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Review Article

DNA Barcoding: An Effective Technique in Molecular Taxonomy

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Abstract

Global warming is affecting regional climate, ecosystem and diversity array of species by causing physical and biological changes throughout the planet. Therefore, there is a need to develop a technique which can identify organisms and differentiate between very closely related species in order conserve species diversity. Classical taxonomy has accelerated its progress with the adoption of new molecular techniques like DNA barcoding to cope with the huge population of organisms and biodiversity available in this planet. DNA barcoding uses short gene sequences which are well classified portion of the genome. With the advent of high throughput sequencing technology such as Next-Generation Sequencing (NGS) technology the DNA barcoding has become more accurate, fast and reliable in the last decade. The Consortium for the Barcode of Life (CBOL) has given a platform for taxonomists across all the countries to collaborate, identify and preserve the biodiversity across the globe. In this review we summarized the recent advances and developments in the DNA barcoding attempts across animals, plants, bacteria, fungi, viruses and protists. We have also attempted to present the popular tools used in DNA barcoding in a chronological order of their development.

Keywords: DNA barcoding; Bioinformatics tools; Barcoding region; Cytochrome C oxidase (*COI*); Maturase K (*matK*); Internal transcribed spacer (*ITS*)

Introduction

A DNA barcode is one or few relatively short gene sequences present in the genome which is unique enough to identify species. DNA barcoding is a useful tool for taxonomic classification and identification of species by sequencing a very short standardized DNA sequence in a well-defined gene. In this technique, complete information of the species can be obtained from a single specimen irrespective to morphological or life stage characters. It is an effective technique in which extracted DNA from the collected sample is processed following the standard protocol (Figure 1). Identification of the species is carried out by amplifying highly variable region i.e., DNA barcode region of the nuclear, chloroplast or mitochondrial genome using Polymerase Chain Reaction (PCR). Region widely used for DNA barcoding include nuclear DNA (e.g. *ITS*), chloroplast DNA (e.g. *rbcL, trnL-F, matK, psbA, trnH, psbK*) and mitochondrial DNA (e.g. *COI*) (Figure 2). DNA barcodes can be used as a tool for grouping unknown species based on barcode sequence to earlier known species or new species. It can also be used for grouping specimens to known species in those cases where morphologic features are missing or misleading. It can also be used as a supplement to other taxonomic datasets in the process of delimiting species boundaries [1]. The set of DNA barcode markers have been applied to specific taxonomic groups of organisms and are proving invaluable for understanding species boundaries, community ecology, functional trait evolution, trophic interactions and the conservation of biodiversity [2]. The application of NGS technology had expanded the versatility of DNA barcodes across the 'Tree of Life', habitats and geographies as new methodologies are explored and developed [3].

In order to characterize species, CBOL has selected few genes as ideal for DNA barcoding. Ideally, one gene sequence would be used to identify species in all of the taxa (taxonomic groups) from viruses to plants and animals. However, that ideal gene has not yet been found, so different barcode DNA sequences are used for animals, plants, microbes and viruses. Research using cytochrome c oxidase barcoding techniques on zoological specimens was initiated by Hebert and his group [4]. From 2004, CBOL (currently hosted at http://www.barcodeoflife.org/) started to promote the use of a standardized DNA barcoding approach, consisting of identifying a specimen based on a single universal marker i.e., the DNA barcode sequence. An ideal DNA barcode region or locus should have low intra-specific and high inter-specific divergence (creating a "barcode gap") and easy to amplify from most or all species in the target group using universal primers. Reference barcodes must be derived from expertly identified vouchers deposited in biological collections with online metadata and validated by available online sequence chromatograms [4].

DNA Barcode of Animals

For animals, the mitochondrial cytochrome C Oxidase I (*COI*) locus appears to satisfy the desired criteria for most groups [5,6]. This

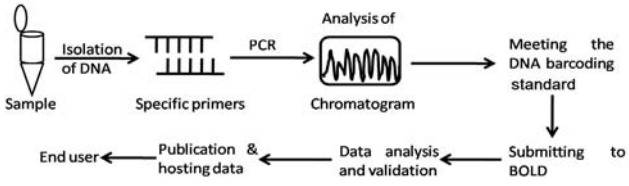

Figure 1: A general DNA barcoding process flow sheet.

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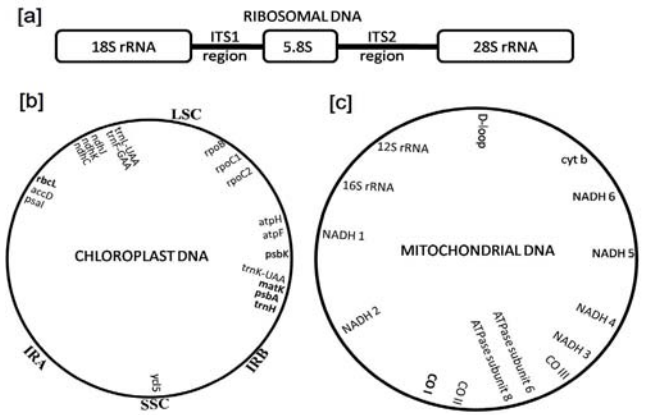

Figure 2: The DNA Barcoding Locus [a] Ribosomal DNA showing ITS region [b] Chloroplast DNA [c] Mitochondrial DNA.

DNA sequence has been found in a mitochondrial gene inherited mainly through the maternal line, which effectively discriminates between most of the animal species (Table 1). Initially, 650 base long segment of the cytochrome C oxidase gene has been elevated to the status of "the barcode of life" for identifying animal species [6]. Later, a 100-base fragment of the original barcode was reported to be effective in identifying archival specimens and potentially useful for all taxa of the eukaryotes [7]. DNA barcoding using *COI* region is now widely used for molecular evaluation of diversity, as it has good potential for identifying cryptic species and improving our understanding of marine biodiversity [8,9].

In animals, mitochondrial DNA occurs as a single double-helical circular molecule containing 13 protein-coding genes, 2 ribosomal genes and several tRNAs (Figure 2c) [10]. Mitochondrial genes are preferred over nuclear genes because mitochondrial genes lack introns, they are generally haploid and exhibit limited recombination [6,11]. Furthermore, each cell has several mitochondria and each mitochondrion contains several such circular DNA molecules and therefore, several complete sets of mitochondrial genes. Thus, when sample tissue is limited, the mitochondrion offers a relatively abundant source of DNA.

DNA Barcode of Plants

DNA barcoding in plants have been a more challenging task than those in animals. Unlike animals, plant mitochondrial genes perform unsatisfactory as a candidate gene for DNA barcoding. The generally low rate of nucleotide substitution in plant mitochondrial genomes precludes the use of *COI* as a universal plant barcode [21]. However, potential candidates have been reported in the chloroplast genome. The most satisfactory results have come from the gene maturase K (*matK*) and *matK* in association with other genes (Table 2). This has been used to resolve the flora of biodiversity hot spots [22]. The *matK* barcode has been claimed to have discriminated 90 percent of plant species [23].

MatK is nested in the group II intron of the chloroplast gene for transfer RNA lysine (trnK), and includes a domain for reverse transcriptase [22]. Group II intron is a class of intron found in rRNA, tRNA and mRNA of organelles in fungi, plants, protists and some mRNA in bacteria. Group II introns are self-splicing *in vitro* but employ maturase proteins *in vivo* [24]. Multi-locus markers such as ITS along with *matK*, *rbcL*, *trnH*, etc. have been assumed to be more successful in species identification (Table 2). However, studies to date demonstrated that these are also inadequate for universal plant identification [25,26]. Despite significant recent effort, the development of single-locus barcodes has stalled, placing plant DNA barcoding at a crossroads. Fortunately, developments in DNA sequencing allowing cost-efficient plastid sequencing are driving plant identification into a post-barcode era [27].

The use of two or more chloroplast barcodes has been advocated for the best discrimination in estimating biodiversity, and impressive progress has been made in using chloroplast DNA barcodes for identifying plant species [28].

DNA Barcode of Bacteria

Bacterial 16S rRNA gene is described as an important marker for soil and marine ammonia oxidizing bacteria [71], mountain lakes [72], rice paddy soil microcosms [73] and human clinical samples [74]. So, it is apparent that 16S rRNA gene is highly conserved for each and every species of bacteria and it can be used as a marker for DNA barcode for different species [75]. A microbial diversity picture in this planet is still not clear as many microbes are difficult to culture. It is very crucial to understand microbe association with different environments and their function in those environments. Gene sequences of 16S rRNA from different samples especially environmental samples have reformed our understanding of microbial diversity and it helps us in cataloguing the vast diversity

Table 1: Gene/locus selected for the study of DNA barcoding in animals.

Gene/Locus	Organism (s)	Reference (s)
COI	*Holothuria edulis*	[09]
	Leptorhynchoides thecatus	[12]
	Pomphorhynchus tereticollis	[13]
	Acanthocephalus lucii	[14]
	Allolobophora chlorotica	[15]
	Polymorphus brevis	[16]
	Axiothella constricta, Deosergestes corniculum, Caprella andreae, Microcosmus squamiger, Microcosmus squamiger, Nucula sulcata, Leptoplana tremellaris	[17]
	Synecdoche constellate, Bruchomorpha beameri, Cixius nervosus	[18]
	Diopatra neapolitana	[19]
	Andrena humilis, Andrena fulvida	[20]

Table 2: Gene/locus selected for the study of DNA barcoding in plants.

Gene/Locus	Organism (s)	Barcodes used in combination	Reference (s)
matK	*Rhubarb*		[29]
	Puerariacandollei, Butea superb, Mucunacollettii		[30]
	Galpemia spp.	*rbcL*	[31]
	Dendrobium spp.	*rbcL*	[32]
	Angelica spp.	*rbcL, ITS ,psbA-trnH*	[33]
	Rhododendron spp.	*rbcL, ITS , psbA-trnH*	[34]
	753 genera	*rbcL, ITS , psbA-trnH*	[35]
	Lonerica spp.	*rbcL, ITS, psbA-trnH, trnL-F*	[36]
	Solanum spp. & adulterants	*rbcL, ITS, psbA-trnH, trnL-F*	[37]
	Ginseng genus	*rbcL, ITS, psbA-trnH, trnL-F, rpoB, rpoC1*	[38]
	Astragalus spp. & adulterants	*rbcL, ITS*	[39]
	Various medicinal roots	*ITS, psbA-trnH, rpoC1*	[40]
rbcL	*Scutellaria spp. Astragalus spp.* and *adulterants*	*matK, psbA-trnH*	[41]
	Lamiaceae	*matK, psbA-trnH*	[42]
	Various medicinal plants	*matK, psbA-trnH*	[43]
	Sabia spp.		[44]
	Pteridophytes		[45]
psbA-trnH	*Paris spp.* and *adulterants*		[46]
	Senna spp.		[47]
	Smilax spp.		[48]
	Phyllanthus spp.		[49]
	Cistance spp.		[50]
	Vitex spp.	*matK*	[51]
	Sideritis spp.	*matK*	[52]
ITS	*Various medicinal plants*		[53]
	Various medicinal plants		[54]
	Boerhavia spp. Astragalus spp. and *adulterants*		[55]
	Sedum spp. Astragalus spp. and *adulterants*		[56]
	Rubus spp.		[57]
	Hypericum spp.		[58]
	Ochradenus spp.		[59]
	Rehmannia spp.		[60]
	Medicinal vines (22 genera)		[61]
	Dipsacus spp.		[62]
	Dendrobium spp.		[25]
	Dendrobium spp.		[63]
	Paris spp.		[64]
	Citrus spp.		[65]
	Ruta spp.		[66]
	Astragalus spp.		[67]
	Meconopsis spp.		[68]
	Orobanche spp. and *adulterants*		[69]
	*Taraxacum*and *adulterants*		[70]

Table 3: Gene/locus selected for the study of DNA barcoding in bacteria.

Gene/Locus	Organism (s)	Reference (s)
COI	*Wolbachia*	[78]
rpoB	*Streptococcus sp.*	[81]
16S	*Rickettsia sp., Ehrlichia sp.*	[82]
cpn60	*Lactobacillus johnsonii, Streptococcus sp.*	[80]
tuf	*Candidatus phytoplasma sp.*	[83]
RIF	*Xanthomonas*	[84]
gnd	*Buchnera sp.*	[85]

Table 4: Gene/locus selected for the study of DNA barcoding in fungus.

Gene/ Locus	Organism (s)	Reference (s)
ITS	*Fusarium virguliforme*	[94]
	Colletotrichum sp., Aureobasidium sp., Pseudocercospora sp.	[95]
	Gomphus floccosus *Lactarius sp.* *Cantharellus cibarius* *Tricholoma viridi-olivaceum* *Laccaria vinaceo-avellanea, Ramaria maculatipes*	[96]
***RPB1* (LSU)**	*Ramaria rubella,*	
***RPB2* (LSU)**	*Ramaria stuntzii,*	
***18S* (SSU)**	*Gomphus floccosus*	
	Gautieria otthii	

of microorganisms on earth [76]. *COI* gene was also used to develop the DNA barcode for 22 species pathogen [77]. Smith and his group developed the DNA barcode for Wolbachia, a common endo-symbiotic bacterium, using *COI* gene, and this gene is one of the five multi-locus sequence typing genes which was applied for categorizing Wolbachia [78]. They have found very few overlap with the eukaryotic DNA barcode area. This study corroborates that the *COI* gene can be a DNA barcode marker for bacteria. Chaperonin-60 (cpn60), also known as GroEL and Hsp60, is a molecular chaperone conserved in bacteria. Conversely, for evaluating the barcoding targets for Archaea including 16S rRNA, type II chaperonin (ortholog of cpn60) was found to be another option [79]. Links and his group suggested that cpn60 can be a common target for bacteria barcode [80]. Some other genes are used for bacterial identification such as *rpoB* gene which may be used as a barcode marker gene for bacteria [81]. So, we can infer that 16S rRNA, *COI* gene, and *cpn60* can normally be used as markers for developing DNA barcode (Table 3).

DNA Barcode of Fungi

A region of the mitochondrial gene encoding the cytochrome C Oxidase (*COI*) is the barcode for animals [4,6] and the default marker adopted by the Consortium for the Barcode of Life for all groups of organisms, including fungi [1]. In Oomycota, part of the kingdom Stramenopila historically studied by mycologists, the de facto barcode Internal Transcribed Spacer (ITS) region is suitable for identification, but the default *COI* marker is more reliable in a few clades of closely related species [86].

COI performs well in Penicillium and other fungi [87], but in few other groups it may not be equally promising, and cloning may often be required [88]. The degenerate primers for many Ascomycota may not be easy to assess as amplification failures may not reflect priming mismatches [89]. Extreme length variation occurs because of multiple introns [87,90], which are not consistently present in a species. Multiple copies of different lengths and variable sequences occur, with identical sequences sometimes shared by several species [89]. Most interestingly, some fungal clades, such as Neocallimastigomycota may lack mitochondria [91]. Most fungi are microscopic and inconspicuous and many are unculturable. Thus robust, universal primers are required to detect a truly representative profile where *COI* seems to have many challenges from other candidates like ITS [88]. The large subunit of the nuclear ribosomal RNA (LSU), a favoured phylogenetic marker among many mycologists, had virtually no amplification, sequencing, alignment or editing problems and the barcode gap was superior to the Small Subunit of rRNA (SSU). However, across the fungal kingdom, ITS was generally superior to LSU in species discrimination and had a more clearly defined barcode gap [92]. ITS have been reported to perform as a close second to RNA polymerase II largest subunit (RPB1) as the protein-coding marker (Table 4). However, the much higher PCR amplification success rate for ITS may pose a critical difference in its performance as a barcode [88]. It had been reported that all primer sets have a range of biases therefore an appropriate solution may be to use more than one primer combination [93].

Table 5: Gene/locus selected for the study of DNA barcoding in protists.

Gene/Locus	Organism (s)	Reference (s)
ITS	*Bigelowiella natans,* *Chlorarachnion reptans,* *Gymnochlora stellata ,* *Lotharella amoeboformis,* *Lotharella globosa,* *Lotharella oceanic,* *Lotharella vacuolata,* *Norrisiella sphaerica,* *Partenskyella glossopodia,*	[107]
COI	*Colpoda sp.,* *Blepharisma sp.,* *Spirostomum ambiguum,* *Spirostomum teres,* *Stentor coeruleus,* *Drepanomonas revolute,*	[124]
rbcL	*Chaetoceros decipiens sp. ,* *Chaetoceros diademasp,* *Thalassiosira anguste-lineata,* *Thalassiosira pacifica,*	[125]
	Pseudo-nitzschia delicatissima, *Odontella aurita,* *Fragilaria sp.,*	[121]
18S	*Tabularia fasciculata*	[126]
28S	*Thalassiosirales,* *Chaetocerotales*	[125]

DNA Barcode of Virus

Probably, viruses are the most abundant biological creature on earth. It has been estimated that the total number of virus particles is more than 10 times the total number of cells. The molecular diversity of viruses is complicated, as virus molecular diversity of genome is also complex. The "molecular entity" and virus species still remain as a debate for several scientists. The identification and explanation of molecular entities of virus should be the major objective of DNA barcoding. Very few works have been incited to identify the pathogenically important viruses. Therefore, researchers may aim to develop the barcode for the detection or identification of the virus.

Recently, few works have been found in this direction [97]. Wei and his group described the k-mer-based barcode image to identify significant pathogenic Human Entero-Viruses (HEVs) [98]. In this process, the condition of 1<k<7 for a fixed k and a genome barcode was described in terms of the k-mer frequency distribution across the whole genome for all combinations of k-mers. Bluetongue Virus (BTV) is an animal virus which affects the different mammals such as cattle, buffalo, sheep, deer, goats, etc [99]. For the detection of BTV, ultrasensitive technique Bio-Barcode Amplification Assay (BCA) method was developed. This method was used for the specific detection of the outer-core protein VP7 of BTV. Though they have produced protein bases bio-barcode, however, signal DNA annealed to DNA strands bound with the gold nanoparticles which were released by heating and characterized by PCR and real-time fluorescence PCR [100]. To detect Avian Influenza Virus (AIV), a fluorescent DNA barcode-based immunoassay was developed based on the application of sandwich immunoassay and fluorophore-tagged oligo-nucleotides as representative barcodes [101]. To understand the viral biodiversity and development of DNA barcode, no marker DNA or RNA has been developed for viruses. However, it is the time to understand the viral biodiversity with DNA barcoding.

DNA Barcode of Protists

Protists are a diverse and loose grouping of disparate eukaryotic microorganisms. They are unicellular, or they are multi-cellular without specialized tissues with relatively simple organization. This simple cellular organization distinguishes the protists from other eukaryotes. They diverged after Archaea and Bacteria evolved but before plants, animals, or fungi appeared on Earth. The Protist Working Group (ProWG), initiated by the Consortium for the Barcode of Life (CBOL) has assessed the efforts to identify the barcode regions across all protist lineages and now introduced a two-step barcoding approach to assess protistan biodiversity. Various protistan DNA barcodes have been proposed (Table 5). The D1–D2 and/or D2–D3 regions at the 5′ end of 28S rDNA have been positively tested in ciliates [102], haptophytes [103], and acantharians [104] and are also promising for diatoms [105]. Zimmermann and his group have also shown that V4 sub-region on the 18S rRNA gene may serve as a potential candidate for barcoding diatoms [106]. Ribosomal Internal Transcribed Spacers (ITS1 and/or ITS2 rDNA), which are the main fungal barcodes, are also commonly utilized in oomycetes [86], chlorarachniophytes [107] and green algae [108]. They have also been suggested for dinoflagellates [109] and diatoms [110] with some reserve [111]. The mitochondrial gene coding for cytochrome C Oxidase (*COI*), which has been proposed as the universal barcode for animals, also allows morpho-species identification in red [112] and brown [113] algae, dinoflagellates [114], some raphid diatoms [115], Euglyphida [116], lobose naked [117] and shelled [118] amoebae, coccolithophoridhaptophytes [119] and some ciliates [120]. Other group-specific barcodes include the large subunit of the ribulose-1,5-biphosphate carboxylase–oxygenase gene (*rbcL*) and the chloroplastic 23S rRNA gene for photosynthetic protists [105,121] and spliced leader RNA genes for trypanosomatids [122]. Clearly, the choice of group-specific barcodes is often a question of tradition or ease of use. The studies systematically comparing the resolution power of different protistan DNA barcodes are rare [123].

Tools to Identify DNA Barcode

Innovative computational approaches for DNA barcoding have been developed in the past based on Compensatory Base Changes (CBCs), Operational Taxonomic Units (OTUs), DNA metabarcoding, locus specific tools [127,128], tool for representing barcode symbology, techniques of neural networks, machine learning, data mining [129], composition vector, etc (Table 6). The two new computational methods used in DNA barcoding have been proposed by using barcode sequences of bacteria, archaea, animals, fungi and land plants [130].

Compensatory Base Changes (CBCs) are mutations of nucleotide at both positions of a paired structural site, while the pairing is still maintained. CBCs reported in rRNA ITS2 have been successfully used for the verification of closely related species. When there is even one CBC at a conserved paired site in the ITS2 secondary structure, they are found to be sexually incompatible [131]. CBCs thus have been successfully used for the discrimination of closely related species. Software based on CBCs is CBC Analyzer [132]. It has been observed that when the number of informative sites are not large, character methods are often less efficient than distance methods [133]. When OTUs with highly unequal evolutionary separation are included in the data set none of the approaches perform well. "Molecular Operational Taxonomic Units" (MOTU) are clusters of sequences derived by grouping the DNA sequences of a conserved gene or gene fragment. The sequence clusters act as representatives of the genomes from which they are derived. A dataset of sequences can be classified into MOTU at a number of different similarity cut-offs, the cut-off value acting as a parameter to the clustering algorithm. Tools like jMOTU and Taxonerator [134], Taxon Gap [135] and CLOTU are based on this concept.

DNA metabarcoding is a process in which environmental sample is used for identifying a number of organisms simultaneously. Environmental sample or eDNA refers to any DNA collected from soil, water or air. It mostly contains degraded DNA and many of the times only short barcodes can be used from it. Therefore, highly conserved and versatile primers are required in metabarcoding studies along with other constraints imposed by this technique. ecoPrimers [136] and OTUbase [137] are tools for DNA metabarcoding studies.

The Back Propagation (BP) neural network approach has been applied for inferring species membership *via* DNA barcoding. To implement this approach, Zhang and his group have developed a computer program BPSI (BP Species Identification) and demonstrated the power of machine learning in DNA barcoding [138]. This method in combination with bioinformatics especially aimed at identifying species with non-coding barcodes and it is also advantageous compared to their previously proposed BP neural network approach [139]. The method can be applied through the data mining software BLOG (Barcoding with LOGic formulas) [140]. Alignment also becomes the rate limiting step for constructing profile trees for DNA barcoding purposes. Thus unaligned rRNA sequences can be used as barcodes based on the Composition Vector (CV) approach [141] without the need for sequence alignment. The Composition Vector (CV) method is an alignment free approach, especially suitable for non-protein-coding sequences used as barcodes [142]. CVTree is software for constructing phylogeny trees using a CV approach and includes only prokaryotic proteome data.

Table 6: Methods used in various DNA barcoding tools and their web address.

Tools	Launch Year	Method	Available at
TaxI	2005	Distance based	axel.meyer@uni-konstanz.de
CBCAnalyzer	2005	Phylogenies based on CBC	http://cbcanalyzer.bioapps.biozentrum.uni-wuerzburg.de/cgi-bin/index.php
4SALE	2006	RNA alignment and editing	http://4sale.bioapps.biozentrum.uni-wuerzburg.de/
CodonCode Aligner	2007	Codon based	http://www.codoncode.com/index.htm
BPSI	2008	Back Propagation (BP) neural networks	zhangab2008@yahoo.com.cn
SAP	2008	Bayesian phylogenetics	http://ib.berkeley.edu/labs/slatkin/munch/StatisticalAssignmentPackage.html
CAOS	2008	Character Based	http://sarkarlab.mbl.edu/CAOS
TaxonGap	2008	Operational Taxonomic Unit (OTU) based	http://www.kermit.ugent.be/software.php?navigatieId=37&categorieId=17
BioBarcode	2009	Sequence based	http://www.asianbarcode.org
BLOG	2009	Data mining approach	http://dmb.iasi.cnr.it/blog-downloads.php
B	2010	Sequence quality and contig overlap	http://www.nybg.org/files/scientists/dlittle/B.html
OFBG	2010	Spp. Discrimination using oligonucleotide frequencies	http://www.nbri.res.in/ofbg.php
OTUbase	2011	Operational Taxonomic Unit (OTU) based	http://www.bioconductor.org/packages/release/bioc/html/OTUbase.html
jMOTU	2011	Multiple Operational Taxonomic Unit (MOTU) based	http://www.jmotu.com-about.com/
Taxonerator	2011	OTU and taxonomy data based	http://www.taxonnerator.com-about.com/
CLOTU	2011	Amplicon and taxa data	http://www.mn.uio.no/ibv/bioportal/
Eco Primers	2011	Barcode markers and primer based	http://www.grenoble.prabi.fr/trac/ecoPrimers
PTIGS-Idit	2011	psbA-trnHIntergenic Spacer (PTIGS) based	http://psba-trnh-plantidit.dnsalias.org
BRONX	2011	Sequence Identification Incorporating Taxonomic Hierarchy	http://www.nybg.org/files/scientists/dlittle/BRONX.html
Spider	2012	Analysis of species identity and evolution	http://spider.r-forge.r-project.org/SpiderWebSite/spider.html
ISHAM	2013	Mycological classification	http://www.isham.org/
LV barcoding	2013	Locality sensitive hashing-based	http://msl.sls.cuhk.edu.hk/vipbarcoding/
Excali BAR	2014	Calculate intra- and interspecific distances	http://datadryad.com/resource/doi:10.5061/dryad.r458n
VIP Barcoding	2014	Vector-based software	http://msl.sls.cuhk.edu.hk/vipbarcoding/
Q-Bank	2015	identification and detection reference database	http://www.q-bank.eu/
obitools package	2015	NGS data based	http://metabarcoding.org/obitools

Limitation of DNA Barcodes

DNA-based species identification depends on distinguishing intra-specific from inter-specific genetic variation. The ranges of these types of variation are unknown and may differ between taxa. It seems difficult to resolve recently diverged species or new species that have arisen through hybridization. There is no universal gene for DNA barcoding, no single gene that is conserved in all domains of life and exhibits enough sequence divergence for species discrimination. The validity of DNA barcoding therefore depends on establishing reference sequences from taxonomically confirmed specimens. This is likely to be a complex process that will involve cooperation among a diverse group of scientists and institutions. Barcode sequences are, in general, short (approx. 500–1000 bp) and this fundamentally limits their utility in resolving deep branches in phylogenies. Some controversy exists over the value of DNA barcoding, largely because of the perception that this new identification method would diminish rather than enhance traditional morphology-based taxonomy, and species determinations based solely on the genetic divergence could result in incorrect species recognition [1]. However, we must keep open the possibility that the barcode sequences per se and their ever increasing taxonomic coverage could become an unprecedented resource for taxonomy, systematic biology and diagnosis, and may be equally useful [143].

Conclusion

As the advantages and limitations of barcoding become apparent, it is clear that taxonomic approaches integrating DNA sequencing, morphology and ecological studies will achieve maximum efficiency at species identification [144]. DNA barcoding may help speed the work of taxonomists and others interested in species identification. The evidence from a number of studies largely confirms the feasibility of such a system [145]. Despite some drawbacks of using DNA barcoding, the reported success of using the barcoding region in distinguishing species from a range of taxa and to reveal cryptic species is remarkable. Efforts should therefore be made to develop nuclear barcodes to complement the barcoding regions that are currently in use.

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