: Biological Sciences, 359(1444), 669–679. https://doi.org/10.1098/rstb.2003.1447
18. Buddhachat, K., Osathanunkul, M., Madesis, P., Chomdej, S., & Ongchai, S. (2015). Authenticity analyses of Phyllanthus amarus using barcoding coupled with HRM analysis to control its quality for medicinal plant product. Gene, 573(1), 84–90. https://doi.org/10.1016/j.gene.2015.07.046
19. Bullerwell, C. E., & Lang, B. F. (2005). Fungal evolution: The case of the vanishing mitochondrion. Current Opinion in Microbiology, 8(4), 362–369. https://doi.org/10.1016/j.mib.2005.06.009
20. Cabelin, V. L. D., & Alejandro, G. J. D. (2016). Efficiency of matK, rbcL, trnH-psbA, and trnL-F (cpDNA) to molecularly authenticate Philippine ethnomedicinal Apocynaceae through DNA barcoding. Cmrims J. Res. and Dev., 1(1), 84–90. https://doi.org/10.3109/19401736.2014.913138
21. Che, J., Chen, H. M., Yang, J. X., Jin, J. Q., Jiang, K., Yuan, Z. Y., Murphy, R. W., & Zhang, Y. P. (2012). Universal COI primers for DNA barcoding amphibians. Molecular Ecology Resources, 12(2), 247–258. https://doi.org/10.1111/j.1755-0998.2011.03090.x
22. Chen, J., Jiang, Z., Li, C., Ping, X., Cui, S., Tang, S., Chu, H., & Liu, B. (2015). Identification of ungulates used in a traditional Chinese medicine with DNA barcoding technology. Ecology and Evolution, 5(9), 1818–
1825. https://doi.org/10.1002/ece3.1457
26. Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE*, 5(1), 1–8. https://doi.org/10.1371/journal.pone.0008613
27. Cho, Y., Qiu, Y. L., Kuhlman, P., & Palmer, J. D. (1998). Explosive invasion of plant mitochondria by a group I intron. *Proceedings of the National Academy of Sciences of the United States of America*, 95(24), 14244–14249. https://doi.org/10.1073/pnas.95.24.14244
28. Christenhusz, M. J. M., & Byng, J. W. (2016). *Phytotaxa*. *Phytotaxa*, 261(3), 201–217. https://doi.org/10.11646/phytotaxa.261.3.1
29. Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2016). GenBank. *Nucleic Acids Research*, 44(D1), D67–D72. https://doi.org/10.1093/nar/gkv1276
30. Costion, C., Ford, A., Cross, H., Crayn, D., Harrington, M., & Lowe, A. (2011). Plant DNA barcodes can accurately estimate species richness in poorly known floras. *PLoS ONE*, 6(11), 1–9. https://doi.org/10.1371/journal.pone.0026841
31. Crisci, J. V., Katinas, L., Apodaca, M. J., & Hoch, P. C. (2020). The End of Botany. *Trends in Plant Science*, 25(12), 1173–1176. https://doi.org/10.1016/j.tplants.2020.09.012
32. De Boer, H. J., Ghorbani, A., Manzanilla, V., Raclariu, A. C., Kreziou, A., Ounjai, S., Osathanunkul, M., & Gravendeel, B. (2017). DNA metabarcoding of orchid-derived products reveals widespread illegal orchid trade. *Proceedings of the Royal Society B: Biological Sciences*, 284(20171182), 1–9. https://doi.org/10.1098/rspb.2017.1182
33. Dixon, A. (2012). Conservation of the Saker Falcon *Falco cherrug* and the use of hybrids for falconry. *Aquila*, 119, 9–19.
34. Doyle, J. J., & Doyle, J. L. (1987). *Doyle_plantDNAextractCTAB_1987.pdf*. In *Phytochemical Bulletin* (Vol. 19, Issue 1), pp. 11–15. https://webpages.uncc.edu/~jweller2/pages/BINF8350f2011/BINF8350_Readings/Doyle_plantDNAextractCTAB_1987.pdf
35. Drake, J. W., Charlesworth, B., Charlesworth, D., & Crow, J. F. (1998). Rates of Spontaneous Mutation. *Genetics*, 148, 1667–1686.
36. Drew, L. W. (2011). Are we losing the science of taxonomy? *BioScience*, 61(12), 942–946. https://doi.org/10.1525/bio.2011.61.12.4
37. Du, G., Wu, F., Mao, Y., Guo, S., Xue, H., & Bi, G. (2014). DNA barcoding assessment of green macroalgae in coastal zone around Qingdao, China. *Journal of Ocean University of China*, 13(1), 97–103. https://doi.org/10.1007/s11802-014-2197-1
38. Elansary, H. O., Ashfaq, M., Ali, H. M., & Yessoufou, K. (2017). The first initiative of DNA barcoding of medicinal plant species. *Molecular Marine Genomics*, 8. https://doi.org/10.1371/journal.pone.0008613
39. Fazekas, A. J., Burgess, K. S., Kesnakurti, P. R., Graham, S. W., Newmaster, S. G., Husband, B. C., Percy, D. M., Hajibabaei, M., & Barrett, S. C. H. (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE*, 3(7), 1–12. https://doi.org/10.1371/journal.pone.0002802
40. Fazekas, A. J., Kesnakurti, P. R., Burgess, K. S., Percy, D. M., Graham, S. W., Barrett, S. C. H., Newmaster, S. G., Hajibabaei, M., & Husband, B. C. (2009). Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? *Molecular Ecology Resources*, 9(SUPPL. 1), 130–139. https://doi.org/10.1111/j.1755-0998.2009.02652.x
41. Feitosa, L. M., Martins, A. P. B., Giarrizzo, T., MacEdo, W., Monteiro, I. L., Gemaque, R., Nunes, J. L. S., Gomes, F., Schneider, H., Sampaio, I., Souza, R., Sales, J. B., Rodrigues-Filho, L. F., Tchaicka, L., & Carvalho-Costa, L. F. (2018). DNA-based identification reveals illegal trade of threatened shark species in a global elasmobranch conservation hotspot. *Scientific Reports*, 8(1), 1–11. https://doi.org/10.1038/s41598-018-21683-5
42. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294–299. https://doi.org/10.1071/ZO9660275
44. Francis, C. M., Borisenko, A. V., Ivanova, N. V., Eger, J. L., Lim, B. K., Guillén-Servant, A., Kruskop, S. V., Mackie, I., & Hebert, P. D. N. (2010). The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. *PLoS ONE*, 5(9), 1–12. https://doi.org/10.1371/journal.pone.0012575

45. Gao, T., Yao, H., Song, J., Zhu, Y., Liu, C., & Chen, S. (2010). Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family. *BMC Evolutionary Biology*, 10(1), 324. https://doi.org/10.1186/1471-2148-10-324

46. Gao, Z., Liu, Y., Wang, X., Wei, X., & Han, J. (2019). DNA Mini-Barcoding: A Derived Barcoding Method for Herbal Molecular Identification. *Frontiers in Plant Science*, 10(August). https://doi.org/10.3389/fpls.2019.00987

47. Gilmore, S. R., Gräfenhan, T., Louis-Seize, G., & Seifert, K. A. (2009). Multiple copies of cytochrome oxidase 1 in species of the fungal genus Fusarium. *Molecular Ecology Resources*, 9(SUPPL. 1), 90–98. https://doi.org/10.1111/j.1755-0998.2009.02636.x

48. Giudicelli, G. C., Mäder, G., & de Freitas, L. B. (2015). Efficiency of ITS sequences for DNA barcoding in Passiflora (Passifloraceae). *International Journal of Molecular Sciences*, 16(4), 7289–7303. https://doi.org/10.3390/ijms16047289

49. Grant, D. M., Brodinie, O. B., Evankow, A. M., Ferreira, A. O., Fontes, J. T., Hansen, A. K., Jensen, M. R., Kalayci, T. E., Leeper, A., Patil, S. K., Prati, S., Reunamo, A., Roberts, A. J., Shgidel, R., Tyukosova, V., Bendiksby, M., Blaalid, R., Costa, F. O., Hollingsworth, P. M., … Ekrem, T. (2021). The future of DNA barcoding: Reflections from early career researchers. *Diversity*, 13(7), 1–11. https://doi.org/10.3390/di13070313

50. Gu, H. F., Xia, Y., Peng, R., Mo, B. H., Li, L., & Zeng, X. M. (2011). Authentication of Chinese Crude Drug Gecko by DNA barcoding. *Natural Product Communications*, 6(1), 67–71. https://doi.org/10.1177/1934578x1100600117

51. Gu, W., Song, J., Cao, Y., Sun, Q., Yao, H., Wu, Q., Chao, J., Zhou, J., Xue, W., & Duan, J. (2013). Application of the ITS2 Region for Barcoding Medicinal Plants of Selaginellaceae in Pteridophyta. *PLoS ONE*, 8(6), 2–9. https://doi.org/10.1371/journal.pone.0067818

52. Habib, K. A., Rahman, M., Oh, J., Lee, Y. H., & Kim, C. G. (2021). DNA barcoding of brackish and marine water fishes and shellfishes of Sundarbans, the world’s largest mangrove ecosystem. *PLoS ONE*, 16(8 August), 1–17. https://doi.org/10.1371/journal.pone.0255110

53. Hajibabaei, M., Smith, M. A., Janzen, D. H., Rodriguez, J. J., Whitfield, J. B., & Hebert, P. D. N. (2006). A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes*, 6(4), 959–964. https://doi.org/10.1111/j.1471-2229.2006.01470.x

54. Hall, J. D., Fučíková, K., Lo, C., Lewis, L. A., & Karol, K. G. (2010). An assessment of proposed DNA barcodes in freshwater green algae. *Cryptogamie, Algologie*, 31(4), 529–555.

55. Hassel, K., Pedersen, B., & Söderström, L. (2005). Changes in life-history traits in an expanding moss species: Phenotypic plasticity or genetic differentiation? A reciprocal transplantation experiment with Pogonatum dentatum. *Ecography*, 28(1), 71–80. https://doi.org/10.1111/j.0906-7590.2005.03910.x

56. Hassel, K., Segreto, R., & Ekrem, T. (2013). Restricted variation in plant barcoding markers limits identification in closely related bryophyte species. *Molecular Ecology Resources*, 13(6), 1047–1057. https://doi.org/10.1111/j.1755-0998.12074

57. Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

58. Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and using a plant DNA barcode. *PLoS ONE*, 6(5). https://doi.org/10.1371/journal.pone.0019254

59. Hollingsworth, P. M., Li, D. Z., Van Der Bank, M., & Twyford, A. D. (2016). Telling plant species apart and using a plant DNA barcode. *Frontiers in Plant Science*, 7590.2005.03910.x

60. Huang, X. C., Ci, X. Q., Conran, J. G., & Li, J. (2015). Application of DNA barcodes in Asian tropical trees - A case study from Xishuangbanna Nature Reserve, Southwest China. *PLoS ONE*, 10(6), 1–17. https://doi.org/10.1371/journal.pone.0129295

61. Irimi, L., Lackner, M., de Hoog, G. S., & Meyer, W. (2016). DNA barcoding of fungi causing infections in humans and animals. *Fungal Biology*, 120(2), 125–136. https://doi.org/10.1016/j.funbio.2015.04.007

62. Iyiola, O. A., Nnej, L. M., Mustapha, M. K., Nzech, C. G., Oladipo, S. O., Nnej, I. C., Okeyoyin, A. O., Nwani, C. D., Ugwumba, O. A., Ugwumba, A. A. A., Faturoti, E. O., Wang, Y. yu, Chen, J., Wang, W. Z., & Adeola, A. C. (2018). DNA barcoding of economically important freshwater fish species from north-
63. Jackson, A. S., & Nijman, V. (2020). DNA barcoding of primates and the selection of molecular markers using african great apes as a model. Journal of Anthropological Sciences, 98, 15–26. https://doi.org/10.4436/jass.98017
64. Janarthanan, S., Bhuvanaragavan, S., & Prabakaran, S. (2020). Molecular markers in taxonomic studies: a review. National Symposium on Insect Diversity and Conservation, February, 137–154.
65. Jiang, K. W., Zhang, R., Zhang, Z. F., Pan, B., & Tian, B. (2020). DNA barcoding and molecular phylogeny of Dumasia (Fabaceae: Phaseoleae) reveals a cryptic lineage. Plant Diversity, 42(5), 376–385. https://doi.org/10.1016/j.pld.2020.07.007
66. Jun, J., Han, S. H., Jeong, T. J., Park, H. C., Lee, B., & Kwak, M. (2011). Wildlife forensics using mitochondrial DNA sequences: Species identification based on hairs collected in the field and confiscated tanned Felidiae leathers. Genes and Genomics, 33(6), 721–726. https://doi.org/10.1007/s13258-011-0080-7
67. Kanz, C., Aldebert, P., Althorpe, N., Baker, W., Baldwin, A., Bates, K., Browne, P., van den Broek, A., Castro, M., Cochrane, G., Duggan, K., Eberhardt, R., Faruque, N., Gamble, J., Garcia Diez, F., Harte, N., Kulikova, T., Lin, Q., Lombard, V., … Apweiler, R. (2005). The EMBL nucleotide sequence database. Nucleic Acids Research, 33(DATABASE ISS.), 29–33. https://doi.org/10.1093/nar/gki098
68. Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30(14), 3059–3066. https://doi.org/10.1093/nar/gkf436
69. Kazi, M. A., Reddy, C. R. K., & Jha, B. (2013). Molecular phylogeny and barcoding of caulera (bryopsidales) based on the truF, rbcL, 18S rDNA and ITS rDNA genes. PLoS ONE, 8(12). https://doi.org/10.1371/journal.pone.0082438
70. Kellermann, A. J., & Kloft, C. (2011). Is there a risk of bleeding associated with standardized Ginkgo biloba extract therapy? A systematic review and meta-analysis. Pharmacotherapy, 31(5), 490–502. https://doi.org/10.1592/phco.31.5.490
71. Khan, B., Muhammad, K., Hamayun, Z., Ahmad, H., Ur Rehan, S., Majid, A., Que, Y., Nadeem, M. S., & Kaleem, I. (2019). Phylogenetic study of living fossil psilotum nudum l. From Himalayan range of Pakistan using DNA barcodes. Bangladesh Journal of Botany, 48(3), 457–466. https://doi.org/10.3329/BJB.V48I3.47725
72. Kim, H. M., Oh, S. H., Bhandari, G. S., Kim, C. S., & Park, C. W. (2014). DNA barcoding of orchidaceae in Korea. Molecular Ecology Resources, 14(3), 499–507. https://doi.org/10.1111/1755-0998.12207
73. Koh, Y. H., & Kim, M. S. (2018). DNA barcoding reveals cryptic diversity of economic red algae, Pyropia (Bangiales, Rhodophyta): description of novel species from Korea. Journal of Applied Phycology, 30(6), 3425–3434. https://doi.org/10.1007/s10811-018-1529-8
74. Krachunov, M., Nisheva, M., & Vassilev, D. (2019). Machine learning models for error detection in metagenomics and polyploid sequencing data. Information (Switzerland), 10(3), 1–16. https://doi.org/10.3390/info10030110
75. Kress, W. J., & Erickson, D. L. (2007). A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. PLoS ONE, 2(6), 1–10. https://doi.org/10.1371/journal.pone.000508
76. Kress, W. J., & Erickson, D. L. (2012). DNA Barcodes: Methods and Protocols. In Methods in Molecular Biology (Vol. 858, Issue February 2008, pp. 3–8). https://doi.org/10.1007/978-1-61779-591-6
77. Kundy, S., Laskar, B. A., Venkataraman, K., Banerjee, D., & Kumar, V. (2016). DNA barcoding of Nilssonia congeneris corroborates existence of wild N. nigricans in northeast India. Mitochondrial DNA, 27(4), 2753–2756. https://doi.org/10.3109/19401736.2015.1046176
78. Kuzmina, M. L., Braunmann, T. W. A., & Zakharov, E. V. (2018). Finding the pond through the weeds: eDNA reveals underestimated diversity of pondweeds. Applications in Plant Sciences, 6(5), 1–8. https://doi.org/10.1002/aps3.1155
79. Lakra, W. S., Singh, M., Goswami, M., Gopalakrishnan, A., Lal, K. K., Mohindra, V., Sarkar, U. K., Punia, P. P., Singh, K. V., Bhatt, J. P., & Ayyappan, S. (2016). DNA barcoding Indian freshwater fishes. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis, 27(6), 4510–4517. https://doi.org/10.3109/19401736.2015.1101540
80. Lane, C. E., Lindstrom, S. C., & Saunders, G. W. (2007). A molecular assessment of northeast Pacific Alaria species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. Molecular Phylogenetics and Evolution, 44(2), 634–648. https://doi.org/10.1016/j.ympev.2007.03.016
81. Li, Q. J., Wang, X., Wang, J. R., Su, N., Zhang, L., Ma, Y. P., Chang, Z. Y., Zhao, L., & Potter, D. (2019). Efficient Identification of Pulsatilla (Ranunculaceae) Using DNA Barcodes and Micro-Morphological Characters. *Frontiers in Plant Science, 10*(October), 1–16. https://doi.org/10.3389/fpls.2019.01196

82. Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2015). Plant DNA barcoding: from gene to genome. *Biological Reviews of the Cambridge Philosophical Society, 90*(1), 157–166. https://doi.org/10.10111/brv.12104

83. Li, Y., Gao, L. M., Poudel, R. C., Li, D. Z., & Forrest, A. (2011). High universality of matK primers for barcoding gymnosperms. *Journal of Systematics and Evolution, 49*(3), 169–175. https://doi.org/10.1111/j.1759-6831.2011.00128.x

84. Little, D. P., & Gulick, P. (2014). Authentication of Ginkgo biloba herbal dietary supplements using DNA barcoding. *Genome, 57*(9), 513–516. https://doi.org/10.1111/gen-2014-0130

85. Littlefair, J. E., Zander, A., de Sena Costa, C., & Clare, E. L. (2019). DNA metabarcoding reveals changes in the contents of carnivorous plants along an elevation gradient. *Molecular Ecology, 28*(2), 281–292. https://doi.org/10.10111/mec.14832

86. Liu, J., Shi, L., Han, J., Li, G., Lu, H., Hou, J., Zhou, X., Meng, F., & Downie, S. R. (2014). Identification of species in the angiosperm family Apiaceae using DNA barcodes. *Molecular Ecology Resources, 14*(6), 1231–1238. https://doi.org/10.11111/1755-9098.12262

87. Liu, M., Zhao, Y., Sun, Y., Wu, P., Zhou, S., & Ren, L. (2020). Diatom DNA barcodes for forensic discrimination of drowning incidents. *FEMS Microbiology Letters, 367*(17), 1–8. https://doi.org/10.1093/femsle/fnaa145

88. Liu, Y., Yan, H. F., Cao, T., & Ge, X. J. (2010). Evaluation of 10 plant barcodes in Bryophyta (Mosses). *Journal of Systematics and Evolution, 48*(1), 36–46. https://doi.org/10.1111/j.1759-6831.2009.00063.x

89. Liu, Z.-F., Ma, H., Ci, X.-Q., Li, L., Song, Y., Liu, B., Li, H.-W., Wang, S.-L., Qu, X.-J., Hu, J.-L., Zhang, X.-Y., Conran, J. G., Twyford, A. D., Yang, J.-B., Hollingsworth, P. M., & Li, J. (2021). Can plastid genome sequencing be used for species identification in Lauraceae? *Botanical Journal of the Linnean Society, 197*(1), 1–14. https://doi.org/10.1093/botlinnean/boab018

90. Liu, Z. F., Ci, X. Q., Li, L., Li, H. W., Conran, J. G., & Li, J. (2017). DNA barcoding evaluation and implications for phylogenetic relationships in Lauraceae from China. *PLoS ONE, 12*(4), 1–20. https://doi.org/10.1371/journal.pone.0175788

91. Liu, Z., Jiang, Z., Fang, H., Li, C., Mi, A., Chen, J., Zhang, X., Cui, S., Chen, D., Ping, X., Li, F., Li, C., Tang, S., Luo, Z., Zeng, Y., & Meng, Z. (2016). Perception, price and preference: Consumption and protection of wild animals used in traditional medicine. *PLoS ONE, 11*(3), 1–19. https://doi.org/10.1371/journal.pone.0145901

92. Lücking, R., Aime, M. C., Robbertse, B., Miller, A. N., Ariyawansa, H. A., Aoki, T., Cardinale, G., Crous, P. W., Druzhinina, I. S., Geiser, D. M., Hawksworth, D. L., Hyde, K. D., Irinyi, L., Jeewon, R., Johnston, P. R., Kirk, P. M., Malosso, E., May, T. W., Meyer, W., … Schoch, C. L. (2020). Unambiguous identification of fungi: Where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus, 11*(4), 1–32. https://doi.org/10.1186/s43008-020-00033-z

93. Luo, K., Chen, S. L., Chen, K. L., Song, J. Y., Yao, H., Ma, X., Zhu, Y. J., Pang, X. H., Yu, H., Li, X. W., & Liu, Z. (2010). Assessment of candidate plant DNA barcodes using the Rutaceae family. *Science China Life Sciences, 53*(6), 701–708. https://doi.org/10.1007/s11427-010-4009-1

94. LV, Y. N., YANG, C. Y., SHI, L. C., ZHANG, Z. L., XU, A. S., ZHANG, L. X., LI, X. L., & LI, H. T. (2020). Identification of medicinal plants within the Apocynaceae family using ITS2 and psbA-trnH barcodes. *Chinese Journal of Natural Medicines, 18*(8), 594–605. https://doi.org/10.1016/S1875-5364(20)30071-6

95. Ma, X. Y., Xie, C. X., Liu, C., Song, J. Y., Yao, H., Luo, K., Zhu, Y. J., Gao, T., Pang, X. H., Qian, J., & Chen, S. L. (2010). Species identification of medicinal pteridophytes by a DNA barcode marker, the chloroplast psbA-trnH intergenic region. *Biological and Pharmaceutical Bulletin, 33*(11), 1919–1924. https://doi.org/10.1248/bpb.33.1919

96. Malati, S. N. L. S., & Rao, G. M. N. (2020). Distribution of Pteridophytes along the Eastern Ghats of India - A Review. *IOSR Journal Of Pharmacy And Biological Sciences, 15*(2), 43–45. https://doi.org/10.9790/3008-1502044345

97. Mashima, J., Kodama, Y., Fujisawa, T., Katayama, T., Okuda, Y., Kaminuma, E., Ogasawara, O., Okubo, K., Nakamura, Y., & Takagi, T. (2017). DNA Data Bank of Japan. *Nucleic Acids Research, 45*(D1), D25–D31. https://doi.org/10.1093/nar/gkw1001

98. Mat Jaafar, T. N. A., Taylor, M. I., Mohd Nor, S. A., de Bruyn, M., & Carvalho, G. R. (2012). DNA
Barcoding Reveals Cryptic Diversity within Commercially Exploited Indo-Malay Carangidae (Teleostei: Perciformes). *PLoS ONE*, 7(11), 1–16. https://doi.org/10.1371/journal.pone.0049623

99. Matthes, N., Pietsch, K., Rullmann, A., Näämann, G., Pöpping, B., & Szabo, K. (2020). The Barcoding Table of Animal Species (BaTAnS): a new tool to select appropriate methods for animal species identification using DNA barcoding. *Molecular Biology Reports*, 47(8), 6457–6461. https://doi.org/10.1007/s11033-020-05675-1

100. McDevit, D. C., & Saunders, G. W. (2009). On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. *Phycological Research*, 57(2), 131–141. https://doi.org/10.1111/j.1440-1835.2009.00530.x

101. McLeod, D. S., Horner, S. J., Husted, C., Barley, A., & Iskandar, D. (2011). “Same-same, but different”: An unusual new species of the Limnonectes kuhlii Complex from West Sumatra (Anura: Dicroglossidae). *Zootaxa*, 64(2883), 52–64. https://doi.org/10.11646/zootaxa.2883.1.4

102. Meier, R., Shiyang, K., Vaidya, G., & Ng, P. K. L. (2006). DNA barcoding and taxonomy in diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology*, 55(5), 715–728. https://doi.org/10.1080/10635150600969864

103. Meier, R., Zhang, G., & Ali, F. (2008). The use of mean instead of smallest interspecific distances exagerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology*, 57(5), 809–813. https://doi.org/10.1080/10635150802406343

104. Meusnier, I., Singer, G. A. C., Landry, J. F., Hickey, D. A., Hebert, P. D. N., & Hajibabaei, M. (2008). A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics*, 9(214), 4–7. https://doi.org/10.1186/1471-2164-9-214

105. Meyer, C. P., & Paulay, G. (2005). DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology*, 3(12), 1–10. https://doi.org/10.1371/journal.pbio.0030422

106. Mezzasalma, V., Ganopoulos, I., Galimberti, A., Cornara, L., Ferri, E., & Labra, M. (2017). Poisonous or non-poisonous plants? DNA-based tools and methods for accurate identification. *International Journal of Legal Medicine*, 131(1), 1–19. https://doi.org/10.1007/s00414-016-1460-y

107. Moniz, M. B. J., & Kaczmar, I. (2010). Barcoding of Diatoms: Nuclear Encoded ITS Revisited. *Protist*, 161(1), 7–34. https://doi.org/10.1016/j.protis.2009.07.001

108. Morajkar, S., & Hegde, S. (2021). DNA Barcoding Identifies a Potential New Ecotype of Chinese Brake Fern, Pteris vittata L. Nano. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, 91(2), 335–341. https://doi.org/10.1007/s40011-021-01231-4

109. Mower, J. P., Touzet, P., Gummow, J. S., Dearenkov, K., Sjökvist, E., & Kristiansson, E. (2009). The ITS region as a target for identification of emerging sequencing technologies. *FEMS Microbiology Letters*, 296(1), 97–101. https://doi.org/10.1111/j.1574-6968.2009.01618.x

110. Nilsson, R. H., Ryberg, M., Abarenkov, K., Sjökvist, E., & Kristiansson, E. (2009). The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters*, 296(1), 97–101. https://doi.org/10.1111/j.1574-6968.2009.01618.x

111. Nithaniyal, S., Majumder, S., Umapathy, S., & Parani, M. (2021). Forensic application of DNA barcoding in the identification of commonly occurring poisonous plants. *Journal of Forensic and Legal Medicine*, 101. https://doi.org/10.1016/j.jflm.2021.102126

112. Nitta, J. H., & Ebihara, A. (2019). Virtual issue: Ecology and evolution of pteridophytes in the era of molecular genetics. *Journal of Plant Research*, 132(6), 719–721. https://doi.org/10.1007/s10265-019-01139-1

113. Nitta, J. H., Meyer, J. Y., Taputuara, R., & Davis, C. C. (2017). Life cycle matters: DNA barcoding reveals contrasting community structure between fern sporophytes and gametophytes. *Ecological Monographs*, 87(2), 278–296. https://doi.org/10.1002/ecm.1246

114. Nneji, L. M., Adeola, A. C., Ayoola, A. O., Oladiupo, S. O., Wang, Y. Y., Malann, Y. D., Anyaene, O., Nneji, I. C., Rahman, M. M., & Olory, C. S. (2020). DNA barcoding and species delimitation of butterflies (Lepidoptera) from Nigeria. *Molecular Biology Reports*, 47(12), 9441–9457. https://doi.org/10.1007/s11033-020-05984-5
117. Oyebanji, O. O., Chukwuma, E. C., Bolarinwa, K. A., Adejobi, O. I., Adeyemi, S. B., & Ayoola, A. O. (2020). Re-evaluation of the phylogenetic relationships and species delimitation of two closely related families (Lamiaceae and Verbenaceae) using two DNA barcode markers. *Journal of Biosciences*, 45(1), 1–15. https://doi.org/10.1007/s12038-020-00061-2

118. Pang, X., Song, J., Zhu, Y., Xie, C., & Chen, S. (2010). Using DNA barcoding to identify species within euphorbiaceae. *Planta Medica*, 76(15), 1784–1786. https://doi.org/10.1055/s-0030-1249806

119. Pang, X., Song, J., Zhu, Y., Xu, H., Huang, L., & Chen, S. (2011). Applying plant DNA barcodes for Rosaceae species identification. *Cladistics*, 27(2), 165–170. https://doi.org/10.1111/j.1096-0031.2010.00328.x

120. Parmesan, C., Ryholm, N., Stefanescus, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A., & Warren, M. (1999). Poleward shifts in geographical ranges of butterfly species. *Nature*, 399(June), 579–583. www.nature.com

121. Parveen, I., Techen, N., & Khan, I. A. (2019). Identification of Species in the Aromatic Spice Family Apiaceae Using DNA Mini-barcodes. *Planta Medica*, 85(2), 139–144. https://doi.org/10.1055/a-0664-0947

122. Patel, M., & Reddy, M. N. (2018). Discovery of the World’s Smallest Terrestrial Pteridophyte. *Scientific Reports*, 8(1), 1–7. https://doi.org/10.1038/s41598-018-24135-2

123. Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S. S., Cepicka, I., Decelle, J., Dunthorn, M., Fiore, M. A., Gile, G. H., Holzmann, M., John, R., Jirků, M., Keeling, P. J., Kudryavtsev, A., Lara, E., … de Vargas, C. (2012). CBOL Protist Working Group: Barcoding Eukaryotic Species from Hainan island, China. *PLoS Biology*, 10(11), 1–5. https://doi.org/10.1371/journal.pbio.1001419

124. Pentinsaari, M., Blagoev, G. A., Hogg, I. D., Levesque-Beaudin, V., Perez, K., Sobel, C. N., Vandenbrink, B., & Borisenko, A. (2020). A DNA barcoding survey of an arctic arthropod community: Implications for future monitoring. *Insects*, 11(46), 1–18. https://doi.org/10.3390/insects11010046

125. Penton, E. H., Hebert, P. D. N., & Crease, T. J. (2004). Mitochondrial DNA variation in North American populations of Daphnia obtusa: Continentalism or cryptic endemism? *Molecular Ecology*, 13(1), 97–107. https://doi.org/10.1046/j.1365-294X.2003.02024.x

126. Piper, A. M., Batovska, J., Cogan, N. O. I., Weiss, J., Cunningham, J. P., Rodoni, B. C., & Blacket, M. J. (2019). Prospects and challenges of implementing DNA metabarcoding for high-throughput insect surveillance. *GigaScience*, 8(8), 1–22. https://doi.org/10.1093/gigascience/giz092

127. Pleijel, F., Jondeius, U., Norlinder, E., Nygren, A., Oxlund, B., Schander, C., Sundberg, P., & Thollesson, M. (2008). Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution*, 48(1), 369–371. https://doi.org/10.1016/j.ympev.2008.03.024

128. Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, 21(8), 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

129. Pulgarín-R, P. C., Olivera-Angel, M., Ortíz, L., Nanclares, D., Velásquez-Restrepo, S., & Díaz-Nieto, J. F. (2021). DNA barcodes of birds from northern Colombia. *Biodiversity Data Journal*, 9(e64842), 1–14. https://doi.org/10.3897/BDJ.9.e64842

130. Ran, K., Li, Q., Qi, L., Li, W., & Kong, L. (2020). DNA barcoding for identification of marine gastropod species from Hainan island, China. *Fisheries Research*, 225(January), 1–7. https://doi.org/10.1016/j.fishres.2020.105504

131. Ratnasingham, S., & Hebert, P. D. N. (2007). The Barcode of Life Data System BOLD. *Molecular Ecology Notes*, 7(3), 355–364. https://doi.org/10.1111/j.1471-8286.2006.01678.x

132. Reid, B. N., Le, M., Mccord, W. P., Iverson, J. B., Georges, A., Bergmann, T., Amato, G., Desalle, R., & Naro-Maciel, E. (2011). Comparing and combining distance-based and character-based approaches for barcoding turtles. *Molecular Ecology Resources*, 11(6), 956–967. https://doi.org/10.1111/j.1755-0998.2011.03032.x

133. Römkle, J., Aima, M., Backeljau, T., Breugelmans, K., Domínguez, J., Funke, E., Graf, N., Hajibabaei, M., Pérez-Losada, M., Porto, P. G., Schmelz, R. M., Vierna, J., Vizcaíno, A., & Pfenninger, M. (2016). DNA barcoding of earthworms (Eisenia fetida/andrei complex) from 28 ecotoxicological test laboratories. *Applied Soil Ecology*, 104(2016), 3–11. https://doi.org/10.1016/j.apsoil.2015.02.010

134. Ruiz-Arrondo, I., Hernández-Triana, L. M., Ignjatović-Cupina, A., Nikolova, N., Garza-Hernández, J. A., Rodríguez-Pérez, M. A., Oteo, J. A., Fooks, A. R., & Lucientes Curdi, J. (2018). DNA barcoding of blackflies (Diptera: Simuliidae) as a tool for species identification and detection of hidden diversity in the eastern regions of Spain. *Parasites and Vectors*, 11(1), 1–7. https://doi.org/10.1186/s13071-018-3046-7
135. Saddhe, A. A., Jamdade, R. A., & Kumar, K. (2017). Evaluation of multilocus marker efficacy for delineating mangrove species of West Coast India. *PLoS ONE*, 12(8), 1–15. https://doi.org/10.1371/journal.pone.0183245

136. Saddhe, A. A., & Kumar, K. (2018). DNA barcoding of plants: Selection of core markers for taxonomic groups. *Plant Science Today*, 5(1), 9–13. https://doi.org/10.104719/pst.2018.5.1.356

137. Särkinen, T., Staats, M., Richardson, J. E., Cowan, R. S., & Bakker, F. T. (2012). How to Open the Treasure Chest? Optimising DNA Extraction from Herbarium Specimens. *PLoS ONE*, 7(8), 1–9. https://doi.org/10.1371/journal.pone.0043808

138. Sass, C., Little, D. P., Stevenson, D. W., & Specht, C. D. (2007). DNA barcoding in the Cycadales: Testing the potential of proposed barcoding markers for species identification of Cycads. *PLoS ONE*, 2(11), 1–9. https://doi.org/10.1371/journal.pone.0001154

139. Saunders, G. W. (2005). Applying DNA barcoding to red macroalgae: A preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360(1462), 1879–1888. https://doi.org/10.1098/rstb.2005.1719

140. Saunders, G. W. (2009). Routine DNA barcoding of Canadian Gracilariales (Rhodophyta) reveals the invasive species Gracilaria vermiculophylla in British Columbia. *Molecular Ecology Resources*, 9(SUPPL. 1), 140–150. https://doi.org/10.1111/j.1755-0998.2009.02639.x

141. Schmidt, S., Wolff, M., & Vargas, J. A. (2002). Population ecology and fishery of Cittarium pica (Gastropoda: Trochidae) on the Caribbean coast of Costa Rica. *Revista de Biología Tropical*, 50(3–4), 1079–1090.

142. Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Bolchacova, E., Voigt, K., Crous, P. W., Miller, A. N., Wingfield, M. J., Aime, M. C., An, K. D., Bai, F. Y., Barreto, R. W., Begerow, D., Bergeron, M. J., Blackwell, M., … Schindel, D. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

143. Seifert, K. A. (2009). Progress towards DNA barcoding of fungi. *Molecular Ecology Resources*, 9(SUPPL. 1), 83–89. https://doi.org/10.1111/j.1755-0998.2009.02635.x

144. Seifert, K. A., Samson, R. A., DeWaard, J. R., Houbraken, J., Levesque, C. A., Moncalvo, J. M., Louis-Seize, G., & Hebert, P. D. N. (2007). Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. *Proceedings of the National Academy of Sciences of the United States of America*, 104(10), 3901–3906. https://doi.org/10.1073/pnas.0611691104

145. Shokralla, S., Gibson, J. F., Nikbakht, H., Janzen, D. H., Hallwachs, W., & Hajibabaei, M. (2014). Next-generation DNA barcoding: Using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. *Molecular Ecology Resources*, 14(5), 892–901. https://doi.org/10.1111/1755-0998.12236

146. Sigel, E. M. (2016). Genetic and genomic aspects of hybridization in ferns. *Journal of Systematics and Evolution*, 54(6), 638–655. https://doi.org/10.1111/jse.12226

147. Srirama, R., Gurumurthy, B. R., Senthilkumar, U., Ravikanth, G., Shaanker, R. U., & Shivanna, M. B. (2014). Are mini DNA-barcodes sufficiently informative to resolve species identities? An in silico analysis using phyllanthus. *Journal of Genetics*, 93(3), 823–829. https://doi.org/10.1007/s12041-014-0432-6

148. Srirama, R., Senthilkumar, U., Sreejayan, N., Ravikanth, G., Gurumurthy, B. R., Shivanna, M. B., Sanjappa, M., Ganeshiaha, K. N., & Uma Shaanker, R. (2010). Assessing species admixtures in raw drug trade of Phyllanthus, a hepato-protective plant using molecular tools. *Journal of Ethnomopharmacology*, 130(2), 208–215. https://doi.org/10.1016/j.jep.2010.04.042

149. Srivathsan, A., & Meier, R. (2012). On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*, 28(2), 190–194. https://doi.org/10.1111/j.1041-1632.2011.01076.x

150. Stevens, J. D., Bonfil, R., Dulvy, N. K., & Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science*, 57(3), 476–494. https://doi.org/10.1006/jmsc.2000.0724

151. Sun, W., Li, J. jian, Xiong, C., Zhao, B., & Chen, S. lin. (2016). The potential power of bar-HRM technology in herbal medicine identification. *Frontiers in Plant Science*, 7(MAR2016), 1–10. https://doi.org/10.3389/fpls.2016.00367

152. Suzuki, T., Inoue, Y., & Tsubota, H. (2018). Molecular phylogeny of the genus Fissidens (Fissidentaceae, Bryophyta) and a refinement of the infrageneric classification. *Molecular Phylogenetics and Evolution*, 127(October), 190–202. https://doi.org/10.1016/j.ympev.2018.05.020
153. Taberlet, P., Prud’Homme, S. M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J. C., Melodelima, C., Pompanon, F., & Coissac, E. (2012). Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology*, 21(8), 1816–1820. https://doi.org/10.1111/j.1365-294X.2011.05317.x

154. Tahir, A., Hussain, F., Ahmed, N., Ghorbani, A., & Jamil, A. (2018). Assessing universality of DNA barcoding in geographically isolated selected desert medicinal species of Fabaceae and Poaceae. *PeerJ*, 2018(3), 1–16. https://doi.org/10.7717/peerj.4499

155. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

156. Tan, J., Lim, P. E., Phang, S. M., Hong, D. D., Sunarpi, H., & Hurtado, A. Q. (2012). Assessment of Four Molecular Markers as Potential DNA Barcodes for Red Algae Kappaphycus Doty and Eucheuma J. Agardh (Solieriacaeae, Rhodophyta). *PLoS ONE*, 7(12), 1–15. https://doi.org/10.1371/journal.pone.0052905

157. Theodoridis, S., Stefanaki, A., Tezcan, M., Aki, C., Kokkini, S., & Vlachonasios, K. E. (2012). DNA barcoding in native plants of the Labiatae (Lamiaceae) family from Chios Island (Greece) and the adjacent Çeşme-Karaburun Peninsula (Turkey). *Molecular Ecology Resources*, 12(4), 620–633. https://doi.org/10.1111/j.1755-0998.2012.03129.x

158. Tillmar, A. O., Dell’Amico, B., Welander, J., & Holmlund, G. (2013). A universal method for species identification of mammals utilizing next generation sequencing for the analysis of DNA mixtures. *PLoS ONE*, 8(12), 1–9. https://doi.org/10.1371/journal.pone.0083761

159. Torres, R. A., Feitosa, R. B., Carvalho, D. C., Freitas, M. O., Hostim-Silva, M., & Ferreira, B. P. (2013). Autenticación mediante DNA barcoding de especies de meros legalmente protegidas y en peligro de extinción, sometidas a explotación pesquera, incluyendo el mero Goliat epinephelus itajara. *Scientia Marina*, 77(3), 409–418. https://doi.org/10.3989/scimar.03805.29A

160. Uda, K., Komeda, Y., Fujita, T., Iwasaki, N., Bavestrello, G., Giovane, M., Cattaneo-Vietti, R., & Suzuki, T. (2013). Complete mitochondrial genomes of the Japanese pink coral (Corallium elatius) and the Mediterranean red coral (Corallium rubrum): A reevaluation of the phylogeny of the family Coralliidae based on molecular data. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, 8(3), 209–219. https://doi.org/10.1016/j.cbd.2013.05.003

161. Vilgalys, R., & Gonzalez, D. (1990). Organization of ribosomal DNA in the basidiomycete Thanatephorus pratensis. *Current Genetics*, 18(3), 277–280. https://doi.org/10.1007/BF00318394

162. Vohra, P., & Khera, K. S. (2013). DNA Barcoding: Current Advances and Future Prospects-a review. *Asian Journal of Biological and Life Sciences*, 3(3), 185–189.

163. Wang, T., Zhang, Y. ping, Yang, Z. yu, Liu, Z., & Du, Y. yan. (2020). DNA barcoding reveals cryptic diversity in the underestimated genus Triplophysa (Cypriniformes: Cobitidae, Nemacheilinae) from the northeastern Qinghai-Tibet Plateau. *BMC Evolutionary Biology*, 20(1), 1–15. https://doi.org/10.1186/s12862-020-01718-0

164. Waugh, J. (2007). DNA barcoding in animal species: Progress, potential and pitfalls. *BioEssays*, 29(2), 188–197. https://doi.org/10.1002/bies.20529

165. White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *PCR: Protocols and Applications - A laboratory manual* (Issue May 2014, pp. 315–322).

166. Williams, S. T., & Knowlton, N. (2001). Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus Alpheus. *Molecular Biology and Evolution*, 18(8), 1484–1493. https://doi.org/10.1093/oxfordjournals.molbev.a003934

167. Williamson, J., Maurin, O., Shiba, S. N. S., Van Der Bank, H., Pfab, M., Pilusa, M., Kabongo, R. M., & Van Der Bank, M. (2016). Exposing the illegal trade in cycad species (Cycadophyta: Encephalartos) at two traditional medicine markets in South Africa using DNA barcoding. *Genome*, 59(9), 771–781. https://doi.org/10.1139/gen-2016-0032

168. Xie, L., Wang, Y. W., Guan, S. Y., Xie, L. J., Long, X., & Sun, C. Y. (2014). Prospects and problems for identification of poisonous plants in China using DNA barcodes. *Biomedical and Environmental Sciences*, 27(10), 794–806. https://doi.org/10.3967/bes2014.115

169. Xu, J. et al. (2016). Fungal DNA barcoding - Genome. *Genome*, 59(August), 913–932.

170. Yang, C., Xiao, Z., Zou, Y., Zhang, X., Yang, B., Hao, Y., Moermond, T., & Yue, B. (2015). DNA barcoding revises a misidentification on musk deer. *Mitochondrial DNA*, 26(4), 605–612. https://doi.org/10.3109/19401736.2014.880887

171. Yang, F., Ding, F., Chen, H., He, M., Zhu, S., Ma, X., Jiang, L., & Li, H. (2018). DNA Barcoding for the
Identification and Authentication of Animal Species in Traditional Medicine. *Evidence-Based Complementary and Alternative Medicine, 2018*(April), 1–18. https://doi.org/10.1155/2018/5160254

172. Yao, P. C., Gao, H. Y., Wei, Y. N., Zhang, J. H., Chen, X. Y., & Li, H. Q. (2017). Evaluating sampling strategy for DNA barcoding study of coastal and inland halo-tolerant Poaceae and Chenopodiaceae: A case study for increased sample size. *PLoS ONE, 12*(9), 1–14. https://doi.org/10.1371/journal.pone.0185311

173. Yu, J., Wu, X., Liu, C., Newmaster, S., Ragupathy, S., & Kress, W. J. (2020). Progress in the use of DNA barcodes in the identification and classification of medicinal plants. *Ecotoxicology and Environmental Safety, 208*(November), 1–7. https://doi.org/10.1016/j.ecoenv.2020.111691

174. Yu, J., Wu, X., Liu, C., Newmaster, S., Ragupathy, S., & Kress, W. J. (2021). Progress in the use of DNA barcodes in the identification and classification of medicinal plants. *Ecotoxicology and Environmental Safety, 208*. https://doi.org/10.1016/j.ecoenv.2020.111691

175. Zangl, L., Daill, D., Schweiger, S., Gassner, G., & Koblmüller, S. (2020). A reference DNA barcode library for Austrian amphibians and reptiles. *PLoS ONE, 15*(3), 1–17. https://doi.org/10.1371/journal.pone.0229353

176. Zeng, Y., Wu, Z., Zhang, C., Meng, Z., Jiang, Z., & Zhang, J. (2016). DNA barcoding of Mobulid Ray Gill Rakers for Implementing CITES on Elasmobranch in China. *Scientific Reports, 6*(July), 1–9. https://doi.org/10.1038/srep37567

177. Zhang, J. (2011). Species identification of marine fishes in China with DNA barcoding. *Evidence-Based Complementary and Alternative Medicine, 2011*(February), 1–10. https://doi.org/10.1155/2011/978253

178. Zou, S., Fei, C., Wang, C., Gao, Z., Bao, Y., He, M., & Wang, C. (2016). How DNA barcoding can be more effective in microalgae identification: A case of cryptic diversity revelation in Scenedesmus (Chlorophyceae). *Scientific Reports, 6*(June), 1–13. https://doi.org/10.1038/srep36822