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DNA BARCODING AS A TOOL FOR TAXONOMIC IDENTIFICATION OF LADYBIRD BEETLES (COLEOPTERA: COCCINELLIDAE) FROM PAKISTAN: A REVIEW

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Abstract

Despite having a large number of species and being ecologically diversified, ladybird beetles (family Coccinellidae) have always been difficult for describing due to their poorly understood evolutionary relations. An alternative to morphological identification is molecular identification techniques like DNA barcoding. Barcoding may result in less expensive, quicker, and more precise identifications. In order to identify organisms, DNA barcoding uses a short sequence of DNA that has been adopted by the scientific community. As the "barcode" for animal life, the Folmer region of the mitochondrial cytochrome oxidase subunit 1 (COI) gene has long been recognized. COI is highly variable among animal species, providing good identification power, and it is flanked by conserved regions that make good primer sites. It is also short, only 658 bp, making it easy to sequence. The present study summarizes the current status of insect species' barcoding in Pakistan and identifies any blanks that need filling. This study reveals that very little of the known taxonomy and defined species are represented by barcoded data. In order to carry out the DNA barcoding of all known insect taxa from Pakistan, it is required to significantly speed up insect species discovery and documentation in a cooperative manner between traditional taxonomists and molecular biology researchers.

Keywords: *COI gene, DNA Barcoding, Integrated Pest Management (IPM), Ladybird Beetles, Mitochondrial DNA, Predators*

INTRODUCTION

DNA barcoding is a valid approach for assessing trends of global biodiversity and enabling non-taxonomists to diagnose recognized species (1). By utilizing short DNA sequences, DNA barcoding provides a quick, precise, and consistent method for species-level identification. DNA barcoding is a novel method that Hebert from the Ontario University of Guelph, Canada, suggested in 2003. A study titled "Biological identifications through DNA barcodes" was written by Hebert and his colleagues. They presented a brand-new technique for identifying species, which involves the identification of species using a brief section of DNA from a predetermined area of the genome. Different species can be recognized using that DNA sequence. The standardized methodology of DNA barcoding makes it easier to conduct biodiversity research like species discovery and identification. By combining molecular, morphological, and distributional data, this method aids researchers in understanding genetic and evolutionary linkages (2). DNA barcodes are typically used for identifying species at the level of the genome by recovering a brief DNA sequence from a common region of the genome (3). The barcode sequences of each unknown specimen were then compared to a library of reference barcode sequences acquired from people with known identities (4). DNA barcoding is necessary for species recognition and specimen identification (5). The inventory of all species on Earth benefits greatly from the use of standardized identification methods, especially now that DNA sequencing technology is widely accessible and reasonably priced. The phrase "DNA barcode" implies that Taxa can be identified using standardized DNA sequences. like how the 11-

digit Universal Product Code identifies goods sold in stores Lambert evaluated the potential use of DNA barcoding to estimate the historical diversity of the Earth's biota (6).

The Barcode of Life Data System (BOLD) is a workbench for informatics that facilitates the acquisition, examination, preservation, and dissemination of DNA barcode information. Gathering morphological, distributional, and molecular data connects a conventional bioinformatics opening. Any researcher familiar with DNA barcoding can access BOLD for free. Offering specialized services facilitates the construction of records that comply with the criteria necessary to receive barcode designation in the worldwide sequence databases (4). The BOLD technique of species identification may be used generally, leading users to specialized databases for details on pathogenic strains, endangered species, and disease vector species (7). The Barcode of Life Data System (BOLD), a resource that the scientific community can access online, has helped the DNA barcoding study (http://www.boldsystems.org). This source offers tools that let researchers, among other things, do neighbor-joining clustering, recognize taxa using a current sequence library, and save information on the numerous researched groupings (8). The Consortium for the Barcode of Life (CBOL), founded in 2004, today has more than 170 member groups representing 50 distinct nations, and its mission is to advance DNA barcoding as the industry standard for biological specimen identification.

The richest part of life on earth is insects. Insects have emerged in an enormous range of diverse forms (9). An important aspect of this kingdom is that this kingdom has a vast heterogeneity in basic biological and ecological traits. Cooperation amid pests, pathogens, and plants pretend ecological and economic menace to the wildland ecosystem as well as agriculture. The insects keep ecosystem services sustainable and help minimize the initiation of negative outcomes such as water shortage, famine risks to economic and human health interruption (10). In climate change, ecology, evolution, developmental biology, genetics, biomechanics, and physiology insects are being used in landmark studies.

An insect may be a pest as well as a natural enemy. Insect pests have been a serious problem for farmers throughout the world, about 10,000 species of insects are found to cause serious damage to crops (9). The other category of insects is natural enemies**.** Those insects are considered natural enemies that eat other organisms.

Ladybugs (Class: Insecta, Order: Coleoptera) are insects that are categorized as natural enemies. Despite the name Ladybugs, they are also called Lady Birds and Lady Bird Beetles. Around 6000 species of ladybug are known globally. Ladybugs belong to the family Coccinellidae and are well-known biological control agents in an ecosystem. On the other hand, some species of Ladybugs may cause harm to many crops and are hence categorized as dangerous pests.

Ladybug is a Predator that acts as a biological pest control agent (11). Ladybugs are also helpful to manage problems pertinent to ecology and evolution. The Ladybugs generally called ladybird beetle is predatory insects. This insect plays a key role as a natural enemy against many pests.

It is estimated that a ladybird beetle can eat up to five thousand aphids throughout its lifetime. Its appetency towards hazardous insects such as aphids, and ladybugs provide an effective control agent concerning IPM (Integrated Pest Management). Coccinella septempunctata's effectiveness as a biological control agent has been reported for adults and larvae of aphids, mealy bugs, sweet potato whitefly, and scale insects in varieties of crops (12).

MOLECULAR TAXONOMY

Protein sequencing, electrophoresis, isozyme research, and immunological procedures were some of the molecular techniques utilized in the previous few decades for species recognition (13). Additionally, several DNA markers, including sequencing, denaturing gradient gel electrophoresis (DGGE), single-stranded conformation polymorphism (SSCP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and PCR specific primers, were used to identify species (14). In recent years, PCR and sequencing technological advancements have accelerated development, reduced chemical and operational costs, and made molecular-based species identification a straightforward process. This methodology is touted by INS (Forensically

Informative Nucleotide Sequencing) as the most reliable way to identify animals. Molecular genetics uses the analysis of certain genes from nuclear and mitochondrial DNA (18S, 28S, 5.8S, and 5S rRNA) to identify animal species. Ribosomal RNA (rRNA) is a slowly evolving gene that typically does not differ among closely related organisms, even though it is required for resolving ancient relationships and offering insights into cellular life (3). As a result, mitochondrial DNA and ribosomal RNA were chosen as suitable targets for the study. Molecular taxonomy and animal identification using the COI gene, a mitochondrial fragment, became accepted in 2003. The COI gene in vertebrates has a total length of 1,540 bp, of which 650 bp are known as the "Barcode of Life" region because they are located close to the beginning of the COXI reading frame (15).

MOLECULAR PHYLOGENY

Before the development of DNA sequencing technologies, phylogenetic trees were mostly used to indicate relationships between species in systematics and naming. To explain the relationships between prologues in a gene family, phylogenetic techniques are now frequently utilized in the area of biology. Today, a key technique for genomic analysis is molecular phylogenetics. The methods employed to reconstruct phylogenies, especially character- and distance-based ones, are crucial. The distance-based method calculates the detachment between each sequence match, and the resulting distance matrix is used to build phylogenetic trees (14).

DNA BARCODING

In the field of study known as taxonomy, living things are classified according to their morphological characteristics. The taxonomy-based classification of living things was begun by naturalists to catalog the enormous number of species. DNA barcoding is currently the most popular approach worldwide for quickly taxonomically identifying species (9). A small portion of the mitochondrial cytochrome C oxidase subunit I (COI) gene is sequenced as part of DNA barcoding. When using DNA barcoding to offer species-level identification, we compare the barcodes of known species that are already stored in barcode libraries to taxonomically unknown specimens to determine their identity. According to Hebert, DNA barcoding is also emerging as a useful method for the cataloging and analysis of animal species (3). The capacity to distinguish species depending on morphology has been rapidly deteriorating (3, 16-18) likewise, funding from local and national governments for taxonomic scientific research has decreased. In light of these circumstances, Hebert estimated that to identify the 10-15 million hidden species, approximately 15,000 taxonomists might be needed (3). It will be challenging to identify the new species because of their morphological ambiguity and environmental identities. By evaluating and developing the benchmark database for the management and conservation of maritime resources, the Census of Marine Life (CoML) program made a gallant attempt to catalog the abundance and dispersion of specific species found in the ocean world. In place of fundamental disciplines like taxonomy, ecology, and biology, researchers are now using advanced molecular technologies (genetic tools) for finding and eliminating resources.

In turn, investigations on taxonomic identification have increasingly been used using molecular techniques like mtDNA barcoding, which uses a short DNA sequence (3, 18, 19). Many different kinds of taxonomic taxa, such as sponges, birds, mollusks, mammals, bats, fishes, Polychaeta, etc., are currently being studied using these techniques (20). The biodiversity assessment has been greatly improved as a result of this initiative's assistance in the identification of novel species and complements traditional taxonomy, several of which are morphologically ambiguous and cryptic (21-23).

Hebert developed the micro genomic identification technology known as DNA barcoding in 2003 (3). It distinguishes life by analyzing a small section of the genome to determine biological diversity. Each unidentified species' barcode sequence is compared to a known sequence from a library of known barcode sequences. If a specimen's barcode sequence closely resembles a species that is already listed in the barcode library, it can be identified; if not, the species adds a new record and acts as a benchmark for subsequent sequences from the same collection. To enable DNA barcoding as an international standard for eukaryote sequence-based identification, the Barcode of Life initiative was created. This program was started by the

Consortium for the Barcode of Life (CBOL) to enhance effective DNA barcoding technologies and create a diverse DNA barcode library.

LADY BIRD BEETLE DIVERSITY STATUS IN PAKISTAN

The importance of coccinellids has grown significantly. As a result of biological control efforts commencing in 1957 in Pakistan, work on coccinellids has also been conducted from the beginning. Although there is a paucity of comprehensive information in Pakistan, there is sporadic data on taxonomy and economic significance. Only a few studies on the species composition of coccinellid beetles in Pakistan were found whenever the literature was retrieved with no specific mention of the previous record from the Balochistan Province of Pakistan. However, Predatory coccinellid species from Pakistan were originally listed as 71 species by Irshad, but he amended this list in 2003 and reduced the number to 53 species (24). However, concurrent taxonomic work was also done during this time. The taxonomic study comprises Ahmad's (1968) description of the new species Pseudoscymnus murriensis (25). Ahmad's (1973) description of a new tribe (26), and Ahmad and Ghani's (1966a) description of a new genus (27). Mubashir Hussain in 2018 reported 14 species, 6 genera, and 3 subfamilies in Irrigated and Rainfed Fields of Gujrat, Punjab, Pakistan (28). Rahat Ullah in 2012 for the first time reported two species Hypraspis rahatiana and Cryptogonus from District Dir Lower (29). Kausar Saeed identified eleven genera, three subfamilies (Subfamily Chilocorinae, Subfamily Coccinellini, and Subfamily Epilachnini), and thirteen species of ladybird beetles in 2016. These species are Brumoidus suturalis, Coccinella septempunctata, Coccinella transversalis, Menochilus were maculatus, Propylea dissecta, Coelophora bissellata, Oenopia sauzeti, Aiolocaria hexaspilota, Psyllobora bisoctonotata, Harmonia dimidiata, Henosepilachna vigintioctopunctata, Henosepilachna septima and Afidentula manderstjernae. The numerical data of these species showed that C. septempunctata, H. dimidiata, C. sexmaculatus, and H. vigintioctopunctata were highly abundant and were collected from all localities, while the other species, except A. hexaspilota, are moderately abundant (30). Muhammad Adnan Bodlah recorded seven genera naming Coccinella septempunctata Linnaeus, 1758; Brumoides suturalis (Fabricius, 1798); Henosepilachna vigintioctopunctata (Fabricius, 1775); Henosepilachna elaterii (Rossi, 1794); Hippodamia variegata (Goeze, 1777); Scymnus (Pullus) quadrillum for first time from district Layyah Punjab (31, 32).

CURRENT DNA BARCODE STATUS OF INSECTS FROM PAKISTAN

Most nations have proved how quickly generating DNA barcode data can be done by generating highly targeted, group-specific barcode libraries (32). Only a few previous researches from Pakistan used DNA sequences to identify certain insect groups' species, For the 658 bp barcode region of the cytochrome c oxidase 1 (COI) gene, which contains sequence variation, Muhammad Ashfaq and his team between 2010-2019 collected insect specimens from 1,858 locations across Pakistan between 2010 and 2019 for further analysis. 6,590 Barcode Index Numbers (BINs), an indicator for species, were allocated to sequences from around 49,000 specimens. The majority of these BINs (88%) also had a representative image on the Barcode of Life Data System (BOLD). Every BIN was allocated to an order (19) and the majority (99.8%) of them (362) were placed to a family by combining morphological inspections with barcode matches on BOLD (33). From locations in north-central Pakistan, DNA barcodes have been obtained for 81 butterfly species belonging to 52 genera (34). In north-central Pakistan, 471 thrips from a variety of plant hosts were examined for barcode sequence variation (35) Muhammad Ashfaq Assembled a Pakistani spider' (Arachnida: Araneae) DNA barcode reference library (36). Khadim Hussain identified a sugarcane black bug (*Cavelarius excavates*) from Pakistan using the cytochrome C oxidase I (COI) gene as a DNA barcode (37) Muhammad Ashfaq conducted a DNA barcode- Based analyses of Pakistani butterfly species suggest regional endemism (34).

The review of the literature reveals that the information on the diversity and rearing of coccinellid beetles from Pakistan is scanty and no works have been so far reported from Balochistan. With national-level campaigns, Pakistani researchers should concentrate on specimen-based group-specific DNA barcode libraries.

DISCUSSION

The DNA barcoding study results mentioned above of predatory insects like Ladybird Beetles in Pakistan are extremely small. According to Hickerson, the significance of DNA barcoding is contested. (38). No matter how DNA barcoding has been criticized (17), it serves as a global bio identification. But to distinguish between "species" that are quite similar, DNA barcoding is expanding in accordance. Barcoding is a technique that can be used to organize novel compilations stranded on barcode sequences, and it may be successful for previously defined species (39). According to the aforementioned findings, this field of research on predatory insects has to be developed especially concerning Ladybird Beetles to use distinct molecular markers as "barcodes" to bridge the untreated group chapter.

CONCLUSION

Due to its economic importance, Coccinellids have become incredibly important worldwide. Although scattered information is available on the taxonomy and economic importance of predatory Ladybird beetles, detailed information is lacking. Considering the importance of these useful predators and their blank chapter in Pakistan. Based on the analysis of survey data from the literature, it is clear that additional taxonomic research must be done to precisely determine the biodiversity of Pakistan's insects, with special attention to Predatory ladybird beetles. The paper reviewed here examines current breakthroughs in DNA barcoding, a popular substitute method for taxonomic identification and species delineation. DNA barcoding-based Research on predatory Ladybird Beetles is very limited and still in its infancy in Pakistan. Therefore, further research in this area is necessary for subsequent studies. in Pakistan, the discovering phase will get a boost from DNA barcoding of predaceous and economically important insects like the ladybird beetles of Pakistan.

From the DNA Barcoding point of view, most of the work has been done Worldwide. This review illustrates only a limited number of species of predatory insects like Ladybird Beetles have been studied in Pakistan. Recently barcoding has become relatively easy and the results obtained from DNA Barcoding are unquestionable. So, there is a scope for study in this area which has manifold benefits to important ones including new findings in evolution. The DNA barcoding of the ladybugs will provide plenty of data that can be compared across the BLAST and BOLD analyses. It is practical to distinguish between closely associated ladybug species by building the NJ, MP, and BI trees. Therefore, more research with in-depth analysis is advised, and it will be beneficial for integrated pest management as long as natural enemies play an essential role in minimizing the pest population. The discovery of new beneficial predator species, revision of phylogenetic trees, and the holistic collection of data will improve integrated pest management (IPM) Programs in Pakistan.

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