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Review Article

INSECT SYSTEMATICS, BIOSECURITY AND LIMITATIONS USING DNA BARCODING : A REVIEW

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The DNA barcoding method, which was first presented 18 years ago, has gained a great deal of attention. DNA barcoding offers a lot of potential as a method for identifying species since it is simple to apply, affordable, and commonly shows species-level separations. Millions of species have already been DNA barcoded as part of ongoing attempts to barcode all major animal groups, including insects. To expedite taxonomic identification, DNA barcoding is now being studied as a potential alternative method for detecting insect biodiversity in India and around the globe. In this review, we have covered its use, potential limitations, and efficiency in terms of biosecurity.

The majority of life on earth is comprised of insects, which have evolved into an enormous variety of various species. Taxonomists only described 10% of the projected total number of species after approximately 200 years. In this situation, identifying insects has proven to be a gigantic effort that necessitates the availability of more professionals and funding. Naturalists developed the concept of categorizing living things based on taxonomy, a field of science that aids in the description of a living entity based on physical characteristics, to catalog the enormous number of species.Traditional taxonomy focuses on the visual observation-based meristic (countable trait) and morphometric characterization of animal, fish, and insect taxa. 250 years after Darwin and Linnaeus, the DNA barcoding, a technology of DNA-based taxonomy,is used to identify known and unknown species based on their DNA's nucleotide organization (Novotny *et al.*, 2002). In addition to assisting in the finding of new species, DNA barcoding involves the use of a short and standardized DNA sequence (in insects, a 658 base pair (bp) fragment of the mitochondrial cytochrome c oxidase (COI) gene).

The "Folmer region" at the 5' end of cytochrome C oxidase 1 (COI, Cox1, or*cox1*) gene, which has 658 bp fragment, was suggested as the universal marker for all eukaryotes. It could be utilized when classifying unidentified individuals as a species or in detecting cryptic and polymorphic species (Hebert *et al.*, 2003; 2004; Herbert and Gregory,2005). This DNA sequence proved to be successful in his pioneering and following investigations on insects, fish, and birds. For the quick and precise identification of several species, short standardized gene segments (DNA barcodes) have been used, such as the 5.8 S ribosomal RNA gene and flanking internal transcribed spacers (ITS) 1 and 2 region (Shenoy *et al.*, 2007; Schoch *et al.*, 2012).

With millions of species and wide differences in their life stages, taxonomy finds it difficult to make an accurate identification. However, because of recent scientific developments, it is now possible to distinguish between known and undiscovered species using a technique called DNA barcoding, which is based on DNA-based taxonomy. Taxonomists can classify specimens and identify species more quickly thanks to DNA barcoding. To ensure quick and precise identification of a variety of biological specimens, Paul Hebert (Jinbo *et al.*, 2011) suggested this technique in 2003. It involves employing a primer set to amplify a 648 bp area of the mitochondrial cytochrome-c oxidase subunit 1 (COI) gene. Based on various investigations using this area on numerous taxa, the 5' fragment of the COI gene has been regarded as the standard barcoding region in animals (Hebert *et al.*, 2003a). These COI gene regions have been regarded as being particularly instructive from an evolutionary and speciation perspective. There are roughly 59,000 insect species described in India. However, the number of barcodes generated from India represents only 4.6 per cent of known species, whereas the corresponding global scenario represents about 16 per cent of described species (Jalali *et al.*, 2015). As a result, much efforts are needed to catch up with the global scenario. The official repository for DNA barcode data, the BOLD system (https://boldsystems.org/index.php/TaxBrowser_Home), now has more than a million records available. Molecular diagnostic technologies offer important help for the quick and precise identification of alien species that are morphologically difficult to distinguish (Armstrong and Ball, 2005). DNA barcoding is regarded as a "tremendous tool" to hasten the finding of new species and to begin their descriptions. It has arisen and established itself as a crucial tool for phylogenetic research and species identification.

According to Wilson (2012), the voucher specimens from which library barcodes are derived have a close connection to other data, notably Linnaean names, locations of collections, and morphology in the form of digital photographs. From ardent taxonomists to recent graduates in molecular biology, this instrument is well acknowledged throughout the world and is being used to catalog every species on Earth by governmental and non-governmental organizations. Identification of different life forms, including insects, has become swift, accurate, and simple with the development of molecular biology and molecular tools. Simple, fast, and accurate methods are

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needed to categorize such vast diversity. DNA barcoding meets all the aforementioned requirements for species-level identification. rDNA ITS-2 (Ashok Kumar *et al.,* 2009), COX 1, NADH dehydrogenase subunit 1 (*nadh1*), and cytochrome b (*cytb*) markers utilized in recent molecular analyses have advanced our knowledge of insect phylogeny. Molecular researchers throughout the world utilize COX 1 to distinguish insect species.

It is considered effective to use species identification markers from the mitochondrial COX 1 region for molecular identification and phylogeny. The quick collection of molecular data is the primary benefit of DNA barcoding (Monaghan *et al.,* 2005). The energy-producing organelles known as mitochondria are present in almost all cells of virtually all plant and animal species. Animal mitochondrial DNA (mtDNA) has a relatively fast mutation rate, resulting in the generation of diversity within populations over relatively short evolutionary timescales. Because it's present in all eukaryotic creatures, the mitochondrial genome is important for tracking evolution (thousands of generations). A 658-bp fragment of the COI gene was typically presented as a potential "barcode" DNA in mammals. Different inheritance patterns can be seen in nuclear and mitochondrial genomes. Different inheritance patterns can be seen in nuclear and mitochondrial genomes (Behura, 2006).

Because mtDNA is inherited from the mother, it evolves quickly, and the majority of nucleotide alterations occur at neutral locations, mitochondrial markers are employed to indicate phylogenetic relationships across related groups. The COI marker gene amplification sequence data was used to study the intra- and inter-phylogenetic interactions related to this genetic marker. In coordinated evolution, mutations rapidly spread to all members of a gene family, preserving relative homogeneity (Arnheim 1983; Gerbi 1985; Tautz *et al*., 2002). Mitochondrial DNA was long assumed to be a neutral marker that represented species history. However, Ballard and Whitlock (2004) and Bazin*et al*. (2006) argue that mitochondria are typically under severe selection and develop under distinctive rules compared to other genomes. Selection may influence mtDNA directly or indirectly, according to Hurst and Jiggins (2005).

DNA barcoding is a standard and practical method for identifying insect species, developmental stages, food webs, and biotypes, but morphology-based taxonomy may not. Morphological data are typically time-consuming and require specialists. Molecular data do agree with morphological theories, according to statistical taxon separation analysis and tree-based taxon clustering.As a result, it was demonstrated that the examined taxa could be identified as species using DNA sequence analysis. For scientists, DNA barcoding has the potential to be a useful tool. It has helped develop cutting-edge techniques for species diagnosis, such as primers for tea mosquito bugs (Rebijith *et al.,* 2012).

A DNA barcode is created in what way?

After extracting the total DNA from the animal, fish, or insect sample, universal primers are employed in a Polymerase Chain Reaction (PCR) to amplify the COI region. After sequencing and analysis, the amplified product is subsequently submitted to databases,The Barcode of Life Databases (BOLD) (Fig. 1).

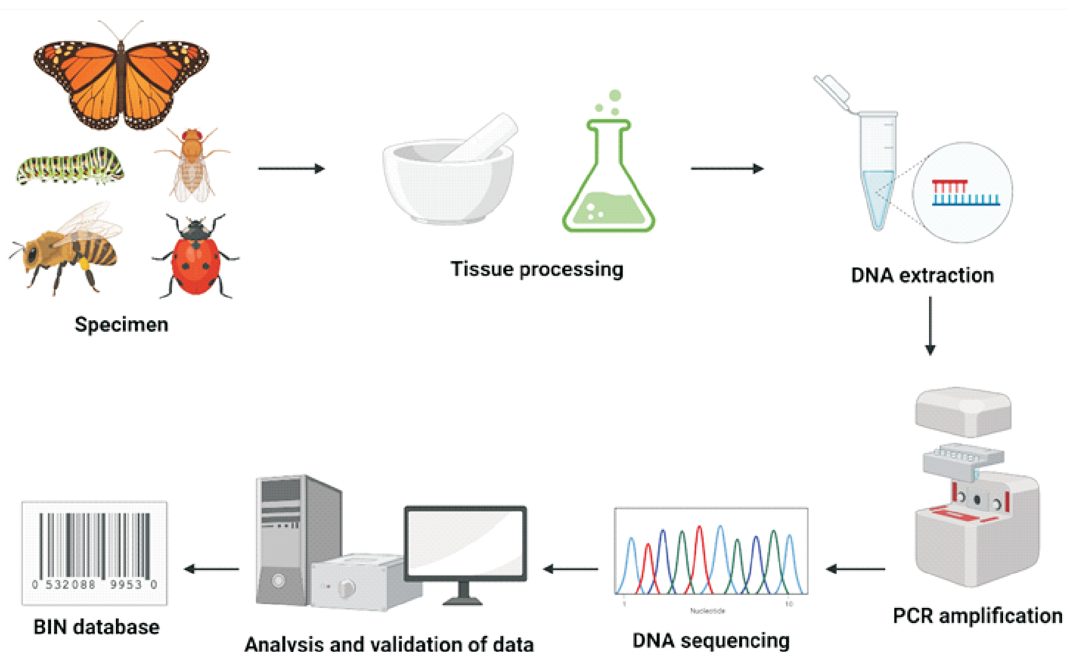

Figure 1. Stepwise DNA barcoding process from samples through barcode data analysis

BOLD is an online database and workbench (http://www.boldsystems.org/). DNA barcode records can be acquired, stored, analyzed, and published with its assistance. It is openly accessible. It helps with record assembling so that records can be designated as BARCODE in the five global sequence databases.

Taxonomy using a DNA barcode approach

Paul D.N. Hebert of the University of Guelph in Ontario, Canada, began working on a public database of DNA barcodes that would be associated with particular specimens in 2003. The power of this library would expand with greater taxonomic coverage and quicker, less expensive sequencing since it would "give a new master key for identifying species." The creation of a sizable, openly accessible sequence database is the aim of a DNA barcoding library. The DNA barcode initiative's participants come in a variety of forms, including consortia, databases, networks, labs, and projects with scales ranging from local to international.

The largest consortia are:

The International Barcode of Life (iBOL) Project. The Biodiversity Institute of Ontario at the University of Guelph created this 25-nation partnership with assistance from Genome Canada.

The Consortium for the Barcode of Life (CBOL) is an international project that aims to establish DNA barcoding as a universal taxonomy standard. Despite not producing any barcode data, CBOL promotes barcoding via conferences, outreach programs, working groups, and workshops. The IBOL working group for outreach and cooperation has CBOL as its designated lead organization. The National Museum of Natural History of the Smithsonian Institution in Washington, DC, USA is home to CBOL. In 40 different countries, CBOL has more than 100 institutional members.

The European Distributed Institute of Taxonomy (EDIT), which was founded as part of the research infrastructure activities, launched the European Consortium for the Barcode of Life (ECBOL).

DNA barcode database

There are two main databases for DNA barcodes. The University of Guelph's BOLD is a public workbench for barcoding projects. Before submitting their data records to the International Nucleotide Sequence Database Collaboration, which is made up of GenBank, EMBL, and DDBJ, researchers can assemble, test, and analyze them in the BOLD. They are the long-term public archives for records including barcode information.Barcoding initiatives can be planned, involve multiple nations through initiatives such as FISH-BOL or the ten International Barcode of Life project (iBOL) working groups, or just be the work of a single person or a small research team. The requirement for voucher specimens that have been identified by knowledgeable taxonomists and adherence to the Barcode data standard is what unites all barcoding efforts.

Barcoding program for DNA

DNA barcoding software requires a Field Information Management System (FIMS), Laboratory Information Management System (LIMS), sequence analysis tools, workflow tracking to integrate field and laboratory data, database submission tools, and pipeline automation for eco-system-size projects.The sequence analysis components of Geneious Pro, Bioinformatics Software for Sequence Data Analysis (https://www.geneious.com/), can be used, and the MooreaBiocode Project's two freely downloadable plugins such asThe Biocode LIMS (https://github.com/biocodellc/biocode-lims)plugin and GenBank Submission (https://www.geneious.com/plugins/genbank-submission-plugin/) plugin handle interface with FIMS, LIMS, workflow tracking, and database submission(s).

Any researcher can register online for free access to the BOLD. The outcome is presented in tables with the most connected species and taxa. DNA barcode collection, maintenance, analysis, and use are all facilitated by the BOLD, an online workbench. It was created by the Canadian Centre for DNA Barcoding as official informatics for the Barcode of Life project (Ratansingham and Hebert, 2007). BOLD's three sections (MAS, IDS, ECS) serve distinct barcoding communities.

Barcode records can be stored in the BOLD-MAS (Management and analysis) repository, which also offers analytical tools. It serves as the DNA barcode community's online workbench.

To identify species, BOLD-IDS (identification engine) provides a tool that receives DNA sequences from the barcode region and, when feasible, produces a taxonomic assignment to the species level.

Web developers and bioinformaticians can create tools and workflows that can be linked with the BOLD framework using BOLD-ECS (external connection). BOLD-ECS offers REST services that enable access to open sequence and specimen data.

Hebert (2003a, b) suggested a method to quickly and precisely identify a variety of biological specimens by amplifying a 648bp area of the mitochondrial cytochrome-oxidase subunit 1 gene. This process was given the moniker "DNA barcoding." The Barcode of Life initiative was then put forth to support DNA barcoding as a universal standard for eukaryotic sequence-based identification. The CBOL was established in 2004 to formally launch this initiative with the goals of creating a standard DNA barcoding methodology and a large DNA barcode collection.With the recent introduction of the International Barcode of Life initiative, the Barcode of Life project has entered a new phase (iBOL, 2010). In terms of insects, campaigns have been launched for the Lepidoptera, Trichoptera, ants (Formicidae), bees, and other taxa (Jinbo, 2011). The BOLD, created by the Canadian Center for DNABarcoding, serves as the project's official informatics workbench (Ratnasingham and Hebert, 2007). (CCDB).The BOLD

provides DNA barcode data storage, an identity support system, and internet services for developers.

Molecular-based identification is not a novel concept. One molecular identification approach employs SrRNA sequences to identify bacteria, among other molecular identification systems (Busse, 1996). However, DNA barcoding has several benefits over earlier techniques. Its accessibility is one benefit. For identifying species, a piece of the common DNA barcode area is particularly effective. For the majority of animal groupings, this area has strong discrimination abilities. The all-purpose primer, which was initially created for marine invertebrates, can be used on any kind of animal (Hebert 2003a, b; 2004a). The information in a 648-bp fragment is sufficient and can be directly sequenced using a sequencer. This is a COI region that codes for proteins, so the alignment process is not challenging. By determining if the obtained sequence can be translated, errors can be found. The region was chosen as the default DNA barcode because of these advantageous characteristics. Another advantage of DNA barcoding is the verifiability of identification of voucher specimens via links to taxonomy.

Because DNA barcoding is easy to use, inexpensive, and frequently reveals species-level separations that correspond to accepted taxonomy, it has enormous potential as a technique for identifying species. Molecular data may be used to identify morphologically distinct individuals of the same species, such as those at different developmental stages, social insect castes, and sexually dimorphic individuals (Kathirithamby 2010; Murria 2010; Pauls *et al.,* 2010). The benefits of DNAbarcoding include primer universality, the compilation of data on a broad variety of taxonomic groups, and its connection to taxonomy, in addition to the characteristics of normal non-barcode genetic markers. These advantages may facilitate the study of ecologically fascinating insect phenomena such as aphid host switching, significant sexual dimorphism, and the heterotrophic heteronomy of Strepsiptera (Kathirithamby, 2010).

The use of some stag beetles as pets and the use of birdwing butterflies as ornaments are examples of unlawful trade in endangered or protected insects that can be detected by DNA barcoding. Rapid identification techniques would make it easier to monitor disease vectors like mosquitoes for epidemiological purposes. DNA barcoding has the potential to replace other methods of species identification in these areas (Floyd, 2010). For pest control, quick identification of pest species' larvae is crucial. Doskocil (2008) used a DNA barcode to examine the species makeup and seasonal distribution of turfgrass-infesting beetle larvae (Coleoptera: Scarabaeidae) and based on their findings offered an effective management method. The taxonomic perspective of molecular data, particularly DNA barcoding, is highly contested (Meier, 2008).The two main problems are species finding and identification. The type specimen of a species is the most trustworthy source for a DNA barcode that accurately represents that species (Brown *et al.*, 2003). Species discovery, on the other hand, is the taxonomic process of identifying a group of individuals or populations as a single species. The DNA barcode can hasten the discovery of new species. First, cryptic species that have hitherto gone unnoticed can be identified via DNA barcoding (Hebert, 2004a; Janzen *et al.*, 2005).

Second, DNAbarcode data aids in grouping all samples of related taxa, particularly when taxonomic studies of these taxa are insufficient (Smith *et al.*, 2008). All specimens for undescribed species may not be found by DNA barcoding, particularly for recently divergent populations. It is clear that neither the DNA barcode alone, nor the information it provides, is sufficient to classify unidentified specimens as a new species (i.e., the barcode alone cannot be used to define a species). As well as providing other crucial supporting data (such as distribution, life history, host plants, and place of origin of species) for taxonomic research such as integrative taxonomy, the results of barcoding can only indicate new species possibilities (Yoshitake *et al.*, 2008;Schlick-Steiner *et al.*, 2010).Based on type specimens, species descriptions utilizing barcodes will likely become increasingly prevalent and significant. The precision of species identification provided by DNA barcoding is one of the most important difficulties with this technology. In general, the accuracy is mostly dependent on how much intra-specific variation and inter-specific divergence overlap. In other words, the more successful the species identification, the wider the "gap" between intra- and interspecific variances in the genetic distance (Hebert, 2004b). DNA barcoding-based identification is ineffective when intra- and inter-specific distances are greatly overlapping (Elias *et al.*, 2007; Wiemers and Fiedler, 2007).

Numerous circumstances, such as high genetic diversity within a species or paraphyly or polyphyly between species (De Salle *et al.*, 2005), can result in the overlap. Species may show up as polyphyletic or paraphyletic in phylogenetic analyses as a result of introgression, insufficient lineage sorting of mitochondrial DNA, or inconsistent morphological species definitions. In these circumstances, additional investigations in conjunction with additional features, such as nuclear genes, are necessary (Hebert, 2003a). The discrepancy between molecular data and the conventional concept of species, particularly when a group is poorly investigated taxonomically, is another aspect that may cause overlap (Meyer and Paulay, 2005). These conditions could benefit from integrated taxonomic revisions that make use of genetic and morphological data (Kehlmaier and Assmann, 2010).The coverage and dependability of the available barcode libraries are the most significant factors influencing the accuracy of species identification (Ekrem *et al.*, 2007).

As was already established, barcodes are insufficient for identifying species when intra- and interspecific distances are heavily overlapping. However, by fusing additional molecular information with barcodes,

samples can be identified. It's not surprising that insect DNA barcoding has so far yielded conflicting results. It is a reliable tool for the molecular identification of Lepidoptera, Hymenoptera, Coleoptera, and Diptera species, according to several studies (Burns, 2008; Smith *et al.*, 2008; Fisher and Smith *et al.*, 2008). A few researchers Smith *et al.* (2006, 2007), Trewick *et al.* (2007) and Elias *et al.* (2007) questioned the effectiveness of DNA barcoding in Lepidoptera and Orthoptera, while Meier *et al.* (2006) reported a shockingly low identification success (400 taxa) for Diptera. The usage of GenBank sequences, which allegedly contain a significant proportion of incorrectly recognized sequences, was suggested as the cause of the low success of DNA barcoding demonstrated by Meier (2006) and Ward *et al.* (2009). Virgilio *et al.* (2010) reported that Type II errors (queries lacking conspecifics in databases) cause poor identification success. Virgilio *et al.* (2010) suggested that Type II mistakes may be avoided by using insect DNA barcoding to validate a query's lack of concordance with a list of correctly referenced target species (e.g., insect pests). The total number of insect specimens barcoded in BOLD was 8,933,257 (Table 1) shows the insect collecting locations.

Table 1. Current status of the insect DNA barcoding library (in the BOLD system)

Sr. No.	Insect order	Specimens with sequences	Specimens with barcodes	Species	Species with barcodes	Public records	Public species
1.	Archaeognatha	4,015	3,829	49	48	4,435	42
2.	Blattodea	33,611	31,718	2,139	1,742	28,331	1,422
3.	Coleoptera	719,319	623,364	48,563	30,059	640,509	39,285
4.	Dermaptera	2,918	2,715	156	58	2,928	79
5.	Diptera	4,733,390	4,622,796	39,689	33,149	3,246,238	29,450
6.	Embioptera	621	597	73	67	715	72
7.	Ephemeroptera	36,110	32,677	2,666	2,372	30,051	2,343
8.	Hemiptera	528,254	480,006	17,522	12,191	456,417	14,680
9.	Hymenoptera	1,405,575	1,260,728	56,540	43,793	1,220,240	38,779
10.	Lepidoptera	1,645,831	1,567,149	120,255	101,582	1,378,333	75,766
11.	Mantodea	4,628	4,333	614	438	2,758	392
12.	Mecoptera	2,761	2,428	285	207	1,198	158
13.	Megaloptera	2,531	2,218	154	113	2,259	132
14.	Neuroptera	18,160	16,580	1,064	834	15,544	776
15.	Notoptera	24	7	8	3	24	7
16.	Odonata	32,538	25,693	3,412	2,773	22,685	2,026
17.	Orthoptera	53,734	47,948	4,123	3,208	49,826	3,493
18.	Phasmatodea	2,960	1,191	647	253	2,756	582
19.	Plecoptera	19,868	18,971	1,491	1,368	17,624	1,302
20.	Psocodea	86,596	81,911	1,526	521	81,952	1,398
21.	Raphidioptera	617	577	33	25	586	27
22.	Siphonaptera	3,208	2,980	211	124	2,107	196
23.	Strepsiptera	807	745	85	43	929	77
24.	Thysanoptera	39,221	37,090	547	443	36,962	416
25.	Trichoptera	72,903	64,168	6,292	4,978	64,226	5,162
26.	Zoraptera	24	24	1	1	18	1
27.	Zygentoma	945	814	126	114	658	96
	Total	9,451,169	8,933,257	308,271	240,507	7,310,309	218,159

(Source: https://boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=82, Accession date: 22nd July 2022)

One of the primary difficulties connected to the incorporation of molecular information into taxonomy elements of biology that has yet to be explored in depth is the optimal approach to scan barcodes. Currently, DNA barcodes are used for two functions. The first is using DNA to discriminate between species while the second is using

DNA to identify new species. Both tasks demand different kinds and amounts of data.

DNA barcoding as a means of facilitating biosecurity

Biosecurity is one of the most critical concerns confronting the worldwide community. It protects against biological risks that endanger ecosystem stability, producer livelihoods, and consumer trust (Cock, 2003; Armstrong and Ball, 2005). It has historically been linked to dangers from infectious illnesses, LMOs, and biological weapons. However, in its broadest definition, it also refers to reducing risk through "biological harm" (Meyerson *et al.,* 2002). Morphological identification approaches seem to have challenges with invasive species control, which demands quick and precise species identification but limited taxonomic expertise. Also, morphological features make it difficult to identify immature taxa (Besansky *et al.,* 2003; Darling and Blum, 2007). Identifying intercepted specimens to the species level is crucial for prediction and monitoring (Armstrong and Ball, 2005). Since the pioneering article on COI as a DNA barcode by Hebert *et al.* in 2003, this short gene region has become the instrument of choice for various biological applications, most notably animal taxonomy and biomonitoring (Coleman and Radulovici, 2020). Invasive alien species (IAS) pose a financial danger to ecological stability, producer livelihoods, and consumer confidence (Cock, 2003). Changes in climate and land use also have an impact on this risk, which is made possible by the spread of exotic species through rising international travel and trade. One per cent of species introduced to new settings are predicted to become invasive and have significant negative economic effects (Williamson, 1996).The IAS puts up to 80% of the world's vulnerable and endangered species in jeopardy (Mandal, 2011). Studies on the cactus moth, the European poplar shoot borer, nocturnal moths, agromyzid leaf miners, ants, siricid wasps, true bugs, and tephritid fruit flies demonstrate the effectiveness of DNA barcoding as applied to invasive species detection and determination of native provenance (deWaard, 2010). A second barcoding investigation found four previously undiscovered alien species in a Vancouver, Canada, urban park (deWaard, 2009).Armstrong (2010) examined the effectiveness of identification using three significant Lepidopteran pest groups, a species group of Lymantria yellow peach moths (Crambidae: Conogethes), and fall webworms (Arctiidae: Hyphantria), with positive findings. In a study conducted by Jinbo (2011), fruit flies(Diptera: Tephritidae) and tussock moths, *Orgyia* sp. (Lepidoptera: Lymantriidae) were examined to see if DNA barcoding might be utilized . Integrating traditional taxonomy, DNA-based technologies, and bioinformatics enhances the possibility of bridging knowledge gaps (Honeycutt, 2021). In recent reports from India, using genetic approaches alone or with conventional taxonomical tools, researchers could quickly detect various invasive pests and advise effective intervention solutions (Firake and Behere, 2021) such as elephant beetle (*Xylotrupes siamensis* Minck) (Firake *et al.,* 2013); fruit fly species (*Bactrocera aethriobasis* (Hardy), *B. thailandica* Drew and Hancock and *B. tuberculata* (Bezzi)) (Manger *et al.,* 2018); cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) (Joshi *et al.,* 2020). Integration of DNA barcoding into national bio-surveillance systems took time, but some agencies have adopted it to diagnose invasive species (deWaard *et al.,* 2010). The most serious danger to biodiversity is biosecurity, which makes early and accurate identification of invasive species essential.Given worldwide concerns about biodiversity, barcodes will likely be used for species discovery. DNA barcoding has revolutionized inventorying biodiversity (Jansen and Hallwachs, 2016), conservation biology (Shapcott *et al.*, 2015), and biosecurity risk assessment from invasive species (Molnar *et al.*, 2008; Madden *et al.*, 2019). The DNA barcode library's global coverage is very important for this use. Boykin *et al.* (2012) analyzed genetic data coverage and availability for 88 invasive insects included in the Global Invasive Species Database (data are documented in either BOLD or GenBank for seven of those species). BOLD is lacking data for 37 (42%) species or includes inaccessible private data, whereas 9 (8%) species have no data in GenBank. A review of the BOLD ver. 3.0 Barcode Identification Number (BIN) system revealed that more than one species was present in the BIN in 41% of the cases. This was caused by the BINs' 1% delimitation thresholds, which could lead to misidentifications. Overall, GenBank provides more information on the 88 invasive species listed in the Global Invasive Species Database. However, quality screening is necessary to verify that the material gleaned from GenBank is accurate and helpful. deWaard *et al.* (2010) evaluated the taxonomic and geographic coverage of the DNA barcode reference library to identify species/subspecies and determine the geographic origin of tussock moth populations in Canada. COI barcodes were collected from 518 individuals and 36 species. 90 per cent of species groupings have significant bootstrap support, according to a maximum likelihood tree that was created. The effectiveness of barcoding was also compared to that of the widely used NB restriction digest system, which is also based on COI. COI barcode sequences provide improved resolution and universality since they cover more unique haplotypes across all Lymantria species.According to deWaard *et al.* (2010), the success of DNA barcodes for identifying species supports the idea that the method is an under utilized resource with significant potential for biosecurity and surveillance. The biomonitoring organizations currently using the NB restriction digest system would gain more information by switching to DNA barcoding, a transition that could be made relatively seamlessly since the same gene region underlies both protocols. Boykin (2012) discussed and advocated a novel "tip to root strategy" in which careful evaluation of species delimitation is necessary to support important biosecurity decisions based on precise species identification. They applied the method for defining the species of two extremely invasive insect pests, the sweet potato whitefly, and the Asian gypsy moth. Both species provide biosecurity

concerns due to their extraordinarily high levels of intraspecific genetic variability and the presence of a number of unclear subspecies, but they also exhibit the most extreme phylogenetic resolutions, which present the most difficult delimitation issues for biosecurity. They tested many analytical alternatives to determine whether they might be utilized to provide stricter species delimitation measures and more defendable species assignments and unknown identification for biosecurity. Various analytical methodologies were employed on DNA barcode datasets. In the species instances analyzed, the findings show that further gene sampling is needed to confirm the new cohort of species or to identify the existing subspecies taxonomy of *Lymantria dispar* (Linnaeus). The Geneious software plugins forspecies delimitation or exploration make it simple to utilize, thus similar analysis of such multi-gene datasets would be supported. Since its inception, DNA barcoding has grown in popularity and will soon be a common method of identifying different kinds of organisms. Despite several drawbacks, the method has shown to be invaluable for quickly identifying a wide range of species, including insects. It has been demonstrated that DNA barcoding can speed up and improve the effectiveness of ecological processes including trophic ecology and biodiversity assessment. The barcode libraries need to be strengthened through international cooperation to be widely used for biosecurity objectives.

Limits of previous molecular biosecurity diagnostics

To identify regulated pest species, multiple independent molecular approaches are available. Immunological (e.g., Symondson *et al*., 1999; Trowell *et al*., 2000) or protein-based (Miles 1979; Soares *et al*., 2000) techniques are not often used since they need high-quality, fresh tissue, and are exceedingly taxon-specific, difficult to change, and subject to environmental impacts. Instead, DNA studies based on Polymerase Chain Reaction (PCR) technology make up the majority of molecular diagnostic techniques, which are not constrained in this way. More than a decade or more into the use of molecular diagnostics for biosecurity and other identifying purposes, there are still two significant barriers to the development of a more intelligent, integrated, and anticipatory IAS identification system.

I. The finite range of taxa

One to about fifty species can be accommodated by each approach, however, the number of taxa that can be accommodated varies. Due to the relative cost of technology development and other practical considerations, the strategy has been to construct tests for those taxa thought to be the most prospective invaders, i.e., for species known to be invasive and expanding elsewhere (Cock *et al.,* 2003). Examples include leafroller moths (Dugdale *et al*., 2002), tussock moths (Armstrong *et al.,* 2003), fruit flies (Haymer *et al.,* 1994; Armstrong *et al.,* 1997a), and thrips (Toda and Komazaki, 2002). Different countries prioritize different taxa in accordance with factors such as how well they match the host and environment, their accessibility to entry points that are already in place, and their potential economic impact. It might not be feasible to change protocols to accommodate new species as needed or to conform to the needs of other industries, nations, or sectors. When the diagnosis depends on a small number of single nucleotide polymorphisms, such as those used to build oligonucleotide arrays, polymorphic restriction sites, or specific PCR primers, this can be particularly challenging. It can be difficult to find more informative polymorphisms using these techniques.

The ability to deal with unexpected arrivals is likewise compromised by methods created out of necessity for a predetermined range of taxa. When compared to species that are tightly related to their host material, such as fruit flies, the entry of species found on inanimate items, such as cars (Armstrong *et al.,* 2003) or solid wood packaging (Wittenburg and Cock, 2001), is more challenging to anticipate (Armstrong *et al.,*1997a). Some may not be anticipated because they are harmless or only minor pests in their natural habitat. They may, however, develop into a serious annoyance in a setting where there are no obvious natural opponents or rivals. This has become a major problem in places such as North America, where only the European strain of the gypsy moth was recognized as a pest in its native region out of the six most destructive forestry pests introduced (Cock *et al.,* 2003). Similar to New Zealand, an eradication operation was started after the painted apple moth,*Teia anartoides*Walker (Lepidoptera: Lymantriidae), an unwelcome visitor from Australia where it is a small local pest, unexpectedly arrived in 1999. The cost to New Zealand if it is not eradicated is anticipated to be equivalent to EUR33-205 million over the next 20 years (Case study 3, 2002). A significantly more flexible and anticipatory diagnostic system is required to provide timely support for the management of these events.

II. Diverse methodologies

Because they are benign or just minor pests in their natural habitat, some might not be predicted. Having no natural competitors or opponents might be a huge inconvenience. This is a major concern in North America, where just one of the six most destructive forestry pests imported, the European strain of the gypsy moth, was a pest in its original place (Cock *et al.,* 2003). The painted apple moth,*T. anartoides*, unexpectedly arrived from Australia in 1999. An eradication effort was undertaken, as in New Zealand. Additionally, the gene or genes used to identify species are not uniform. The *cox1*, the non-transcribed region between *cox1* and tRNALeu (Stauffer 1997), the 16S rDNA (Brown *et al.,* 2002), and cytochrome B are a few examples of mtDNA that has been used to identify insects (Khemakhem *et al.*, 2001, 2002). Nuclear gene regions have been identified using ITS1 plus ITS2 (Armstrong *et al.,* 1997a,b), ITS1 alone (Chiu *et al.,* 2001), ITS2 only (Pfeifer *et al.,* 1995), an actin gene intron (He and Haymer, 1997), and randomly amplified polymorphic DNA (RAPD)(Kengne *et al.,* 2001). The taxonomic range involved and the gene's

proper evolutionary rate play a role in the decision to some extent, but convenience factors like the primers that are readily available and in-house expertise may also come into play.Phylogenetics experts have acknowledged that the implications of this mismatch are a problem with much wider implications (Caterino *et al.,* 2000).

Therefore, molecular diagnostic tests still have very few applications, despite growing acceptance as a necessary and unavoidable part of the biosecurity toolbox (Martin *et al*., 2000). They are not adaptable enough to handle the rising number of IAS, spot unexpected arrivals, or make use of others' efforts who together work over a wide variety of taxonomic ranges.

Limitations of DNA barcoding

When other molecular marker genes are included, such as cytochrome oxidase b(Cytb), COI seldom fails to reveal interspecies differences. A short DNA sequence from the mitochondrial genome has become the standard barcode for unidentified animals and a technique for discovering new species. Because mitochondrial DNA is inherited from the mother and may induce interspecific hybridization or endosymbiont infections, DNA barcoding requires analytical skills. Bioinformatic techniques enable DNA sequence analysis. Pseudogenes, often called nuclear mitochondrial DNA (NUMTs), are a barrier in discriminating species based on mitochondrial DNA. Transposable elements or short dispersed repeats were expected to be involved in NUMT integration into the nuclear genome. However, a systematic analysis of NUMT loci shows a lack of similar traits at integration sites (Bensasson *et al*., 2001). According to Whitworth *et al.* (2007), maternally transmitted bacteria may distort mitochondrial diversity patterns. 12 species of the bird blowfly genus *Protocalliphora* Hough, known to be infected with Wolbachia, a intracellular bacteria infecting many arthropod species, were barcoded. The results showed very limited success.For example,assignment of unknown individuals to species is impossible for 60% of the species, and using the technique to identify new species would significantly underestimate the number of species in the genus. Smith *et al*. (2012) analyzed more than two million insect COI trace data on the BOLD and found Wolbachia in 0.16 per cent of cases. Standard insect primers can yield Wolbachia COI, but it was never confused with the host's COI. The presence of Wolbachia DNA in insect genomic extracts is unlikely to impact the accuracy of the DNA barcode library. Large-scale insect barcoding projects can and should incorporate regular Wolbachia presence and type testing. Wolbachia alleles discovered were predominantly from Supergroup A and broadly spread.

Future perspectives

A paradigm that can be applied to many fields and will enable analytical needs to be scaled to meet the size of the present biodiversity crisis is the combination of traditional taxonomy with sequencing data. Taxonomic studies will be greatly aided and enhanced by DNA barcoding. It will aid in locating and protecting the evolutionary mechanisms that produce and maintain biodiversity. The potential use of DNA barcoding for populations where identification can be challenging is enormous. Conservation organizations can respond more swiftly to habitat loss and degradation because of DNA barcoding's fast identification of important functional components of extremely diverse arthropods. Insect diversity should offer crucial fine-scale maps for evaluating biodiversity at a scale at which conservation choices are made. Insect diversity may be evaluated by DNA barcoding in cooperation with traditional taxonomists. Insect species may be identified,thanks to COI barcoding. Taxonomists may systematically progress in the area of conservation with the use of modern resources and collaborations. Despite its limitations, DNA barcoding has had a tremendous influence on science and is now a widely used technology. Numerous significant aspects of homogeneity and generalization set it apart. The technique has to be understood critically to be used successfully. Researchers may now upload and get DNA sequence and specimen data from all insect orders utilizing a number of online sites for phylogenetic and barcoding analyses. More funding should be allocated to the repository 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. It will be possible to detect more endangered species and invasive species in the future through biomonitoring using more available barcodes. In the future, many DNA barcoding constraints will be solved as biological data science is progressing rapidly with the availability of exclusive bioinformatic tools.

Conclusion

Systematics plays a vital role in the conservation of biodiversity which is at stake due to environmental degradations and anthropogenic activities. Because of the ever-increasing decline of classical taxonomic expertise, there is a need to develop molecular marker-based tools drives 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 that involves species identification and naming, has quickened by its integration with an increasingly 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. DNA barcoding may be employed in a variety of ways to verify insect species in addition to conventional morphometrical analysis. Phylogeographic analysis, processed food identification, taxonomy, evolutionary study, and species complex identification are just a few of the fields where DNA barcode analysis is applied

owing to its broad range of applications and high level of accuracy. DNA barcoding is a well-established technique that is expanding its uses despite a lot of criticism. The traditional direct methodology is also constrained by the slow speed of morphological analysis, although DNA sequencing techniques have significantly improved since their inception. The reverse strategy which, without presorting and sample identification, enables this process to be significantly accelerated by next-generation sequencing (NGS) techniques. As a result, DNA barcoding can be used to achieve stated global goals without explicitly referencing taxonomy.

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