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# DNA BARCODING A REVIEW: MODERN TOOL FOR SPECIES IDENTIFICATION OF INSECTS

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## ABSTRACT
Insects are essential for pollination and aid in the reproduction of various plant species. They aid in the transfer of pollen as they forage from one flower to another, enabling plants to produce fruits and seeds. This vital ecosystem function not only preserves the diversity of plant life, but also has a direct bearing on the abundance and variety of fruits, vegetables, and nuts that are an important component of the diets of both humans and other animals. Knowing the vital roles that these tiny creatures play helps to emphasize how crucial it is to preserve and protect them for the ecosystems' long-term health and stability. DNA barcoding is a molecular technique based on the examination of a specific genetic marker such as the cytochrome c oxidase subunit I "(COI) gene which has transformed the process of insect identification. The purpose of this study was to demonstrate the efficacy of DNA barcoding using the COI gene in the rapid and accurate identification of insects. A wide range of insect specimens from various taxonomic groups were collected and subjected to DNA extraction and COI gene PCR amplification. Bioinformatics tools assessed the resulting DNA sequences for intra- and interspecific genetic variations. The COI gene provided a reliable and robust basis for insect identification. Sequence comparison revealed distinct genetic differences between species, allowing for accurate species identification. The intraspecific genetic variability was usually noticed as low, confirming the suitability of the COI gene as a stable molecular marker for discrimination at the species level. DNA barcoding is useful in identifying cryptic species and taxa that are morphologically similar. This technique can also identify life stages that are difficult to distinguish using traditional taxonomic identification methods, such as larvae. The creation of a large DNA barcode reference library improved the efficiency and accuracy of insect identification. Furthermore, the availability of such a library aided in the discovery of potential new species among the specimens collected. This study illustrates the importance of DNA barcoding technology as a powerful tool for insect identification, as well as its potential applications in biodiversity assessments, pest management, and conservation efforts. The COI" gene's standardized and accessible nature makes it an ideal candidate for large-scale insect identification projects and it significantly contributes to our understanding of insect diversity and ecology.

***Keywords:*** *Barcoding, Bioinformatics, Cytochrome oxidase, Insects, Pollination*

## INTRODUCTION
Insects are the pollinators for flowering plants, making them a very important group of organisms (Michener, 2007). Bees have changed the modern terrestrial environment by providing pollination services to sexually reproducing plants (Grimaldi *et al.,* 2005; Novacek, 2007). Bees are considered the most effective pollinators among the other insects (Kevan *et al.,* 1983). It is estimated that there are between 20000 and 30000 species of bees found around the world (Michener, 2007). Bees also serve as an indicator of environmental stress, so environmental stress can be assessed by estimating their abundance (Kevan *et al.,* 1997). Pollination is essential for about 85% of the total flowering plants of the world, the majority of which are insects (Ollerton *et al.,* 2011). Insect pollination is indispensable for food security. It is estimated that about 35% of global crop production is carried out with the help of animal pollinators (Klein *et al*., 2007; Eilers *et al.,* 2011). Insect pollinators are also an important component of

most terrestrial ecosystems, as they are required for plant reproduction and play an important role in wildlife food webs (Kearns *et al*., 1997; Potts *et al.*, 2010). Bees, wasps, flies, beetles, butterflies, and moths are considered the most predominant pollinators, but some bird and bat species also pollinate flowering plants (Potts *et al.*, 2016). It is considered that all insects play a crucial role in plant reproduction but bees are very important for pollinating crops and temperate wild plants because of their adaptable morphology (McGregor, 1976; Garibaldi *et al.*, 2013; Potts *et al.*, 2016). It was reported that about forty per cent of invertebrate pollinators are at risk of extinction and these are declining faster globally due to anthropogenic activity (IPBES, 2016). Threats to habitat include habitat loss, degradation, and fragmentation (Potts *et al*., 2010; Kremen, 2002; Williams, 2007). Insects are the most diverse and largest group of organisms on the Earth, but due to the existence of cryptic species and morphological similarities, it is very difficult to identify accurately even for specialists, and there are currently no keys available for all species (Whitfield, 1997). Traditional identification methods were based on morphological characters but these methods are inappropriate for the fragmented and incomplete specimens. It is believed that morphological and DNA barcoding combined approaches are more effective strategies for species identification (Hebert *et al*., 2003; Tyagi *et al*., 2017; Kundu *et al.*, 2018). Because DNA is found in all biological tissues and may persist even in material that has not been preserved to its best ability, DNA barcoding is an alternative strategy in this instance (Post *et al.*, 1993; Drabkova, 2014; Peterson *et al.*, 2014). DNA barcoding offers a powerful solution for species identification by utilizing standardized genetic markers (Hebert et al., 2003). DNA barcoding identifies a unique DNA sequence for each species which is specific for a particular insect species and by analyzing this specific region of an organism's DNA, species can be identified. DNA barcoding was introduced in 2003 as a molecular approach for taxonomic identification (Herbert *et al.*, 2003). Mitochondrial DNA is used for DNA barcoding because it is more susceptible to genetic drift than nuclear markers (Filipova *et al.*, 2011). In the DNA barcoding process, a short standardized region of the mitochondrial genome is sequenced to generate a unique DNA barcode that is used to distinguish different insect species. Phylogenetic relationships of insect pollinators can be determined by using partial 28S rDNA, ITS2 (internal transcribed spacer 2 for rDNA) and cytochrome oxidase subunit I (Erasmus *et al.*, 2006). The mitochondrial cytochrome c oxidase subunit 1 (COI) gene is used for this purpose for animal species because this genetic marker is universally present in all animals and can be easily amplified and sequenced (Hebert *et al.*, 2003). Cytb-gene is also can be used to distinguish various mammals such as badgers (Koepfli *et al*., 1998; Hsieh *et al.*, 2001). COI gene has proven successful in discriminating various animal groups because of its high competency in species identification for this purpose a universal primer can be used (Folmer *et al.*, 1994). Cytb-gene provided valuable genetical information that can be used in the study of population genetics and intr-specific analysis due to its high polymorphism, rapid evolution nature, and ease of amplification and sequencing (Xu *et al.*, 2011). DNA barcoding was found incapable for some animal groups in distinguishing them at the species level (Hebert *et al.*, 2003) because the COI gene very slowly evolved in some groups of benthic Coelenterates (France et al., 2002; Shearer et al., 2002). As a result, some scientists questioned the COI gene as a target region of selection (Erpenbeck *et al*., 2006), while others have suggested sequencing a larger portion of the gene (Roe *et al.*, 2007). This is regarded as an essential tool by the majority of animal groups. This method has transformed insect taxonomy and has a wide range of applications in areas such as biodiversity assessment, conservation biology, and pest management. DNA barcoding is gaining popularity because it is more accurate than other taxonomy methods (Pradhan *et al*., 2015). DNA barcoding is widely regarded as a reliable, low-cost, and straightforward molecular identification method with broad applicability across metazoan taxa (Hajibabaei *et al.*, 2006). It has gained widespread acceptance and recognition in the field of science. There are numerous advantages to DNA barcoding for insects. It enables rapid and accurate species identification, even with cryptic or morphologically similar species. It also allows for the discovery of new species and the evaluation of biodiversity in various ecosystems. Furthermore, DNA barcoding can aid in the monitoring of invasive species, the detection of pest infestations, and the support

of conservation efforts. The DNA barcode is a useful tool for identifying large carnivores such as lion and leopards using specific body parts and wildlife forensics (Verma *et al*., 2014; Khedkar *et al*., 2016). DNA barcoding accurately identifies species and is the most promising application in studies of biological diversity within regional and habitat-specific biotas (Smith *et al.,* 2005). This has significant implications for taxonomy. Through the identification of molecular operational taxonomic units, DNA barcoding has shown great promise in assessing and understanding the extent of diversity in various groups that have proven difficult to classify using traditional morphological taxonomic methods (Floyd *et al.,* 2002; Blaxter 2004; Smith *et al.,* 2005). This study's main objective is to assess how well this method works for identifying different insect species and to demonstrate how DNA barcoding might be a useful tool for identifying insects and other flora.

***TARGET GENE***

It was reported in an investigation that Subunit I of the cytochrome c oxidase "(COI) gene was used as a target gene for insect DNA barcoding (Hebert et al., 2003). The COI gene has enough variation to distinguish between closely related species while also having conserved regions for primer design. COII and other mitochondrial genes have also been used in specific insect groups (Smith et al., 2006). Furthermore, nuclear genes like 18S rRNA and ITS2 have been used for DNA barcoding in some insect taxa (Hajibabaei et al., 2005). The COI gene plays a central role in the DNA barcoding of insects. The COI gene is commonly used as the DNA barcode region for insect barcoding for the following reasons:

**a. Universal presence**: The COI gene is found in the mitochondria of nearly all animals, including insects. It is a haploid and maternally inherited protein coding region with a high presence in each cell (Hebert *et al*., 2003; Fazekas *et al.,* 2009; Hollingsworth *et al*., 2011). COI has been given priority as compared to other mitochondrial genes because of its high specificity and a high degree of accuracy to retrieve the 5' end of target DNA (Folmer *et al*., 1994). Mitochondrial DNA shows a high mutation rate due to its smaller size than nuclear barcodes (Drake *et al.,* 1998; Waugh, 2007). This region of mitochondrial DNA is a highly conserved coding region and this region of variation can discriminate animals up to species level which made it suitable for species identification across a wide range of insects. Its universal presence allows for standardized DNA barcoding protocols applicable to diverse insect groups.

**b. Inter-species variation**: The COI gene shows sufficient sequence variation between different insect species, making it an effective tool for species identification and delineation. The levels of COI sequence divergence are typically higher among species than within species, facilitating the differentiation of closely related species and the identification of cryptic species complexes. The 5'-end of COXI and COI (600-1000 bp) of mitochondrial DNA sequences is used as a universal barcode for the identification of different animal species and it is considered fit for the identification of interspecific variability (Kress and Erickson, 2012). COI shows less than 10 per cent intraspecific variations and deletion and insertions are rare mutations (Blaxter, 2004).

**c. Amplification and sequencing success:** The COI gene is amenable to PCR amplification, which enables efficient and reliable amplification of the target DNA region from insect specimens. The PCR primers designed for the COI gene, such as the universal primers LepF1 and LepR1 (Hebert *et al*., 2004), have been widely used and have demonstrated high success rates in amplifying the target region. Additionally, the COI gene can be easily sequenced using Sanger sequencing or high-throughput sequencing technologies (Kelly *et al.,* 2018).

**d. Standardized reference database**: The COI barcode region has been extensively studied and sequenced for numerous insect species, leading to the development of a comprehensive reference database. The Barcode of Life Data Systems (BOLD) and GenBank host large repositories of COI barcode sequences, providing a valuable resource for comparing and identifying unknown insect specimens based on their COI sequences (Benson *et al.,* 2002; Ratnasingham *et al*., 2007).

**e. Conserved primer binding sites**: The conserved regions of the COI gene, which flank the variable regions, provide suitable binding sites for PCR primers. This allows for the development of universal primers that can efficiently amplify the target region across a broad range of insect taxa. The conserved primer binding sites contribute to the success and reliability of COI gene amplification in insect DNA barcoding studies (Kress *et al*., 2012).
**f. Standardization and comparability**: The use of a standardized DNA barcode region, such as the COI gene, allows for comparability of results across different studies and laboratories. The uniformity in the target region facilitates data sharing, collaborative research, and the development of global initiatives for biodiversity assessment and species identification, such as the International Barcode of Life (iBOL) project (Santschi *et al*., 2013).
The COI sequence was found able to identify about 70 per cent of species of Diptera by comparing with GenBank sequences (Harris, 2003; Mitchell, 2008). It was observed that many records in this repository are known to derive from misidentified specimens. The COI gene provides species-level resolution in different groups of Diptera such as tachinids (Smith *et al.,* 2006) and Chironomic midges (Carew *et al*., 2007). The COI gene serves as a valuable and widely adopted DNA barcode region for insects. Its universal presence, inter-species variation, successful amplification and sequencing, standardized reference databases, and comparability across studies contribute to its significance in insect DNA barcoding. The use of the COI" gene helps advance taxonomy, species identification, and the understanding of insect diversity and evolution.

## *LABORATORY PROTOCOLS*

DNA extraction methods, PCR amplification, and sequencing techniques are critical steps in insect DNA barcoding. Various DNA extraction protocols, including Chelex-based extraction and commercial kits, have been employed depending on the insect group and the quality of the samples (Janzen *et al*., 2009). PCR amplification of target genes is performed using universal primers designed for COI or specific primers for other gene regions (Pentinsaari *et al*., 2014). Sanger sequencing or high-throughput sequencing platforms are used to obtain DNA barcode sequences (Miller *et al*., 2016).
**a. Specimen collection**: Insect specimens are collected from the field using appropriate sampling methods such as sweep netting, light trapping, baiting, or direct collection. Specimens should be properly preserved using methods such as ethanol, drying, or freezing to maintain the integrity of DNA. Aquatic insects are collected by using kick nets, plankton nets and bottle traps (CBD, 2020). To determine the patterns of COI divergence among species, a single middle leg of an insect was removed (Cory *et al*., 2009). Different tissues, such as whole specimens (for minor invertebrates), fin clips (fish), feathers (birds), and muscles or ear punches (mammals), can be used for DNA barcoding.
**b. DNA extraction**: The DNA extraction step involves isolating the genomic DNA from the collected insect specimens. Various DNA extraction protocols are available, and the choice of method depends on the insect group, specimen type, and available resources. Common methods include silica-based column purification, phenol-chloroform extraction, or commercial DNA extraction kits (Cory *et al*., 2009).
**c. Amplification of the target DNA region**: The standard DNA barcode region for animals, including insects, is a fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. Polymerase chain reaction (PCR) is used to amplify the target DNA region. PCR primers specifically designed for the COI gene, such as the universal primers LepF1 and LepR1, are commonly used. The PCR reaction mixture contains the extracted DNA, PCR primers, nucleotides, and DNA polymerase enzyme. The COI gene is amplified using the forward and reverse primers HCO2198 and LCO2198 (Folmer et al., 1994). Denaturation, annealing, and extension are performed by incubating at 94$^{o}$C (1 minute), five cycles at 94$^{o}$ C (1 minute), 45$^{o}$ C (1.5 minutes), 72$^{o}$ C (1.5 minutes), followed by 30 cycles at 4$^{O}$C (1 minute), 51$^{O}$ (1.5 minutes), 72$^{O}$ (1.5 minutes), and a final incubation at 72$^{O}$C (5 minutes) (Cory et al., 2009). Several primers have been developed and widely used for DNA barcoding of insects. Here are some commonly used primers for amplifying the COI gene region in insect DNA barcoding studies:

**i. LepF1/LepR1**: The LepF1 "(5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3')" primers were originally designed for DNA barcoding of Lepidoptera (butterflies and moths) but have been successfully used across a wide range of insect taxa. They amplify a fragment of approximately 650-700 base pairs within the COI gene (Hebert *et al*., 2004).
**ii. C1-J-1718/C1-N-2191**: These primers, also known as Folmer primers, were among the first primers designed for DNA barcoding and have been widely used in various insect groups. The "C1-J-1718 (5'-GGTCAACAAATCATAAAGATATTGG-3')" and "C1-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC-3')" primers amplify a slightly longer fragment of approximately 650-700 base pairs (Folmer *et al*., 1994).
**iii. LCO1490/HCO2198**: The "LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')" primers are commonly used for insect DNA barcoding and were initially designed for amplifying the COI gene in a broad range of animal taxa. They amplify a fragment of approximately 600-700 base pairs (Folmer *et al*., 1994).
**iv. Jerry/Pat**: The Jerry (5'-CAACATTTATTTTGATTTTTTGG-3') and Pat (5'-TCAACATTTATTTTGATTTTTTGC-3') primers are often used for DNA barcoding of Diptera (flies). It is famous by the name C1-J-2183 (Jerry) and TL2-N-3014 (Pat). They amplify a shorter COI fragment of approximately 300-400 base pairs, which can be advantageous for degraded or low-quality DNA samples (Erasmus *et al*., 2006).
**v. Rep COI-F/Rep COI-R**: 50-TNTTMTCAACNAACCACAAAGA-30 and Rep COI-R: 50-ACTTCTGGRTGKCCAAARAATCA-30 was used as a primer to amplify the partial mitochondrial cytochrome-c-oxidase (COI) gene (Kundu *et al.,* 2020). It is a kind of primer which is mostly used for vertebrates like Reptilia.
**vi. COBU/COBL**: COBU (50 - TYTCAACAAAYCAYAARGATATTGG-30) and COBL (50-TAAACTTCWGGRTGWCCAAARAATCA-30) primers were used to amplify mitochondrial COI gene of Orthoptera insect order (Pan *et al*. 2006)
The selection of primers is carried out based on specific insect groups under investigation, as the success of amplification and sequence quality can vary among different taxa. Additionally, there may be other primers or primers combinations designed for specific insect families or orders that are more tailored to their genetic characteristics (Shashank *et al.,* 2022). Researchers should consider the latest literature and consult established protocols and databases (e.g., Barcode of Life Data Systems, BOLD) to select appropriate primers based on their target insect group and specific research objectives. Optimization of PCR conditions and thorough validation of primers on representative samples are crucial to ensure reliable and accurate DNA barcoding results (Kelly *et al.,* 2018).
**d. Sequencing of target gene:** After the polymerase chain reaction (PCR) amplification of the target DNA barcode region using specific primers, the PCR products are purified to remove residual primers, nucleotides, enzymes, and other reaction components (Bruce *et al.,* 2014). Purification can be achieved using commercially available purification kits or enzymatic methods.
The quality and quantity of extracted DNA can be assessed by Nanodrop ND-1000. The purified PCR products are quantified using fluorometric methods or Spectrophotometry to determine the concentration of DNA in each sample. The DNA samples are then normalized to ensure equal representation and concentration for subsequent sequencing steps. The amount of DNA extracted from the leg of insects was measured by estimating absorbance at wavelengths 260 nano-meter for nucleic acids (Kelly *et al.,* 2018). The purified PCR products are then sent for Sanger sequencing or high-throughput sequencing using next-generation sequencing platforms. The resulting sequence data should cover the target DNA region (COI) of the insect specimen (Hebert *et al*., 2003). It's important to note that specific variations and modifications of the methodology may be required depending on the insect group, research objectives, and available resources. However, the general steps mentioned above provide an overview of the DNA barcoding process for insects.

## *DATA ANALYSIS*

The analysis of DNA barcode data involves comparing the obtained sequences with reference databases to assign species identifications. Distance-based methods, such as pairwise sequence divergence and neighbour-joining trees, are commonly used for species delimitation (Mitchell *et al.,* 2021). Tree-based methods, such as maximum likelihood and Bayesian inference, can provide phylogenetic relationships and aid in species identification. Machine learning algorithms are also being explored for automated species identification (Virgilio *et al.,* 2020).

**a. Data analysis and species identification**: The DNA sequences obtained utilizing the sequencing process are compared with existing reference sequences found at repositories such as GenBank and Barcode of Life Data Systems (BOLD). The DNA barcode cluster was created using the HMM algorithm and then assigned a unique Barcode Index Number (BIN) by the BOLD system (Ratnasingham *et al.,* 2007). Compare the resulting clusters to the taxonomic assignments iteratively (Gibbs, 2009, 2010a).

Sequence comparison tools like BLAST (Basic Local Alignment Search Tool) can be used to match the query sequences with similar or identical sequences in the database. Statistical algorithms and sequence comparison methods are employed to establish species boundaries and assign unknown sequences to known taxa.

**b. Data storage and sharing**: The generated DNA barcode data, including the specimen information, DNA sequences, and associated metadata, should be stored in a database or repository for future reference and public access. This allows for data sharing, further analysis, and comparison with other studies. To obtain good quality DNA sequences, the messy 5' and 3' ends of the sequences are trimmed, and the total length of the finally obtained DNA segments is thought to vary from species to species. Such final sequences are submitted to NCBI's GenBank and given accession numbers (Pongen *et al*., 2023). This method identified cryptic and new species (Seifert *et al.,* 2007; Burns *et al*., 2008).

## *APPLICATION OF DNA BARCODING IN ENTOMOLOGY*

**a. Species Discovery and Delimitation**

DNA barcoding has facilitated the discovery of new insect species and the delimitation of cryptic species complexes. For example, DNA barcoding uncovered hidden species diversity in beetles, moths, and wasps (Pentinsaari *et al*., 2014; Miller *et al*., 2016; Mitchell *et al*., 2021). The approach has been particularly valuable in tropical regions with high insect biodiversity (Janzen *et al.,* 2009).

**b. Cryptic diversity analysis**

It is a powerful approach to detecting and investigating hidden genetic variation within species that may not be apparent based on morphological characteristics alone. Cryptic diversity analysis using DNA barcoding is essential for uncovering hidden species diversity and improving our understanding of evolutionary processes and patterns. It aids in refining species concepts, contributes to conservation efforts, and enhances our knowledge of biodiversity. Due to morphological plasticity in similar and related species, accurate species identification of insect species is not possible (Pigliucci, 2005; Robinson *et al.*, 2002). As a result, advanced identification methods such as DNA barcoding were required (Tautz *et al.,* 2002). It has been reported that DNA barcoding can distinguish cryptic species and aid in phylogenetic analysis (Almeron *et al*., 2018).

**c. Ecological and Conservation Studies**

DNA barcoding provides insights into insect biodiversity, population structure, and interactions with their environment. By examining DNA barcodes from various habitats, researchers can assess insect community composition and dynamics (Virgilio *et al*., 2020). Additionally, DNA barcoding aids in the monitoring of threatened or endangered species, assisting conservation efforts (Mitchell *et al.,* 2021).

**d. Pest Management**

DNA barcoding plays a crucial role in pest management strategies by enabling the accurate identification of insect pests and the monitoring of their distribution. DNA barcoding has been applied to identify invasive insect species, track their spread, and guide targeted control measures (Virgilio *et al.,* 2020).

Insects are both beneficial and detrimental to ecosystems and human activities. DNA barcoding aids in identifying insect pests, distinguishing them from beneficial species, and tracking their spread and population dynamics. This information is vital for developing targeted pest management strategies, implementing early detection and prevention measures, and minimizing the economic and ecological impacts of pest infestations. It also supports biosecurity efforts by assisting in the identification and management of invasive insect species.

**e. Forensic entomology**: In forensic investigations, DNA barcoding of insects collected from crime scenes or associated with human remains provides valuable information for estimating the postmortem interval, determining geographic origin, or establishing links between crime scenes and suspects. The Bavarian State Collection of Zoology created a reference library of arthropods with potential forensic applications for DNA barcoding. Approximately 502 high-quality sequences covering 88 different Arthropod species were data-based using COI (Chimeno *et al*., 2018). It enhances the accuracy and reliability of forensic analyses, assisting law enforcement agencies and the justice system in criminal investigations. If a body has been found in the later stages of decomposition, forensic entomology will play an important role in the investigation (Gennard, 2007). A forensic entomologist may determine the postmortem interval or minimum postmortem interval (Tarone *et al.*, 2017) assuming that arthropod colonization is possible and not hindered by various factors like cold weather. It was assumed that arthropod colonization coincided with the start of death. Accurate species identification is the first crucial step in any death investigation that used an arthropod specimen as a starting point (Joseph *et al*., 2011).

## *CHALLENGES AND LIMITATIONS*

### a. Incomplete Reference Libraries

The success of DNA barcoding relies on comprehensive reference libraries containing DNA barcodes from known species. The lack of complete reference databases poses challenges in accurate species identification, especially for poorly studied or newly discovered species. Efforts to expand and curate reference libraries are ongoing (Mitchell *et al.*, 2021). When using BM, the possibility of incorrect identifications of queries with conspecifics remains relatively low (up to 5.2%) with the reference database. But there is a problem with DNA barcoding of insects in that there are only limited references are available in repositories to compare obtained results (Virgilio *et al*., 2010).

### b. Taxonomic and Identification Challenges

Some insect groups exhibit complex taxonomies, with morphological variations and cryptic species. DNA barcoding may face challenges in accurately identifying species due to incomplete taxonomic knowledge and potential hybridization events (Mitchell *et al*., 2021).

### c. Technical Limitations

DNA barcoding is not without technical limitations. PCR amplification biases, DNA degradation in museum specimens, and limitations of sequencing technologies can impact the success of DNA barcoding in insects. These limitations need to be considered while interpreting DNA barcode data (Mitchell *et al*., 2021).

## CONCLUSION

DNA barcoding is an excellent technique for accurately identifying animals. There are several promising avenues for DNA barcoding in insect research in the future. DNA barcoding is transforming insect taxonomy and improving our understanding of insect biodiversity. It improves traditional morphological taxonomy by introducing a molecular marker capable of distinguishing closely related species, resolving taxonomic ambiguities, and assisting in the discovery of new species. Researchers can estimate species richness, detect changes in biodiversity due to habitat loss, climate change, or invasive species, and identify priority areas for conservation efforts by rapidly identifying species. As a citizen science initiative, DNA barcoding has grown in popularity, allowing non-experts and enthusiasts to contribute to species identification and biodiversity research. This participation raises public awareness and

understanding of the importance of insects, promotes scientific literacy, and encourages active participation in conservation and research efforts.

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