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Current Status of DNA Barcoding as a Tool for Identification of Plants

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***Abstract*:- DNA barcoding is a novel technique where a small segment of DNA is used for species identification. This technique provides the taxonomist an added tool in addition to anatomical and physiological markers DNA barcoding offers a chance to hasten the discovery of new species. Cytochrome C oxidase (COI) gene has been experimentally proven to identify animal taxa at species level, and hence has universally been accepted as the standard barcode for animal species Scientists all across the globe have reported different plastid and nuclear markers as potential DNA barcode for different group of plants. Identification of the species of origin of corpses, meat, or blood is necessary in forensic cases. In forensic wildlife investigations, the COI gene was sequence in identifying the species of unidentified species. Effective and quick identification of confiscated wildlife or wildlife remains is a must in the fight against the illegal wildlife trade. The reliable identification of plant species from herbal remedies using specific DNA markers is now possible because to recent advancements in molecular plant identification utilizing DNA sequence data. Here in this paper we have explored all the research that have been conducted towards optimization for a universal DNA barcode for all plant species.**

Keywords: DNA Barcoding, COI, matK, rbcL, ITS, CBOL

INTRODUCTION:

The "DNA Barcode of Life" initiative attempts to create a uniform, quick, and affordable species identification method that can even be used by non-specialists [1]. DNA barcoding has recently made an appearance in the literature which uses a specific DNA region as a tag for rapid and accurate species identification [2]. At the first worldwide conference on "Barcoding Life," held at the Natural History Museum in London (UK), four decades later, it was claimed that DNA sequencing technology which is portable and identify all life was now in grasp [3]. By employing short, standardized gene sections as internal species markers, DNA barcoding is a revolutionary method for quickly, accurately, and automatically identifying different species[4]. DNA barcoding is the development of short DNA sequences that facilitates species recognition, identification, and discovery in a certain sphere of life [5]. To provide a thorough study, it combines ecological, genetic, and morphological data. It also runs the primary genetic data against other sequences [6]. Barcoding aims to identify a distinctive DNA fragment for each described species so that future taxonomists can conduct extensive biotic surveys without having to memorise or utilise morphological keys [7]. The DNA barcoding enterprise is currently working on a global scale with the start of the iBOL-project, and it is focusing on clades rather than local species assemblages [8].

The Consortium for the Barcode of Life (CBOL, http://barcoding.si.edu), whose goal is to collect DNA barcodes from all species on Earth, was quickly established following the initial success of DNA barcoding [9]. The huge international collaboration known as the iBOL, which involves 26 countries, aims to develop an automatic identification system based on a DNA barcode library of all eukaryotes. The iBOL covers the advancement of technology, such as quicker information systems, new or enhanced informatics, equipment, and protocols for extracting DNA[10]. Additionally, barcoding helps in identifying and then removing species with characteristics that may cause phylogenetic tree reconstruction to be incorrect, such as unusually high rates of evolution or nucleotide compositional biases [11].

The Consortium for the Barcoding of Life (CBOL) established the following standards for the creation of trustworthy barcode data: Candidate loci should be acceptable for a variety of taxa, exhibit considerable interspecific variation, yet be conserved within species, resulting in negligible intra-specific variation [12]. The borders between races, variations, demes, populations, and species are addressed through DNA barcoding, which is a well-known challenging field [13]. The Consortium for the Barcode of Life (CBOL) initiated the formation of a working group with representation from the various research groups/research consortia from the systematics community that had proposed or tested the seven leading candidate barcoding markers in order to facilitate and formalise the selection of a plant barcode [14].

The usage of the cytochrome oxidase subunit I (cox1) has been promoted by DNA barcoding. [15]. The mitochondrial and chloroplast genomes in plants are evolving too slowly to generate adequate variety, the situation is even more challenging [16]. The 5′ of the mitochondrial gene cytochrome c oxidase I is the DNA sequence utilised as a barcode in arthropods and vertebrates (CoI) [17]. The polymorphic D1/D2 domains within the 28S ribosomal subunit and the nuclear ribosomal internal transcribed spacer (ITS) regions were mostly exploited at the species and generic levels. For taxonomic levels above the genus, the 18S ribosomal subunit and the 28S subunit were used [18].

The threshold strategy in barcoding is no longer applicable if there is no barcode gap. Even while more advanced methods can occasionally be used to delineate species with converging genetic divergences, these methods call for additional suppositions and

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are only practical in well-sampled clades [19]. The ideal barcode would also be short enough to be sequenced in one piece using current technology (-700 base pairs [bp] or less), technically straightforward to sequence, challenging DNA polymerases' accuracy, length-constrained to mitigate alignment issues (although indels would undoubtedly provide helpful diagnostic characters), and recoverable from herbarium samples and other degraded DNA samples like alcohol-preserved samples [20]. It is generally recognised that taxonomic biases of one kind or another can limit the focus of ecological and conservation studies to specific species and also happen in taxonomic study [21].

DNA BARCODING IN ANIMALS

The literature now has a sizable number of comprehensive animal studies in which DNA barcoding, utilising a portion of the COI gene, has been evaluated, utilised to help clarify taxonomic ambiguity, or used as part of a taxonomic description [22]. As a universal barcode for animal species, a 658-bp segment of the cytochrome c oxidase I (COI) has been proposed. Due of the bimodality of intra- and interspecific genetic differences, it is intended to employ this genetic marker as a tag to group conspecifics together and distinguish them from other taxa. This approach offers a trustworthy and simple identification of an unknown specimen when used in conjunction with a database that links genetic tags with taxon names [23].

According to a sequence analysis of the COI amplicon, members of a species typically exhibit little sequence variation, averaging 0.43 percent, but congeneric species have mean divergences that are 18 times greater [24]. It is suggested that the ITS2 locus, one of the most widely utilised phylogenetic markers for eukaryota, be employed as a universal DNA barcode for identifying plant species and as a complementing locus for CO1 to identify animal species [25].

In three forensic wildlife cases, it was discovered that the COI gene made it possible to precisely identify the animal species of unidentified samples. One of the most numerous and well-researched groups of vertebrates, birds were used in this study to test the COI barcode's ability to distinguish across species. For 260 species of birds found in North America, we created COI barcodes and discovered that differentiating between species was generally simple. A study's findings showed that two of the incidents did not include the alleged unlawful poaching of wild animals, as both of the unidentified samples contained sequences that matched those of domestic cattle. [26]. There found no shared COI sequence among any of the 260 bird species. The 130 species that were represented by two or more individuals each had a COI sequence that was either identical or remarkably comparable to other sequences from that same species [27]. There were various COI sequences among the 92 bird species. The COI sequences were identical to the other sequences of the same species. [28].

DNA BARCODING IN PLANTS

There is a huge variety of form and function among land plants. In addition to the bryophytes (mosses, hornworts, and liverworts), ferns, and fern allies, they are made up of seed plants (angiosperms and gymnosperms). There are an estimated 380,000 species of land plants, with 352,000 species of angiosperms, 1,300 species of gymnosperms, and 13,000 species each of bryophytes and ferns/fern allies. Authors' estimates of the total species numbers vary greatly, but a estimate suggests that approximately 380,000 species of land plants are there [29].

Even specialists may find it challenging to identify the 295 tree species on Barro Colorado Island (BCI), but because the majority of the 189 genera have just one species on the island, employing this geographic information can make common barcoding issues easier to solve. 97% of the 1035 samples were identified at the species level and 100% to the genus level using rbcL and trnH-psbA. Psychotria, Ficus, Inga, and Piper are four different genera from which the problematic samples all came. New types of investigations are now made possible by this work [30]. According to Taberlet et al. (2007) and Fazekas et al. (2008), rbcL and trnL stood out the best among the potential plant barcodes (ITS, trnH-psbA, rbcL, trnL, rpoC1, rpoB, matK) [31].

ANIMAL DNA MARKER CO1 FAILED IN THE CASE OF PLANT

Plants change too slowly for the typical animal DNA barcode, which is made up in part of the mitochondrial gene CO1, to be useful. This prompted researchers to look for a similar DNA barcode for terrestrial plants. The plastid genome has been the main focus of this study, despite the fact that several scientists have noted that multiple sections are necessary. It has been challenging to choose a standard plant DNA barcode since each candidate locus has unique advantages and disadvantages and no one candidate locus has emerged as the clear front-runner. It should be emphasized that species discrimination levels produced by CO1 in many animal groups are generally higher than those obtained by traditional DNA barcoding loci in plants. This is partly because the plastid genome experiences less nucleotide substitution, but it also results from other factors like hybridization, polyploidy, speciation through breeding system transitions, species defined by very specific taxonomic ideas, large ancestral population sizes, and low intraspecies gene flow for plastid markers. Since these problems are not equally distributed throughout all plant groups, it is anticipated that certain groups will have a reasonable level of resolution at the species level while other groups would have a very low level of resolution [29].

MARKERS FOR PLANTS

Different barcode regions have been suggested by plant researchers. Scientists proposed that the nuclear ITS region and the plastid trnH-psbA intergenic spacer might be useful as universal plant barcodes, although they later advised combining the two. The Royal Botanical Gardens, Kew, UK, as part of a working group, has also suggested other combinations combining three plastid regions. The trnH-psbA area and pieces of two coding regions (matK and rpoC1), or three coding regions (matK, rpoB, and rpoC1), are included in these. The trnH-psbA area and pieces of two coding regions (matK and rpoC1), or three coding regions (matK, rpoB, and rpoC1), are included in these. Other areas, including the plastid trnL-trnF intergenic spacer and a section of the

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plastid 23S rDNA locus, have been proposed. Furthermore, it has been recommended that without more proof, the mitochondrial cox1 locus should not be disregarded as a plant barcode identifier (P.D.N. Hebert, University of Guelph, pers. comm.)[30].
Some researchers employed a combination of markers to identify medicinal plants because single-locus marker sequences don't always provide adequate details for low level identification. The DNA markers matK, rbcL, trnH-psbA, and ITS are the most frequently combined [31]. The core plant barcode had previously been advocated as a two-marker combination of plastid rbcL and matK, to be complemented by other markers such plastid trnH-psbA and nuclear ribosomal internal transcribed spacer (ITS). Using four distinct data analysis techniques, they sampled 6,286 samples in order to evaluate the efficacy and universality of these barcode markers in seed plants.
We have found the following results from the study:
(i)These studies show that I whereas ITS performed well in angiosperms rather than in gymnosperms, the three plastid markers demonstrated high levels of universality; Ascriptions based on ITS and plastid DNA barcodes were incongruent in certain samples for 45.2% of the studied genera when several members of a single species were evaluated. (ii) ITS showed the highest discriminatory power of the four markers in taxonomic groups and (iii) a combination of ITS and any plastid DNA marker was able to discriminate.
rbcL + matK was suggested as the primary barcode for land plants in August 2009 as a result of an international collaboration. This advice was based on the examination of just a small number of species, from which numerous individuals were drawn from a variety of congeneric taxa. Internal transcribed spacer 2 (ITS2) was also proposed as a novel barcode for both plants and animals after the completion of this study. The Third International Barcoding of Life Conference, held in Mexico City in November 2009, emphasised the need to continue evaluating complementary markers from both the nuclear and plastid genomes, such as ribosomal DNA and trnH-psbA, in addition to the suggested core barcode of rbcL and matK, ITS or ITS2)[32].
Despite much research, traditional barcodes still have a number of drawbacks. Here are some examples of these popular single-locus barcodes. Analysis of different markers among all group of plants:
Matk According to the CBOL Plant Working Group (2009), a single pair of primers may successfully amplify the DNA of angiosperms almost 90% of the time. Even with many primer sets, the success was still modest in gymnosperms (83%) and significantly poorer in cryptogams (10%). In the Orchidaceae, Matk can distinguish between more than 90% of species, but just 49% of nutmeg species. Fazekas et al. (2008) attempted to use the matK barcode to identify 92 species from 32 genera but only had a 56% success rate. These results show that the matK barcode is not a sufficient universal barcode on its own [33].
rbcL RbcL is frequently utilised in phylogenetic analyses, and there are more than 50000 sequences of it in Genbank. This gene has the benefit of being an excellent DNA barcoding region for plants at the family and genus levels, as well as being simple to amplify, sequence, and align in most terrestrial plants. However, rbcL sequences change gradually, and this locus in flowering plants has by far the lowest divergence of plastid genes [33].
TrnH-psbA Currently, the most popular plastid barcode is TrnH-psbA. Designing universal primers is made possible by the presence of highly conserved coding regions on both sides, with a single primer pair potentially amplifying almost all angiosperms. The non-coding intergenic region contains the highest rates of insertion and deletion and the largest sequence divergence. With these characteristics, trnHpsbA is an excellent candidate for use as a plant barcode for species differentiation. Extensive barcoding experiments have shown that the trnH-psbA region can identify virtually all species in various terrestrial plant groups, including Hydrocotyle, Dendrobium, and Pteridophytes. The complex chemical development of the trnH-psbA spacer might lead to highly unclear alignment [33].
psbK-psbI M.R. Enan et al., 2016 conducted a case study. The initial evaluation of the chloroplast intergenic spacer psbK-psbI as a DNA barcode for differentiating date palm cultivars took place in this study. Among the 30 cultivars analysed, five haplotypes were found, resulting in a haplotype diversity of 0.685. Date palm cultivar varieties are clearly related to one another, according to a phylogenetic tree created using the unweighted pair group method and the arithmetic mean [34].
rpoB For bacterial genotypic identification, comparison of the sequences of conserved genes—most frequently those producing 16S rRNA—is utilised. The amount of divergence between the rpoB sequences of various strains was discovered to be significantly higher than that between their 16S rRNA genes [35].
atpF-atpH When used as a single locus, the region atpF-atpH performed significantly better than trnH-psbA, rbcL, and ITS2, and marginally better than rbcL and ITS2 [36].
rpoC For 42 Lathyrus species and two Vicia species, two cpDNA regions—rpoC (rpoC1, its intron, part of rpoC2, and their intergenic spacer) and IR (psbA, trnH-GUG, part of ndhF, and their intergenic spacers)—were examined. 109 potentially useful characteristics were produced by PCR (polymerase chain reaction) amplification of rpoC and IR products after they were digested with 31 and 27 restriction endonucleases, respectively [37].

DNA Barcode segregation potential among different group of plants:

Lichens

In association with photosynthesizing organisms (cyanobacteria or green algae), lichens are fungi (Hale, 1974). A thallus is a basic structure produced by the symbiotic partners, the fungus (mycobiont) and the alga (phycobiont). In places where the alga and fungus couldn't live on their own, the lichen thallus grows. For this reason, lichens grow on a wide range of surfaces, including glass, the ground, stone, and even trees (epiphytes). The pH of the substrate determines the initial choice of lichen Fora on stone artefacts. Silicicolous species grow on acidic soils, while calcicolous species grow on neutral and alkaline substrates.

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Because of the weaknesses in the symbiosis between fungus and algae, lichens act as organic sensors of air pollution. High levels of pollution, especially sulphur dioxide, harm the lichen thallus, first causing slowed development and finally mortality. From the edge to the centre of urbanised regions, the number of species tends to rapidly decline, along with the amount of surface area occupied by those species (Seaward, 1976)[38].

Divakar *et al*., 2015 conducted a case study by collecting 14 species of ***Parmelia sensu stricto*** from across all content.For identification they used ITS marker.From their studies they found out a prominent barcode gap which means in their experiment ITS were able to segregate among these 14 species[39].

Marthinsen *et al*., 2019 conducted a case study by presenting 507 species in 175 genera and 25 orders using 1324 DNA barcode sequences (nrITS). 38 new species were added to GenBank, and for 25 more, ITS sequences were made available for the first time. The three genera Cladonia, Ramalina, and Umbilicaria have barcode gap assessments that are provided and discussed. The new combination Bryobilimbia fissuriseda (Poelt) Timdal, Mart were found out [40].

Mark *et al*., 2016 conducted a case study.There, 100 epiphytic lichen species from Switzerland were barcoded utilising high-throughput, long-read 454 pyrosequencing in a GS FLX+ System. Fungal-specific primers were used for amplifying the whole Internal Transcribed Spacer region (ITS). In the investigation, the predicted lichen fungus was successfully sequenced for all samples except one, demonstrating the feasibility of DNA barcoding utilising pyrosequencing. For the generated lengthy reads, alignment options like BLAST were found to be generally sufficient. However, a number of problems were observed, including a high rate of sequencing errors, the occurrence of numerous ITS variants in a genome, and mixed lichen-forming fungi in certain samples [41].

Kelly *et al*., 2011 conducted a case study by taking samples from a variety of species spanning eight orders. The ITS region was amplified for 351 samples, representing 107, 55, and 28 species, genera, and families of lichenized fungi, respectively. In a taxonomic dataset and a floristic dataset, we evaluated the ability of the whole ITS vs. the ITS2 alone to differentiate between species.In the floristic dataset, a barcode gap for ITS is present in 92.1% of species; 96.3% of sequenced samples could be attributed to the proper species using ITS or ITS2. Up to 94.1% of samples were identified to the proper species using BLAST, despite the fact that fewer species (73.3% with ITS and 68.8% with ITS2) have a barcode gap in the taxonomic collection.Their findings shown the potential of employing DNA barcoding in a floristic environment to identify a large percentage of specimens to the proper species, and the remaining to the correct genus, even though differentiating between the most closely related species would remain difficult[42].

Parnmen *et al*., 2013 conducted a case study, they investigated the ability of ITS to distinguish across lineages within the Cladia aggregata complex, a collection of lichenized fungi with extraordinary morphological and chemical diversity.Their most recent research h validated a 12 species delimitation scenario utilising multilocus DNA sequence data and coalescent-based species delimitation methodologies. In this investigation, they assessed the ratio of the genetic distances between these 12 potential species' intra- and interspecific ITS segments.Each of the 12 potential species displayed a decreased ratio of intraspecific to interspecific variance, indicating that these species may represent different lineages[43].

Bryophytes

This article reviews the use of bryophytes as bioindicators and biomonitors in terrestrial and aquatic settings. Bryophytes are good markers for a variety of pollutants. This is a result of various physiological and morphological characteristics, such as the absence of a cuticle or the presence of strong cationic exchange capabilities within the cell wall. Mosses have mostly been utilised as accumulation indicators, particularly for radionuclides, hazardous organic chemicals, and heavy metals. Benefits and the need for additional study are explored after reviewing a wide range of studies on the subject. By examining the number, distribution, fertility, and vitality of bryophyte species and populations, sulphate and nitrogen depositions can barely be analysed using techniques in the field of accumulation monitoring. Global change research focuses on similar methodologies, particularly for the examination of climate warming and the impact of land-use intensity on biodiversity[44].

Bell *et al*., 2012 conducted a case study by using three plastid (matK, rbcL, and trnH-psbA) and one nuclear (ITS) marker, DNA barcoding of a collection of European liverwort species from the genus Herbertus was carried out. The DNA barcode data helped identify a previously undiscovered European Herbertus species, H. norenus sp. nov., and were useful in differentiating amongst the Herbertus species that were sampled. This species is unique visually and displays distinct variances in DNA sequence for various barcode locations. Clarifying the taxonomic relationships between some species from North America and Asia and some European species was another benefit of the DNA barcode data. The most informative region in terms of the discriminatory ability of the several barcode markers was ITS, closely followed by matK. Each species could be identified [45].

Hassel *et al*., 2012 conducted a case study.They looked at the performance of the nuclear ITS2 region and the plastid sections atpF-atpH, rbcL, and trnH-psbA as barcode markers on closely related bryophyte species of specific moss (Bartramia, Distichium, Fissidens, Meesia, and Syntrichia) and liverwort (Blepharostoma) genera from the boreal.Additionally, they assessed the relationship between the length of the sequenced fragment, specimen age, and taxonomic group with the success of herbarium specimen sequencing. Sequencing success was higher for shorter fragments and more youthful herbarium specimens, but it was less successful than anticipated in the genera Distichium and Fissidens, indicating that the primers employed were not completely ubiquitous. When used as a single locus, the area atpF-atpH performed significantly better than trnH-psbA, rbcL, and ITS2, and somewhat better than rbcL and ITS2.In comparison to atpF-atpH alone, concatenated data sets of two and three markers categorised more conspecific sequences into monophyletic groups, although the improvement was not significant. They come to the conclusion that the most promising moss barcode markers are atpF-atpH, rbcL, and ITS2[46].

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Stech *et al*., 2013 conducted a case study based on variable DNA sequence markers from the plastid (rps4-trnT-trnL area) and nuclear (nrITS) genomes, they assessed morphological species circumscriptions in the R. canescens complex, a species complex of the moss genus Racomitrium. In terms of amplification and sequencing success, four molecular marker partitions (rps4-trnT, trnT-trnL, ITS1, ITS2) fared nearly equally well. In particular in complexes of closely related species, ITS1 offered the highest degree of species differentiation and should be taken into consideration as a DNA barcoding marker for mosses [47].
Liu *et al*., 2011 conducted a case by examining the applicability of DNA barcoding to four moss genera of the Grimmiaceae (Racomitrium, Coscinodon, Grimmia, Schistidium) in China using four sections of chloroplast DNA (rbcL-a, rps4, trnH-psbA, and trnL intron). The four loci under investigation had species resolutions of 65% (trnH-psbA), 59% (rps4), 53% (rbcL-a), and 29% (trnH-psbA) (trnL intron). The number of monophyletic species with bootstrap support more than 50% was larger for rps4 (59%) than for trnH-psbA (47%), rbcL-a (35%), and trnL intron (18%). In contrast to the best single locus, multi-locus combinations boosted the percentage of monophyletic species with bootstrap support >50% but were unable to significantly improve species resolution. Racomitrium species may be resolved entirely by rps4 and primarily (>80%) by rbcL-a or trnH-psbA.The plastid regions of the Coscinodon species had distinctive rbcL-a, rps4, trnH-psbA, and trnL intron sequences. In Grimmia and Schistidium, considerable intra-specific variability or inter-specific sequence sharing caused significant barcoding failure[48].
El-Atroush *et al*., 2015 conducted a case by using two DNA barcoding areas, they examined two medicinally significant yet threatened plants (Cleome droserifolia and Iphiona scabra) by using ITS and rbcL. The universality of the ITS and rbcL sections was good, demonstrating the use of these loci as DNA barcodes. The two loci had significant inter-specific genetic diversity and were simple to amplify and sequence, making them suitable DNA barcodes for higher plants. In order to identify two medicinally important endangered species that were gathered from the Abou Galoom Protectorate in South Sinai, Egypt, they examined the potential of the ITS and rbcl markers. The viability and potential of the ITS region in the identification process for the two plants used is more efficient than rbcl, where rbcl confirms the identification of two plants at generic level, whereas ITS at the species level. In this study, ITS and rbcl markers were used to discriminate and confirm the identification of two medicinal endangered plants [49].
Burgess *et al*., 2011 conducted a case based on the rbcL+matK barcode, they created a barcoding database for a temperate flora with a considerable taxonomic breadth in the Koffler Scientific Reserve in Ontario, Canada. They compared the performance of this pairing to that of three other potential supporting locations (the coding region rpoC1 and two non-coding intergenic spacerstrnH-psbA and atpF-atpH). They looked at these markers individually and in combination to assess their ability to distinguish between the 436 species and 269 genera of terrestrial plants.They were able to recover a high-quality sequence from at least one region in 98.2% of the 513 samples screened using high-throughput approaches, and 55% of those samples had full coverage across all five gene regions. RpoC1 had the lowest sequencing success rate (74.5%) and rbcL had the highest (91.4% of the samples collected). In comparison to the non-coding intergenic spacers, the two coding regions rbcL and matK offered a disproportionately large number of high-quality bidirectional sequences, which when combined allowed for the accurate identification of 93.1% of the examined species. Adding the trnH-psbA intergenic spacer (95.3%) or combining all five gene areas (97.3%) led to marginal improvements in species resolution.The identification success rate using rbcL+matK was weakly correlated with the number of species per genus, with 100% for monotypic genera (representing 70.5% of the flora) and 83.6% for polytypic genera. Gymnosperms, bryophytes, lycophytes, and monilophytes, which together make about 5% of the flora, had a greater success rate of identification using the rbcL+matK barcode (100%) than angiosperms (92.7%). Their findings shown that for this and other nearby northern temperate floras, the rbcL+matK barcode can offer an acceptable high rate of species resolution. It accomplishes so efficiently, with only a small amount of laboratory work, and despite the fact that some samples lack data from specific plastid regions [50].

Pteridophyta

Ebihara *et al*., 2010 conducted a case study by collecting 733 taxa of Japanese pteridophytes, including subspecies and variants. For rbcL, DNA sequences were acquired from 689 (94.0%) taxa, and for trnH-psbA, from 617 (84.2%) taxa. While mean K2P distances of each genus shown substantial variation according to systematic location, mean interspecific divergence values across all taxon pairs (K2P genetic distances) did not demonstrate a significant difference in rate between trnH-psbA and rbcL. When rbcL and trnH-psbA were coupled, the minimal fail rate of taxon discrimination in an identification test using BLAST (12.52%) was attained, and it became lower in datasets eliminating infraspecific taxa or apogamous taxa, or both.This study shows that DNA barcodes are generally useful for identifying species in the Japanese pteridophyte flora. Despite the high prevalence of apogamous species in this flora, which make it difficult to identify plants using DNA barcodes, these taxa are confined to a small number of genera and only slightly lower the success rate overall. Routine species identification might not be achievable if a query sequence matches a recognised apogamous genus. The majority of Japanese pteridophytes may be identified by DNA barcoding, which is also expected to be useful for identifying non-hybridizing gametophytes[51].
Nitta *et al*., 2020 conducted a field survey and 176 species from 69 genera and 22 groups of pteridophytes were discovered. The DNA barcode marker plastid rbcL was chosen and found in >95% of the pteridophyte taxa at this location. Two previously unknown taxa that seem to have hybrid origins were discovered through combined genetic and morphological investigations. By calculating minimal interspecific distances, the usefulness of rbcL for species identification was evaluated, and it was discovered to have an 18% failure rate. Finally, they made a comparison between Japan and Tahiti, two other regions that have been the focus

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of pteridophyte molecular surveys, and the distribution of minimal interspecific rbcL distances. Nectandra is more like Japan than Tahiti, according to the comparison, which may be related to the biogeographic history of both floras[52].

Gu *et al*., 2013 conducted a case study.In their study, 103 samples totaling 34 species and nearly all of the Selaginellaceae's medicinal plants were gathered from the primary distribution areas in China. Using common primers and reaction settings, the ITS2 region of the genome was amplified from these samples and sequenced. Their findings demonstrated that the ITS2 regions could successfully identify the species of all Selaginellaceae samples evaluated using the BLAST1 and closest distance approaches. The stem loop number, size, position, and screw angle among the Selaginellaceae medicinal plants showed considerable changes in the secondary structures of ITS2 in the helical regions.Medicinal Selaginellaceae plants can be accurately identified using the ITS2 barcode. The findings offered a rationale for the prudent exploitation of these resources as well as a scientific basis for the exact identification of plants belonging to the Selaginellaceae family [53].

Trujillo-Argueta *et al*., 2021 conducted a case study. By Applying the CBOL criteria for barcoding, they examined three plastid barcodes—rbcLa, matK, and trnH-psbA—in selected pteridophytes, a group that is well-represented in these woods but has received little study in Oaxaca, Mexico. RbcLa and trnH-psbA universal primers were effectively amplified and bidirectionally sequenced, but most species were unable to amplify matK. In BLASTn, rbcLa displayed the strongest species discriminating (66.67%). In comparison to rbcL and rbcLa + trnH-psbA, trnH-psbA displayed greater significant interspecific divergence values (two-sample sign test, P value 2.2e-16). Monophyletic species were effectively resolved (100%) using NJ and ML phylogenetic trees, differing only in support values and showing complete agreement with the most recent fern classification. The highest mean support value (80.95%) was found in ML trees. The Elaphoglossoideae subfamily was only detectable by the barcode trnH-psbA. Utilizing rbcLa + trnH-psbA had no increased effect on species discrimination. RbcL is good for fern barcoding, trnH-psbA is best for phylogenetic analysis, and matK might not be a reliable marker for all types of barcoding[54].

De Groot *et al*., 2011 conducted a case study. They looked at how well rbcL and the non-coding plastid marker trnL-F worked together to identify fern species through DNA. By building a reference library of reliable rbcL and trnL-F sequences for the wild-occurring homosporous ferns of NW-Europe, a regional approach was used. For assessing the two-region barcode's ability to discriminate, parsimony and distance-based methods were combined. 86 small fern gametophytes were utilised as test subjects for the effective extraction of DNA for DNA-based identification. Both markers demonstrated excellent primer universality.Unless they belonged to a pair of Dryopteris species with totally similar chloroplast genomes, all test samples could be recognised to the genus level; however, species identification was not always achievable. Our findings point to a significant possibility for using rbcL and trnL-F together as a two-locus cpDNA barcode for fern species identification. Ecological tests might favour a regional strategy. Here, we present a ready-to-use barcoding method for ferns that paves the way for addressing a wide range of issues in fern gametophyte ecology[55].

Chetia *et al*., 2016 conducted a caes study by choosing 16 species of Assamese pteridophytes, and the variation in their chloroplast rbcL gene sequences was examined. A bryophyte, Funaria hygrometrica, was used as the outgroup sequence in a molecular phylogenetic analysis employing rbcL gene sequences. The findings demonstrated that the out group pteridophytic species Funaria hygrometrica generates a completely distinct branching from the other pteridophytic species. Azolla pinnata and Marsilea minuta, two aquatic pteridophytes, were shown to form a distinct clad from the other pteridophytes, indicating their evolutionary distance from other pteridophytes despite sharing a common ancestor as shown by the root in the tree[56].

Spermatophyta

He *et al*., 2015 conducted a case study .In this work, 144 samples from 35 different species of Ligularia were evaluated for four DNA core barcoding regions (ITS, matK, psbA-trnH, and rbcL). The findings showed that relatively low interspecific variation causes a very low species identification rate in the chloroplast areas (matK, psbA-trnH, and rbcL). In contrast, the ITS sequence demonstrated a greater rate of species identification (60%) and the ability to distinguish between species that are challenging to identify[57].

Li *et al*., 2011 conducted a case study by testing the universality of nine possible matK primers and one rbcL primer using 57 gymnosperm species from 40 genera, 11 families, and four subclasses. Due to its great universality, primer (1F/724R) of rbcL is suggested here as a primer for all gymnosperms. Gym F1A/Gym R1A, one of the nine possible matK primers, is recommended as the best "universal" matK primer for gymnosperms due to its high success rate in polymerase chain reactions and regular production of high-quality bidirectional sequences. In this study, a brand-new matK primer for Ephedra was created, and it worked well on the species that was sampled. For the majority of gymnosperms, the primers for rbcL and matK that are suggested here can be amplified quickly and successfully[58].

Pang *et al*., 2012 conducted a case study.Non-flowering seed plants' DNA barcoding has received less attention, and there has only been a small amount of evaluation of DNA barcodes in this field so far. Here, seven markers from non-flowering seed plants were examined: psbA-trnH, matK, rbcL, rpoB, rpoC1, ITS, and ITS2. Four factors were used to evaluate each region's usefulness: the PCR amplification rate, the difference between intra- and inter-specific divergences, the DNA barcoding gap, and the capacity to distinguish between species. The barcoding of non-flowering seed plants yielded the best results with the ITS2 locus out of the seven evaluated.Additionally, utilising a sizable database of gymnosperms from GenBank, we examined the capacities of the five most suggested markers (psbA-trnH, matK, rbcL, ITS, and ITS2) to identify additional species. In a wide variety of non-flowering seed plants, ITS2 was nevertheless useful for species identification: of the 1531 samples from 608 species of 80 different genera, 66% of them were correctly authenticated at the species level by ITS2. In conclusion, this study will provide important information

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for the barcoding of plant species and the ITS2 region can be used as a barcode to distinguish between non-flowering seed plants[59].

Bolson *et al*., 2015 conducted a case study. 30 commercially relevant woody species across several populations, the effectiveness of each marker and chosen marker combinations were assessed, with an emphasis on Lauraceae species. The capacity to retrieve species-specific clusters, species discrimination rates, and inter- and intraspecific distances were assessed. According to the "best close match" test, ITS was the most effective area and combination for identifying species, while the trnH-psbA + ITS combination also produced good results. Maximum Likelihood analysis showed a better defined topology for internal branches when trnH-psbA + ITS were combined, with 91% of species-specific clusters. There is a high degree of confidence in recognising Ocotea members using DNA barcoding, which has been demonstrated to be a feasible and quick way for identifying important vulnerable woody angiosperms from Araucaria Forests, such as Lauraceae species[60].

Heckenhauer *et al*., 2016 conducted a case study. They created 14 matK primers (seven forward and seven reverse) for multiplex PCR utilising sequences from 178 taxa belonging to 123 genera in 41 families and 18 orders that were already accessible in GenBank. 53 samples from 44 representative angiosperm families in 23 different orders were used to evaluate the universality of these new multiplexed primers. The primers demonstrated significant PCR success and sequencing success. These findings demonstrated that the newly created primers are very efficient for multiplex PCR and can be used in upcoming barcode research including samples from a variety of angiosperm taxa. The price and duration of PCR amplification will be decreased by using multiplex primers for barcoding [61].

CONCLUSION:

Several researchers have already established the potential of COI as the universal barcode for animals, having the capacity to distinguish animals at species level. However, it is evident that scientist could not identify any single marker which can segregate and identify all plant species. Scientists have used several markers, both singly and multilocus approach towards findings an universal DNA Barcode for plants. But none of them so far could be used to segregate different group of plants. Despite these difficulties, plant DNA barcodes will be very beneficial for many applications, including ecological forensics, the identification of traded materials, carrying out identifications in situations where there is a lack of available taxonomic expertise, and assisting in the discovery of new species in some plant groups. In order to identify samples using molecular methods, DNA barcoding has grown to be a common way of choice. Research on herbal pharmacovigilance of adverse reactions to particular products is showing that DNA barcoding is a potent method for determining the species composition in herbal medicines and has the potential to be used as a standard method. DNA barcoding is a method developed by molecular genetics that provides a low-tech but potentially highly effective tool for identifying species. The shortcomings of morphology-based identification call for the incorporation of molecular data. One method that has been suggested for identifying herbal components and spotting adulteration is DNA barcoding. We suggest a strategy that combines DNA-based techniques using genus- or species-specific primers, chemical analysis, microscopic and macroscopic methods, and DNA barcoding of processed plant material instead of universal primers for DNA barcoding of processed plant material as a sole method of species identification based on the information currently available. Population decreases are a result of wildlife animal poaching for both commercial and subsistence uses. DNA barcoding was used in this investigation to identify many poaching cases. Laura Filonzi et al., 2021 by using two different primer sets and mini-COI sequencing, the molecular analysis of 71 commercial fish samples yielded amplification success rates of 87.3 and 97.2%. Xiao-Long Lin et al., 2021 uncovered 14 unknown species and one potential synonym with reference to COI DNA barcodes. V Dincă et al., 2021 created the first high-resolution reference library for European butterflies, with 22,306 COI sequences and 97% taxon coverage (459 species).In forensic fields, molecular identification has become relevant and is frequently used to address some morphological determination problems. In a wide range of animal taxa all over the world, DNA barcoding based on the mitochondrial gene cytochrome c oxidase subunit 1 (cox1) is used as a quick and reliable tool for species identification. The most important stage is compiling sizable DNA sample sets that represent the earth's botanical diversity, supported by voucher specimens, and indexed by DNA sequences. Several studies have identified rbcl +matK+ITS2 has the choice of barcode. However, future detailed research on this aspect will be necessary for assigning this primer combination as the universal barcode for plants. Future technical advancements will likely result in enhancements over current methods. This will serve as the underpinning for both current and future applications in the coordinated use of DNA sequence data to distinguish between plant species.

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