Minireview Article

DNA Barcoding: Accelerating insect species discovery and biodiversity documentation

ABSTRACT

Species identification is essential for recognizing and describing biodiversity. Traditionally, this process has relied on morphological diagnosis through taxonomic studies, which have certain constraints such as subjectivity and time-consuming processes. With the advancement of modern molecular techniques, DNA barcoding has gained global attention. The term "DNA barcoding" refers to the technique of establishing species-level identification by sequencing a short fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, the "DNA barcode," from a specimen that is taxonomically unknown and comparing it to a reference library of barcodes from known species. This review article explores the evolution of DNA barcoding, its universal marker, and its application in insect taxonomy, emphasizing its role in accelerating species discovery and biodiversity documentation. In India, DNA barcoding initiatives have made significant progress, yet there remains a vast opportunity to barcode the country's rich insect diversity. Overall, DNA barcoding emerges as a powerful tool to address the urgent need for efficient species identification and biodiversity conservation in an ever-changing world.

Keywords: Barcode, COI, Sequencing, Biodiversity, Taxonomy.

1. INTRODUCTION

Insects are the most abundant of all life on earth and have evolved into various forms. They represent about 66% of all identified species, making up over three-quarters of the planet's biodiversity. ~~There are~~ Aapproximately 1 million ~~documented~~ insect species have been documented~~,~~ although only 7% to 10% have been scientifically described [1]. Given the many undiscovered species, estimates suggest there could be around eight million insect species globally [2]. According to Mayr and Ashlock [3], it took nearly 200 years for taxonomists to describe 1.7 million species which is only 10 percent of the total number of species estimated. In this context identification of insects has been a monumental task where it calls for the availability of more specialists and funding. But with the dwindling interest in taxonomy and fund availability, classification and identification of various life forms particularly insects have been a major challenge to the scientific community. Naturalists developed the concept of categorizing living things based on taxonomy, a field of science that aids in categorizing a living entity based on morphological features to catalogue the enormous number of species.

A novel technique termed DNA barcoding, a tool of DNA-based taxonomy, is currently being used to identify known and undiscovered species based on the pattern of nucleotide

arrangement in a particular species' DNA fragment [4]. Several researchers have suggested the use of DNA barcoding in taxonomy as a method to achieve rapid species identification in the context of the current biodiversity crisis [5], [6]. With a total land area of around 3,287,263 km^2, India ranks among the world's most biodiverse countries, home to a diversity of habitats from deserts to high mountains and tropical to temperate woods [7]. The current necessity to classify such huge diversity calls for a quick, efficient, and accurate solution. DNA barcoding is the use of a short, standardized fragment of DNA sequence to identify and assign unknown specimens to species identity. Besides, it facilitates the detection of new species based on the differences in DNA barcodes. For insect species, a 658 bp section of the mitochondrial cytochrome c oxidase (COX I) gene is extensively utilized for DNA barcoding. The different barcode libraries *viz*., NCBI, BOLD are gaining value due to the integration of information of a species through voucher specimens, their binomial names, type locality and other collection data, and morphology in the form of digital photographs [8]. This method is widely feasible to catalog all the species on our planet. Over time it is largely accepted by hard-core taxonomists and governmental and non-governmental organisations as well. Since the development of molecular biology and molecular tools, identifying various life forms, including insects, has become simple, fast, and accurate.

Commented [s1]: Reference?

Commented [s2]: Reference?

2. HISTORY OF DNA BARCODING

In 1960, Carl Woese gave this concept for the first time. He utilized rRNA and molecular markers like rDNA and mtDNA for discovering *Archaea* i.e., prokaryotes, and for drawing an evolutionary tree [9]. In 2003, Paul Herbert, a researcher at the University of Guelph, Ontario, Canada proposed “DNA Barcoding” to identify species. He is considered the “Father of DNA Barcoding”. The first article on DNA barcoding was published by Hebert in 2003 with the title “-Biological identifications through DNA barcodes”.

3. UNIVERSAL DNA BARCODE REGION OR UNIVERSAL MARKER FOR DNA BARCODING

Mitochondria are energy-producing organelles, found in nearly every cell in almost every plant and animal species. The mitochondrial genome, which is present in all the eukaryotic organisms and evolves more quickly than nuclear DNA, has proven to be incredibly helpful in tracing evolutionary history. Different inheritance patterns can be seen in nuclear and mitochondrial genomes. Since mitochondrial DNA (mtDNA) is hereditary from the mother, it evolves quickly, and most nucleotide alterations occur at neutral sites. Mitochondrial markers are employed to indicate phylogenetic relationships among related groups. Using the sequence information obtained from the COX 1 marker gene amplification, the intra- and inter-phylogenetic interactions regarding this genetic marker have been investigated. In humans, Cytochrome c oxidase I (COX1), also referred to as mitochondrially encoded cytochrome c oxidase I (MT-CO1), is a protein encoded by the MT-CO1 gene ~~in humans~~. In other eukaryotes, this gene is known as COX1, CO1, or COI. COX1 serves as the primary subunit of the cytochrome c oxidase complex. A region approximately 650 base pairs in length from the 5' end of the Cytochrome c oxidase subunit 1 (COI) gene has been proposed as the universal barcode for animals [10].

Barcodes that are shorter than the full-length barcode are often referred to as “mini-barcodes”. They have the additional advantage that they more easily can be amplified when the DNA is damaged or fragmented, which is common in environmental DNA samples. In addition to the COI gene, some of the other markers such as 16S rRNA, 12S rRNA, and CytB are also being used for metabarcoding. However, the reference libraries for these alternative markers are small in comparison with those for COI [11].

4. NEED FOR NEW MARKERS

An ideal barcoding marker should contain highly conserved sequence regions that allow for the design of universal primers capable of amplifying all taxa of interest in the sample. These conserved regions should flank a highly variable region, which can then be used to differentiate between species. Because of the redundancy in the genetic code and the fact that COI is a protein-coding gene, the third position of most codons is highly variable. This variability complicates the design of primers for metabarcoding that can provide sufficient taxonomic coverage. The sample will inevitably contain a range of mismatches between the primers and the templates, which will result in variable primer affinities for various templates. Less mismatched primer-template combinations will amplify more readily with each cycle, which could lead to a severe overrepresentation of these sequences in the PCR output. These "universal" COI primer biases have been empirically verified in several investigations. LepF1/LepR1 primer biases have been reported [12], [13], [14]. Folmer primers fail to amplify many species of Hymenoptera [15]. Several primer pairs are associated with amplification bias resulting in an overrepresentation of Diptera and Lepidoptera sequences. The bias can be somewhat reduced by using degenerate primers [16], [17]. The amplification performance of four COI primer pairs from Malaise trap samples was investigated for several taxonomic groupings with varying degrees of degeneracy. Degeneracy significantly impacted amplification success, ranging from 5% for primers with little degeneracy to 49% for primers with considerable degeneracy [18].

Many experiments have sought alternative markers ~~because of~~due to the amplification bias associated with COI primers. For studies involving a broad taxonomic range (up to the phylum level), it is common to use a highly conserved and easily amplifiable marker, such as the nuclear small subunit ribosomal RNA (rRNA) gene (18S) [19]. There are several examples of 18S metabarcoding, most of which include eukaryotic microorganisms and soil/sediment biodiversity evaluation. The mitochondrial large subunit rRNA gene (16S) has been evaluated for insect metabarcoding, yielding promising results. When applied to a set of 315 species of insects (representing 264 genera and 23 orders), In silico analyses demonstrated that 200 bp mini-barcodes of the 16S gene identified slightly more species compared to mini-barcodes of the COI gene of the same length. Moreover, the taxonomic coverage was higher for 16S (75%-90%) compared to COI (only 50%) [16]. However, longer COI mini barcodes enhanced the taxonomic resolution between closely related species to nearly 100%, whereas the resolution for 16S reached a maximum of 85%. Surprisingly, the taxonomic coverage and resolution of 16S were constant across the 11 insect orders that were examined, but the best taxonomic coverage of COI was only between 0% and 47% in all other insect orders and slightly above 50% in Diptera and Lepidoptera. 16S amplified more species and more evenly through orders, improving biomass estimation. According to them, COI is still the best option if the objective is to identify the species present in the sample because there are numerous public reference databases available. However, 16S would be a better option if the objective is to assess the biodiversity in terms of numbers rather than species names. A further benefit of 16S metabarcoding over COI is that amplicons cannot be confused for nuclear pseudogenes or Wolbachia [17].
Nuclear rRNA markers present a different scenario. The rRNA sequences of the large and small ribosomal subunits (18S and 28S) include conserved regions that allow for the design of primers with broad coverage, much like mitochondrial rRNA markers. However, the resulting amplicons tend to be highly conserved, resulting in very low taxonomic resolution [20]. The internal transcribed spacer (ITS), a nuclear marker, might be the most useful for metabarcoding. It has the benefit of being flanked by conserved areas (subunits 5.8S and 28S), which makes primer design possible, and is known to provide strong taxonomic resolution. For fungi, the ITS reference database is comprehensive. Unfortunately, this is not the case for insects, which face a similar situation to that of some mitochondrial genes

mentioned here. However, ITS is unquestionably a viable option for a survey when the separation of MOTUs suffices. In addition to ongoing efforts to develop reference databases for entire mitochondrial genomes and specific mitochondrial markers, the entomological community could significantly benefit from establishing reference databases for promising nuclear metabarcoding markers, such as ITS. With low levels of primer degeneracy and stringent PCR conditions, rRNA markers offer considerably broader taxonomic coverage while still resolving most genetically distinguishable species [21].

5. CURRENT STATUS OF INSECT DNA BARCODING IN INDIA

In India, approximately 62,429 insect species across 595 families have been described, but only 2,330 species from 264 families have DNA barcodes i.e., only 3.73%. BOLD contains barcodes for 852,657 different insect species from 12 mega-diverse nations. The country with the highest recorded number of barcodes is Costa Rica, which accounts for 77% of all reported barcodes. South Africa, China, and Mexico each contribute about 5%, while India barely makes up 1.53%. With 13,152 sequences (including 10,570 COI-5P and other suitable barcode markers supported by the Consortium for the Barcode of Life for Animals), representing 2330 species gathered from various geographic regions throughout India, India ranks seventh among the given megadiverse countries [22].

The species belong to 20 different orders, which are (in decreasing number of samples) Lepidoptera, Hemiptera, Diptera, Coleoptera, Thysanoptera, Hymenoptera, Odonata, Blattodea, Ephemeroptera, Orthoptera, Neuroptera, Siphonaptera, Embioptera, Mantodea, Psocoptera, Trichoptera, Zygentoma, Dermaptera, Strepsiptera and Phasmatodea. Among these orders, the largest number of sequences was generated for Lepidoptera (26.08%), followed by Hemiptera (25.87%), Diptera (16.09%), Coleoptera (12.66%), Thysanoptera (6.55%), Hymenoptera (6.40%), Odonata (2.29%), Blattodea (1.78%), Ephemeroptera (1.17%) and the rest of the eleven orders comprise <1%. The highest species coverage was achieved for, Lepidoptera with 687 species (29.48%), followed by Hemiptera with 391 spp. (16.78%), Coleoptera 373 spp. (16.01%), Diptera 332 spp. (14.25%), Hymenoptera 209 spp. (8.97%), Odonata 117 spp. (5.02%), Thysanoptera 89 spp. (3.82%), and Ephemeroptera with 44 spp. (2.22%), while the remaining 12 orders together comprise <3%. The large percentage of known insect species that have not yet been barcoded indicates that there is a tremendous opportunity to work on the barcoding of Indian insects [22].

6. ADVANTAGES OF DNA BARCODING

DNA barcoding offers numerous advantages in insect identification, including accuracy, speed, and the capability to distinguish various species when traditional morphological methods fail. High precision and accuracy are key benefits, as DNA barcoding often surpasses the limitations of conventional morphological methods by, providing a highly accurate and precise means of identifying insect species due to the consistency and objectivity of genetic sequences [23]. Additionally, DNA barcoding is a rapid method that delivers results in a matter of hours to days, making it suitable for extensive ecological investigations and biodiversity assessments [24]. The results of DNA barcoding are objective and reproducible, minimizing the subjectivity in species identification that can arise with traditional taxonomy, especially when dealing with cryptic species [25]. It is useful in pest management by providing rapid and accurate identification of pest species, enabling timely and effective control measures. Moreover, DNA barcoding facilitates the identification of various insect life stages, such as eggs, larvae, or even damaged specimens, which can be challenging to identify using conventional morphological techniques [26]. Importantly, DNA

barcoding has also led to the discovery of previously unrecognized cryptic species that were indistinguishable based on morphology alone, thereby revealing hidden biodiversity [23].

7. LIMITATIONS AND DRAWBACKS OF DNA BARCODING

DNA barcoding faces several limitations, such as the lack of universal primers. Using a single universal identifier does not enable the successful barcoding of all insect groups, necessitating the purpose of multiple markers or specific primers for some taxa, which complicates the study [5]. Incomplete reference databases also pose a challenge, as DNA barcoding relies on comprehensive reference databases, which may not be complete for certain regions or insect groups, leading to difficulties in accurately identifying species. Hybridization and introgression can further complicate matters, as these processes between closely related species can produce mixed or false genetic signatures, making the barcode region unable to clearly identify a species [27]. Superior intraspecific genetic variation within some insect species can also hinder accurate species identification, as DNA barcoding might not effectively differentiate between individuals within a single species [5]. Additionally, insects collected from certain environments may have degraded DNA, making it challenging to obtain high-quality DNA sequences for barcoding, which is particularly relevant for specimens in museum collections [28]. Convergent evolution can result in different species having similar or identical sequences, leading to incorrect identifications [25]. Biological anomalies, such as parthenogenesis or endosymbiont associations in some insects, can affect the interpretation of DNA barcodes. The lack of reference sequences for rare, newly discovered, or undescribed species further complicates identification efforts. Moreover, DNA barcoding can be expensive, requiring access to specialized equipment and expertise in molecular biology techniques. Limited taxonomic coverage in reference databases for some insect groups, particularly non-model organisms, reduces the efficiency of DNA barcoding. These limitations highlight the need to develop new markers, improved techniques, and broader reference databases. While DNA barcoding is an effective tool, it is essential to consider these drawbacks and combine DNA data with other sources of information, such as morphology and ecology, for a comprehensive approach to insect identification and taxonomy [29].

8. ADVANCEMENTS IN DNA BARCODING

A contemporary technique for identifying several species in a mixed sample, such as bulk DNA or environmental DNA (eDNA), is DNA metabarcoding. High-throughput sequencing (HTS) of a specific DNA marker is necessary for this method to work. DNA metabarcoding, in contrast to traditional DNA barcoding, which often uses Sanger sequencing on individual specimens, makes use of the vast amounts of DNA sequence data produced by HTS to rapidly allocate taxonomic classes to a variety of species present in a sample.[30]. It takes DNA barcoding to the next level by allowing the processing of bulk samples and environmental samples. Samples collected from traps such as yellow pan traps, and malaise traps can be processed in bulk without sorting them. Even in situations when the species are not physically present, DNA metabarcoding can be used to extract species DNA from samples such as soil, water, sediments, or other materials. Currently, the most popular high-throughput sequencing (HTS) platform for DNA metabarcoding research is the Illumina MiSeq, located in San Diego, California. DNA metabarcoding helps in the detection of invasive insects in a surveillance context and highlights the unique technical and regulatory challenges that must be considered when implementing high-throughput sequencing technologies into sensitive diagnostic applications. Large-scale species identification using this technology is rapidly becoming an affordable option, particularly when traditional morphology-based identification presents budgetary or logistical difficulties.

NGS technology was initiated for commercial use in 2005. It is also called massively parallel sequencing because it allows the sequencing of millions of DNA fragments from thousands of DNA templates in parallel. Sanger sequencing technology can generate the sole method for sequencing readings of up to 1000 bases for DNA.sequencing for nearly three decades, but next-generation sequencing (NGS) devices are now beginning to dominate the sequencing niche. Next-generation sequencing technologies allow the sequencing of millions of DNA fragments, from thousands of DNA templates in parallel and facilitate the generation of DNA barcodes more quickly and at a lower total cost [31]. As a result, numerous genomics facilities have transitioned to NGS from Sanger sequencers. By using NGS the entire genome can be sequenced in 2 days, and it has greatly revolutionized genomics.

Mobile DNA barcoding is possible through third-generation sequencing platforms such as Oxford nanopore technologies. MinION is the smallest and most user-friendly portable sequencer that can be run outside of the conventional laboratory such as in fields and forest areas. MinION sequencing offers a rapid and cost-effective approach for analyzing smaller samples, making it more suitable for day-to-day border detections. It allows for in-situ species monitoring without having to remove organisms from their habitat. It has the ability to produce full-length DNA barcodes, unlike Illumina and other second-generation sequencing methods. By using MinION sequencing technology, morphology-based identification will be supplemented, allowing for more informed biosecurity decision-making and offering a vastly quicker and less expensive alternative to the current Sanger sequencing molecular identification method [32]. Nanopore sequencing is a unique, scalable technology that enables direct, real-time analysis of long DNA or RNA fragments. It functions by keeping an eye on variations in an electrical current that occur as nucleic acids go through a protein nanopore. To obtain the precise DNA or RNA sequence, the resultant signal is decoded. A single fragment of DNA can produce lengthy reads with about two million base reads.

9. BLAST & BOLD: INTEGRAL PLATFORMS FOR GENETIC ANALYSIS AND DATA MANAGEMENT

BLAST – Basic Local Alignment Search Tool
A bioinformatics tool called BLAST allows you to compare the sequences of two or more proteins or nucleic acid molecules and one sequence to a group of sequences in a database. The NCBI offers a common matching tool that looks for similarities between a query sequence and a sequence library. It breaks the query and database sequence into fragments and seeks matches between them. It can infer the evolutionary and functional links between sequences and identify the individuals who make up gene families.

BOLD – The Barcode of Life Data System
BOLD is an informatics workbench that makes it easier to collect, store, analyze, and publish DNA barcode records. It is a repository for specimen and sequence records. It helps with barcode data administration, quality control, and analysis. By combining flexible security and data entry features with web-based delivery, it offers a means of collaboration between geographically dispersed research communities.

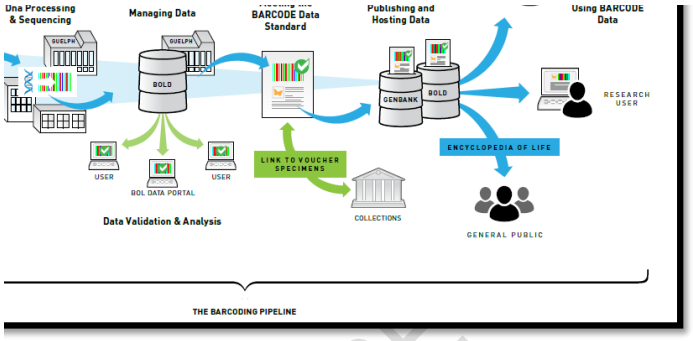

Figure 1: The barcoding pipeline (www.barcodeoflife.org)

10. INTERNATIONAL BARCODE OF LIFE CONSORTIUM

Established in 2008, the International Barcode of Life Consortium (iBOL) is a global research alliance dedicated to transforming biodiversity science. The consortium has initiated three major projects: Barcode 500K, BIOSCAN, and the Planetary Biodiversity Mission [17], [18]. The first major initiative, BARCODE 500K, ran from 2010 to 2015 with the primary goals of delivering DNA barcodes for 500,000 species and developing the necessary informatics tools and analytical protocols for DNA barcoding. Following this, the BIOSCAN project, spanning from 2019 to 2026, aims to deliver DNA barcodes for 2 million species and promote the various purposes of DNA barcoding. Looking further ahead, the PLANETARY BIODIVERSITY MISSION, scheduled from 2026 to 2045, seeks to complete a comprehensive census of all multicellular species, establish a global biosurveillance program, and construct a 'library of life' by preserving DNA extracts from all species. These initiatives reflect the consortium's commitment to enhancing our understanding and documentation of global biodiversity through advanced genetic tools.

iBOL Conference Series: The International Barcode of Life conference series is a biennial event that began in 2005. It serves as a major platform for the international community to discuss advancements and collaborations in DNA barcoding and biodiversity science. The series has grown significantly in participation and scientific scope over time.

11. CONCLUSION

DNA barcoding has arisen as a transformative tool in entomology, providing a standardized and powerful method for identifying insect species. This technology has revolutionized insect taxonomy, biodiversity monitoring, and ecological research by offering rapid, accurate, and objective species identifications. DNA barcoding assists taxonomists in resolving complex species groups and enables a broader range of researchers, including citizen scientists, to participate in research on studies of insect diversity. This technique is pivotal in conserving endangered species and managing invasive pests, thereby supporting global biodiversity

preservation and agricultural sustainability. The impact of DNA barcoding extends beyond traditional taxonomic boundaries. It facilitates the monitoring of insect populations, assists in ecological studies, and boosts our understanding of insect behavior and interactions within ecosystems. The contributions of DNA barcoding to conservation efforts are particularly significant, as it helps in the identification and protection of endangered species, ensuring their survival in the face of environmental changes and human activities.

As technological advancements continue, the expansion of reference databases and the development of more efficient methods will further enhance the capabilities of DNA barcoding. The future of this technology in entomology looks promising, with the potential to reveal new insights into insect diversity, behavior, and ecological roles. This versatile tool is set to remain a cornerstone of entomological research, playing a critical role in efforts to understand, protect, and manage insect populations in a rapidly changing world. Its capacity to offer comprehensive data on insect species will continue to support biodiversity conservation, pest management, and the study of ecological dynamics, making it an indispensable resource for scientists and conservationists alike.

12. FUTURE PERSPECTIVES

As technology and our understanding of DNA continue to advance, DNA barcoding will likely play an increasingly pivotal role in entomological research and insect management. One key area of development lies in the expansion and refinement of reference databases. Efforts to comprehensively catalog the DNA barcodes of insect species from diverse geographical regions will enhance the accuracy and applicability of this tool. This, in turn, can bolster biodiversity conservation and biosecurity efforts, aiding in the rapid identification of invasive species and the protection of endangered ones. Moreover, the incorporation of high-throughput sequencing technologies, for example next-generation sequencing, will accelerate the DNA barcoding process, enabling researchers to analyze large-scale insect datasets more efficiently. This can be particularly valuable in monitoring and understanding complex insect communities and ecological interactions.

DNA barcoding will also continue to serve as a crucial tool for taxonomists, helping to resolve cryptic species complexes and providing an objective basis for species delimitation. In relation to disease vectors and agricultural pests, DNA barcoding will facilitate the development of targeted and effective control strategies, helping to mitigate the economic and health impacts of insect-borne diseases. Furthermore, the integration of machine learning and artificial intelligence with DNA barcoding data will allow for the development of automated identification tools that can be employed by non-experts, including citizen scientists and field workers. These tools will empower a broader community to contribute to insect biodiversity monitoring and research.

REFERENCES

Commented [s3]: Follow the reference style of respective journal

1. Zhang, Z. Q. Animal Biodiversity: An Introduction to Higher-Level Classification and Taxonomic Richness. Zootaxa. 2011;3148:7-12.

2. Samways, M. J. Insect Diversity Conservation. Cambridge University Press, New York. 2005:25-29.

3. Mayr, E., Ashlock, P. K. Principles of Systematic Zoology. McGraw-Hill, Inc., New York. 1991:475 pp.

4. Novotny, V., Basset, Y., Miller, S. E., Weiblen, G. D., Bremer, B., Cizek, L., & Drozd, P. Low host specificity of herbivorous insects in a tropical forest. Nature. 2002;416:841-844.

5. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London A. 2003a;270:313-321.

6. Hebert, P. D. N., Ratnasingham, S., & deWaard, J. R. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B. 2003b;270(Suppl. 1):96-99.

7. Stork, N. E. How many species of insects and other terrestrial arthropods are there on earth? Annual Review of Entomology. 2018;63:31-45.

8. Wilson, J. J. DNA barcodes for insects. Methods Molecular Biology. 2012;858:17-46.

9. Sapp, J., Fox, G. E. The singular quest for a universal tree of life. Microbiology and Molecular Biology Reviews. 2013;77(4):541-550.

10. Park, D. S., Suh, S. J., Oh, H. W., & Hebert, P. D. Recovery of the mitochondrial COI barcode region in diverse Hexapoda through tRNA-based primers. BMC Genomics. 2010;11:1-7.

11. Marquina, D., Andersson, A. F., & Ronquist, F. New mitochondrial primers for metabarcoding of insects, designed and evaluated using in silico methods. Molecular Ecology Resources. 2019;19:90-104.

12. Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A. C., & Baird, D. J. Environmental barcoding: A next-generation sequencing approach for biomonitoring applications using river benthos. PLoS ONE. 2011;6:17497-7.

13. Brandon-Mong, G. J., Gan, H. M., Sing, K. W., Lee, P. S., Lim, P. E., & Wilson, J. J. DNA metabarcoding of insects and allies: An evaluation of primers and pipelines. Bulletin of Entomological Research. 2015;105:717-727.

14. Yu, D. W., Ji, Y., Emerson, B. C., Wang, X., Ye, C., Yang, C., & Ding, Z. Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods in Ecology and Evolution. 2012;3:613-623.

15. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 1994;3:294-299.

16. Clarke, L. J., Soubrier, J., Weyrich, L. S., & Cooper, A. Environmental metabarcodes for insects: In silico PCR reveals potential for taxonomic bias. Molecular Ecology Resources. 2014;14:1160-1170.

17. Elbrecht, V., & Leese, F. Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. Frontiers in Environmental Science. 2017;5:11.

18. Moriniere, J., Cancian de Araujo, B., Wai Lam, A., Hausmann, A., Balke, M., Schmidt, S., ... Haszprunar, G. Species identification in malaise trap samples by DNA barcoding based on NGS technologies and a scoring matrix. PLoS One. 2016;11.

19. Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., ... de Vargas, C. CBOL protist working group: Barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. PLoS Biology. 2012;10.

20. Wangensteen, O. S., Palacin, C., Guardiola, M., & Turon, X. DNA metabarcoding of littoral hard-bottom communities: High diversity and database gaps revealed by two molecular markers. PeerJ. 2018;6.

21. Schoch, C. I., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. I., Levesque, C. A., ... Schindel, D. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences. 2012;109:6241-6246.

22. Shashank, P. R., Naveena, N. L., Rajgopal, N. N., Elliott, T. A., Sreedevi, K., Sunil, S., & Meshram, N. M. DNA barcoding of insects from India: Current status and future perspectives. Molecular Biology Reports. 2022. https://doi.org/10.1007/s11033-022-07628-2

23. Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. Identification of birds through DNA barcodes (Charles Godfray, Ed.). PLoS Biology. 2004;2.

24. Hajibabaei, M., Singer, G. A. C., Hebert, P. D. N., & Hickey, D. H. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics. 2007;23(4).

25. Meier, R., Shiyang, K., Vaidya, G., & Ng, P. K. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Systematic Biology. 2006;55(5):715-728.

26. Burns, J. M., Janzen, D. H., Hajibabaei, M., Hallwachs, W., & Hebert, P. D. N. DNA barcodes and cryptic species of skipper butterflies in the genus Perichares in Area de Conservacion Guanacaste, Costa Rica. Proceedings of the National Academy of Sciences. 2008;105(17):6350-6355.

27. Rubinoff, D., & Holland, B. S. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biology. 2005;54(6):952-961.

28. Meyer, C. P., & Paulay, G. DNA barcoding: error rates based on comprehensive sampling. PLoS Biology. 2005;3(12).

29. Geary, J., Reay, T., & Bubel, T. The impact of heterogeneity in a global knowledge commons: Implications for governance of the DNA barcode commons. International Journal of the Commons. 2019;13(2):909–930.

30. Liu, M., Clarke, L. J., Baker, S. C., Jordan, G. J., & Burridge, C. P. A practical guide to DNA metabarcoding for entomological ecologists. Ecological Entomology. 2020;45:373-385.

31. Shokralla, S., Gibson, J. F., Nikbakht, H., Janzen, D. H., Hallwachs, W., & Hajibabaei, M. Next-generation DNA barcoding: using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. Molecular Ecology Resources. 2014;14:892-901.

32. Abeynayake, S. W., Fiorito, S., Dinsdale, A., Whattam, M., Crowe, B., Sparks, K., Campbell, P. R., & Gambley, C. A Rapid and Cost-Effective Identification of Invertebrate Pests at the Borders Using MinION Sequencing of DNA Barcodes. Genes. 2021;12:1138..