DNA-based identification and control of disease spreading mosquito species: A review

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ABSTRACT

Harms caused by parasites such as mosquitoes are one of the severe health problems of people, particularly in those areas where unhygienic environmental conditions exist. The diseases caused by these insects lead to many severe diseases and even deaths affecting public health along with social economy and welfare. Consequently, the development of the successful implementation of identification and controlling strategies of these parasite species is one of the challenges of health departments of many nations in the globe. However, effective eradication of disease-causing mosquito specimens, especially immature or damaged individuals, is possible by molecular identification. As a result, cytochrome oxydase c subunit I (COI) gene-based method can play a role in identifying and assigning taxa to mosquito species and has worldwide importance. In sequence, in this review, we assessed the occurrence, spread of diseases, and COI gene-based identification status of mosquito species (Anopheles annularis, Armigeres subalbatus, Mansonia annulifera, Mansonia uniformis, Aedes aegypti, Aedes albopictus, Culex tritaeniorhynchus, Culex quinquefasciatus, Anopheles culicifacies, Anopheles subpictus, Culex gelidus, Ochlerotatus sp., and Anopheles fluviatilis T) as well as their control measures along with role of DNA barcoding on global scale.

1. INTRODUCTION

Suffering of humans by blood-consuming parasites is a major issue on the global scale from many years. In addition, scientific communities have been always engaged in overcoming this problem through various ways, although they failed to solve the problem completely. In addition, mosquitoes attracted wide range attention of researchers since they are carriers of multiple bacterial, viral, and protozoan diseases among animals including humans as well as plants such as commercially valuable crops. Moreover, diseases caused by these dipterans lead to severe illness, in which death cases also occur if diagnosis and treatment are not performed within time. Moreover, these can be reduced with efficient controlling strategies detecting disease-carrying mosquito species in the infected regions.

However, for successful control of such diseases, the mosquito species responsible for their outbreak and spread are required to be analyzed through detection and identification processes, which need the development of comprehensive identification strategies that are unproblematic for their implementation. Nevertheless, the morphology-based identification system has certain limitations. Among them, unavailability of characters that are used for identification such as genital, color pattern, especially, in case of larval, immature and damaged stages or availability of only body fragments of the specimen are under study. Nevertheless, this problem can be solved with a greater extent using mitochondrial cytochrome oxydase c subunit I (COI) gene sequence with help of existing DNA sequence database, which can support for the development of vector born disease control operations.

In this review, we assessed the biodiversity, geographical distribution, and dominance of infectious mosquito species (Anopheles annularis, Armigeres subalbatus, Mansonia annulifera, Mansonia uniformis, Aedes aegypti, Aedes albopictus, Culex tritaeniorhynchus, Culex quinquefasciatus, Anopheles culicifacies, Anopheles subpictus, Culex gelidus, Ochlerotatus sp., and Anopheles fluviatilis T). In addition, we analyzed eruption and transmission of diseases due to them, their COI-based identification strategy, and their control measures in infected areas. Furthermore, we assessed the probabilities of DNA barcoding technique for effective eradication of disease spreading mosquito species in the globe making human life free from mosquito-borne infections.
2. BIODIVERSITY

Species of mosquitoes have a wide range of diversity depending on various localities and there are 3200 species which are known [1,2]. Interestingly, hidden species are still being investigated [3]. Vasantha et al. [4] proposed that their 41 genera and 3500 species as well as subspecies are existed in the world and according to Knipling [5], 100 species were detected only in China in 1938. As well, the authors claimed that this number has reached to 390 and leftover species, especially individuals within genera Armigeres, Hezimannia, Topomyia, and Uranotenia, are left to be assigned by their taxonomic names. Interestingly, in accordance with Itahi and Suleman [6], family Culicidae constitutes 3521 species, whereas according to Hiscox et al. [7], the largest tribe, namely Aedini, has 1255 known species. However, in India, less species diversity has been detected. For example, according to Hiscox et al. [7], only 350 species were reported in India.

2.1. Species Distribution and Dominance

Mosquito species are distributed throughout the globe and dominate many geographical areas. This aspect was exemplified by the World Health Organization (2012) [8], which reported 32 species of anophelines group from geographical regions of India. Consequently, this view was supported by Reinert et al. [9], who found A. aegypti and A. albopictus as 14.99% and 6.30% out of 1554 total collected specimens. Moreover, Tripathy et al. [10] observed that A. subpictus was dominant over Anopheles vagus and A. culicifacies in Orissa. Similarly, Khamsi et al. [11] found that A. subpictus was dominantly distributed (29%) than other species in the order of A. vagus (19.7%) > A. culicifacies and Atropheles varuna (11.6%) > A. annularis (9.92%) > A. flaviatilis (8.4%) > Anopheles nigerimurus (4.9%) in Angul. Similar observations were reported by Saleezza et al. [12], who agreed that A. subpictus was widely distributed than A. culicifacies and A. annularis of 10 anopheine species in Allahabad, Uttar Pradesh state of India. This supported occurrence of the species in question in greater extent.

Moreover, Tripathy et al. [10] proposed that A. culicifacies and A. flaviatilis species were comprised as 15.8% and 2.6%, respectively, in Orissa state. It was smaller than 34.9% and 29.2% which was reported by Sharma et al. [13]. It might be owing to either low success in DNA sequencing or changes in habitats [10]. Furthermore, in the case of A. flaviatilis series, A. flaviatilis is dominant over others [10,14,15]. However, A. culicifacies was spread from Ethiopia, Yemen, Iran, Afghanistan, Pakistan, India, Bangladesh, Myanmar, and Thailand to Laos and Vietnam along with Cambodia [16-21] as well as in Nepal, Southern China, and Sri Lanka [22]. More to the point, Sharma et al. [22] stated that A. culicifacies, A. flaviatilis, A. subpictus, and A. annularis are found in Madhya Pradesh, India. Similarly, Thongsripong et al. [23] stated that Kolhapur district was infected by disease-causing mosquito species, for example, A. culicifacies, An. stephensi, A. subpictus, Culex fatigans, A. albopictus, and A. aegypti.

In addition, specimens belonging to this species are dominant over others, which are supported by the fact, that although mosquito species such as An. minimus and An. baimaii are predominantly available vector species [24]; there quantity is lowering down [25]. Besides, these are replaced by A. culicifacies in their locations [26]. In contrast, C. quinquefasciatus was reported by Cook et al. [27] from different Indian cities and by Dhandha et al. [28] in Guwahati, Assam, as the most prevalent species with 29.92% of overall mosquito collection while C. tritaeniorhynchus was the second majorly found species (26.08%) in the same investigation. A similar approach was reported by Ottesen et al. [29], who found that C. quinquefasciatus was the most prevalent mosquito species in urban, semi-urban, and rural areas. To add, this is considered as the most commonly recognized domestic mosquito species. Likewise, this species was predominant (33.7%) in the total collection of mosquito species [30].

In addition, C. gelidus, which is native to the eastern part of South Asia, has its geographical existence ranging from India to Australia, covering nations such as China, Japan, Australia, and New Guinea [31-33]. Similarly, in India, this species has a wide range of distribution in various states, namely, Maharashtra, Goa [34], Kerala [34], Rajasthan [35], Karnataka [36], Tamil Nadu [37], Andhra Pradesh [37], Uttar Pradesh [38], West Bengal [39], and Assam [40]. This explicitly suggested that the species under study has dominated Indian biogeographical areas in a greater degree might be due to accessibility of favorable environmental conditions.

Conversely, in the case of species dominance, Arunachalam et al. [41] stated that individuals of C. tritaeniorhynchus were recorded in more number than C. gelidus in rural area of Andhra Pradesh. In the same way, the supremacy of this species was supported by Ottesen et al. [29] by stating that it is dominant in the Thanjavur region of Tamil Nadu, followed by A. subpictus. Nevertheless, this observation was opposed by Sudeep et al., 2015 [42], who stated that C. tritaeniorhynchus was replaced by C. gelidus gradually in semi-urban and urban regions of Southern India in contrast to the fact that C. gelidus individuals are significantly enlarged in the Kerala state of India. For example, it was found to be 57.9% among total collected mosquitoes during 2012–2013, whereas it was 17% in 2009 [42]. Moreover, Asha and Aneesh [43] found that genus Culex was dominant over other genera such as Aedes, Anopheles, Mansonia, and Armigeres, indicating the increased distribution of this genus.

Nevertheless, Parmasivan et al. [30] cast doubt on this finding and stated that M. uniformis was observed in more numbers as 14.7% in their collection, whereas C. gelidus was found to be 3.3% along with M. annulifera (5.05), A. albopictus (0.2%), and A. culicifacies from Udmari area (4.5%) along with A. annularis (7.4%). Likewise, Reinert et al. [9] observed only 3.66% of M. annulifera in their total collection of 1554 larval individuals of mosquitoes obtained from Guwahati, Assam, and M. uniformis (0.16%) and M. annulifera (0.01%) species were observed by Ottesen et al. [29]. It suggested that they were habituated in a lower number in the given geographical regions. In addition, According to Surendran et al. [44], M. annulifera was found to contain 2.19% of the overall collection of 3005 individuals, and Asha and Aneesh [43] recorded the occurrence of various genera such as Culex (40%), Anopheles (20%), Aedes (27%), Mansonia (10%), and Armigeres (3%) belonging to Irinjalakuda municipal area. Further, Amsan et al. [45] reported survival of Mansonia and Aedes spp. from outside India in Ajana-Liyebi, which is an agricultural area of rice crop belonging to Obafemi-Owode Local Government area and Ikene farm in Ikenne Local Government Area of Ogun state of Nigeria.

Besides, the subgenus Anopheles includes more than 183 species and Culex includes 26 subgenera and 769 species in the world. Green and Miles [2] stated that the genus Lutzia which is divided into 3 subgenera having 8 species is also found on a global scale. In addition, Culex periguenicus species was found to be distributed in Swat, Pakistan [46]. Ashfaq et al. [47] found the existence of sibling species of Anopheles annularis such as A. annularis A and A. annularis B in Punjab as well as Khyber Pakhtunkhwa (KPK) area of Pakistan. Similarly, genus Aedes was also investigated by Rohani et al. [48], who reported that...
is preliminary vector of DENV infection, especially in endemic regions, including Singapore. In the same way, *A. culicifacies*, *A. stephensi*, *A. subpictus*, *C. fatigans*, *A. albopictus*, and *A. aegypti* are found to transmit diseases such as Chikungunya, JE, Dengue, Malaria, and Filaria in Kolhapur district of Maharashtra state [95]. In addition, Samuel et al. [49] reported that in Thanjavur district of Tamil Nadu, the JE infection-causing mosquito species, for example, *C. tritaeniorhynchus* (found in more number) which is followed by *A. subpictus*, *C. gelidus*, *A. subpictus*, *Anopheles petidinaeitius*, *Anopheles barbirostris*, *Anopheles palillardus*, and *Anopheles tessellates*, *A. subalbatus*, *C. tritaeniorhynchus*, *C. gelidus*, *C. quinquefasciatus*, and *Aedes vexans*, *M. uniformis*, and *M. annulifera* are found to cause infections. Above and beyond, another mosquito species known as *A. subpictus* results into infection by JEV [26].

Furthermore, India, which is a developing country, confronted considerable health problems due to the spread of infectious mosquito species from a longer duration, especially in either underdeveloped states or in rural areas. This view was exemplified by Ghosh et al. [59] and Gopalakrishnan et al. [34], who stated that *A. culicifacies* is found in India, causing malaria. According to the report of WHO [75], 60–70% malarial infections were due to this vector species. Likewise, Sharma et al. [19] reported the prevalence of *A. fluviiatis* in the Orissa state of the country, which caused malarial infections [34] with locally important *A. annularis* [52]. More to the point, according to Acharya et al. [96], transmission of malaria among various regions varies since the diversity and allotment of both *Anopheline* and *Plasmodium* members are different. Thus, it is obvious that the spread of mosquito-borne diseases is related to health disasters across the globe, causing severe effects on social welfare.

### 4. NEED OF SPECIES IDENTIFICATION

It is essential to identify the members of disease-causing mosquito species for their effective control and the recent technologies used to achieve this goal may affect other harmless insects causing the greater problem in their food chain, food web, and ecosystems. To lower the spread of vector borne diseases and harm to useful flora and fauna, implementation of specific and effective control programs are required, which is possible only when we could identify the targeted mosquito species [97] reliably and precisely. This approach was supported by Kraemer et al. [58], who stated that identification of Chikungunya spreading mosquito species can be beneficial to assess the probability of infection by disease in question and it is required to detect the locations where these species are prevailed. So that future outbreaks of diseases caused by them can be avoided. Furthermore, Goswami et al., [76] stated that identification of malaria-causing sibling species of *A. culicifacies* is recommended since the spread of infection differs among them. Furthermore, according to Goswami et al. [76], the contribution of the anopheline group for malaria spread can be analyzed by appropriate species identification, which is also necessary for knowing its role in the outbreak of malaria disease and its effective control.

Unfortunately, in many places, the presence of mosquito causing diseases is not detected, particularly before sensitization of outbreak of infection. Moreover, there is a requirement of assessment for the presence of mosquito species carrying dengue virus in the area, where the probabilities of the presence of such virus exist [98]. It can reduce the probability of diseases. For example, the detection of *A. aegypti* spread in the related region with help of meteorology can assist in controlling mosquito-borne diseases [99] and malaria can be controlled.
by analyzing the presence of *A. culicifacies* [100] in the given area. Thus, all these views pointed toward the need of identification of disease spreading mosquito species.

### 4.1. Identification Problems

The identification of mosquitoes at species level using their morphological characters is having barriers especially, in the cases where specimens are available in the forms of eggs, larvae, immature state of life, or in damaged condition [32]. The intensity of such an obstacle is increased by the fact that such a type of taxonomical method requires expert taxonomists for identifying organisms at the rank of species. It is because morphological characters display noticeably small variation among species [101] and are time consuming, especially when less specialists work in this field. Furthermore, this process may not be completed in the case where valuable characteristics like bristles as well as scales are in the damaged form [102] and are enough to avoid reliable species identification. Thus, this circumstance of taxonomic science of mosquitoes requires an alternative method of species identification and differentiation that can be easily employed on a worldwide level. In addition, although images of voucher specimens can be used for species identification, in some situations, it may not be convenient to photograph a given organism. It is, especially, true when the sample is available in damaged, powdered or skin or tissue forms. In such cases, use of images does not work for the identification of the specimens under study.

### 4.2. Need for Genetic Delineation of Mosquito Species

The taxonomy of disease spreading vector species and their hosts may generate unauthentic reports as their taxonomical averages are not always accurate [103]. There are problems in the identification of mosquito specimens, which are responsible for the generation of barriers in their control programs. Similar view was put forth by Golnar et al. [104], who affirmed that obstacles in control of *Culex* spp. may be a source of increased spread of WNV infection in the United States (US). In addition, Samuel et al. [50] proposed that effective monitoring methodologies are required on an urgent basis through the identification of mosquitoes from different areas of India. Daravath et al. [105] stated that disease-causing species of mosquitoes, for example, *C. quinquefasciatus* should be controlled. In addition, in accordance with Hay et al. [106], lowering the number of mosquitoes is a key aspect of controlling the diseases that are originated from mosquitoes acting as their vectors. All these findings reported by various researchers indicate the fact that there is a need of the identification of mosquito species, especially based on DNA analysis rather than morphology, to assure correct species recognition of all types of specimens. In this view, it is important to study mosquito species for their biodiversity genetics since they have the ability to act as vectors [107] of many kinds of diseases. Some studies related to the whole-genome analysis of mosquito species have been conducted by researchers. According to Nene et al. [108], launching such type of project belonging to *C. quinquefasciatus* (WNV carriers) can provide a platform for understanding both generals as well as specific functions of the gene of mosquito species.

Such type of circumstances suggests the use of DNA sequence-based data for taxonomic identification strategies (firstly proposed by Tautz and Arctander [109] of harmful mosquito species and their control. This concept of DNA taxonomy was further expanded by Merget et al. [110], who used nuclear internal transcribed spacer 2 as a molecular marker for species identification. Such an approach was also supported by Sevilla et al. [111] and Shen et al. [112] by developing cytochrome b oxidase gene sequence to identify species. Moreover, in addition to the use of 16S rRNA gene for bacterial identification, Vences et al., 2005 [113] and Chu et al. [114] developed a 12S rRNA-based species identification system. However, geneticists never stopped at that stage and continued their research in finding more molecular markers such as nicotinamide adenine dinucleotide dehydrogenase [115,116] for DNA-based taxonomy. Nonetheless, genuine and pioneering work in the field of molecular marker-based species identification which is globally accepted and appreciated was performed by Hebert et al. [117], who proposed that the short fragment of mitochondrial and maternally inherited COI can be reliably utilized for identification of animals due to its lower intraspecific distance than interspecific variation among investigated animal species. A similar finding was recorded by Cywinska et al. [3], who supported the concept of COI-based taxonomy and agreed that COI gene region can be employed for assigning species-level taxa to 37 mosquito species collected from Ontario and New Brunswick areas of Canada. Likewise, this gene sequence depository of mosquito species collected from China was developed by Wang et al. [32]. Consecutively, mosquito species of Thrirupuvanam village belonging to Tamil Nadu were analyzed by Parmasivan et al. [30] through their identification and investigation of phylogenetic relationship using COI gene of 63 species of mosquitoes from 15 genera collected from India. In the same way, Abigail Chan et al. [64] identified 128 mosquito samples with 45 species and 13 genera collected from Singapore by taxonomy based on the same molecular marker.

In addition, this method of modern taxonomy has advantages over morphological identification system such as damaged specimens of mosquitoes can be identified by this technique [118]. It was also employed by Ashfaq et al. [47] for the taxonomy of mosquitoes belonging to Punjab and KPK areas of Pakistan in addition to analyzing divergence among them and the distribution of mosquito species carrying dengue virus. Further, other authors, for example, Cywinska et al. [3], Kumar et al. [81], Khamis et al. [11], Wang et al. [32], Laurito et al. [56], and Bourke et al. [119], found that DNA sequence can have application in identification of not only mosquitoes but also metazoans. Likewise, in accordance with Brown and Miller [120], Hebert et al. [121], Monaghan et al. [122], and Burns et al. [123], individuals of unknown mosquito species can be identified using COI gene sequences. Accordingly, all these findings support the aspect of COI-based identification system, which can be used on a global scale for the detection of animals, especially mosquitoes.

### 4.3. Genetic Divergence

The mosquito specimens should show enough genetic variation so that these can be easily distinguished among and within species, which is the basic criteria for identifying animals using their standard DNA barcodes. Similarly, Hebert et al. [117] found the genetic divergence between the species as 9.3% in more than 1400 species of order Diptera [Table 1]. However, this genetic distance is very higher than >2.3% recorded by Wang et al. [32] and Ashfaq et al. [47] as >2%. This suggested that the mosquito species collected from various geographical areas of different nations can be distinguished using a set baseline of the cutoff value. However, the threshold value for intraspecific divergence was established as 3% by Rubinoff [124] [Table 1] generated controversies in the identification process (Chan et al., 2014) [64]. It is due to the fact that the authors reported intraspecific divergence values above and
Table 1: Genetic divergence among mosquito species.

| S. No. | Content                                      | Genetic divergence | Reported by          |
|--------|----------------------------------------------|--------------------|----------------------|
| 1      | Between species (Diptera)                    | 9.3%               | Hebert (2003a) [117]  |
| 2      | Between mosquito species in China            | 2.3%               | Wang et al. (2012) [32] |
| 3      | Intraspecific divergence                     | 3%                 | Rubinoff (2006) [124] |
| 4      | Genetic distance within species *Culex quinquefasciatus* | 1.1                | Ashfaq (2014) [47]   |
| 5      | Genetic distance within species *Aedes aegypti* | 0.5                | Ashfaq (2014) [47]   |
| 6      | Genetic distance within species *Aedes albopictus* | 1.3                | Ashfaq (2014) [47]   |

below 3% as the set value. In contrast, Ashfaq et al. [47] proposed that maximum genetic distances within species of *C. quinquefasciatus*, *A. aegypti*, and *A. albopictus* were found to be 1.1%, 0.5%, and 1.3%, respectively [Table 1]. Although Brown and Miller [120] claimed that the database of genetic divergences related with both within and among the population of *A. aegypti* is existed, we believe that more inclusive work is required to be done for a setting platform for maximum intraspecific and minimum interspecific values. This will help determine a reliable barcode gap and species boundaries. This can be achieved by increasing global efforts for the generation of enough COI gene sequence library to ensure appropriate identification and differentiation of unknown taxa.

4.4. Phylogenetic Analysis

The phylogeographic analysis of specimens is important since it supports an inference of both contemporary outlines of transmission of vectors and their maximum routes of import (140) and patterns of distribution of the species in question [58]. Further, a phylogenetic tree is a tree diagramming, which gives an illustration of probable genetic distances and evolutionary relationships among different species of organisms. Typically, the individuals of the same species are placed in a single clade or a group, whereas specimens belonging to different species are clustered into different clusters depending on their DNA sequence similarities and differences, respectively. Moreover, the bioinformatics tools used for tree construction uses various statistical methods (neighbor joining, maximum likelihood, minimum evolution, UPGMA and maximum parsimony method, distance models [p-distance], Kimura 2 parameter model, Tajima-Nei model, LogDet [Tamura-Kumar], and maximum composite likelihood) that determine patterns of clade formation and distances among sequences.

Further, though the use of the statistical method for phylogeny construction varies depending on the data for analysis, neighbor joining method is commonly used for the analysis of mosquito species by various authors such as Krüger et al. [118], Cywinska et al. [3], Wang et al. [32], Ashfaq et al. [47], and Katsuya et al. [126]. The authors used Kimura 2 parameter as a distance model along with bootstrap support. Moreover, nevertheless, NJ tree is majorly used by researchers for mosquito phylogenetic analysis; it has few demerits, which raises the question on the reliability of this tree. For instance, according to Reinert et al. [127], this type of tree is phenetic but not phylogenetic and clades are formed using a holistic base of similarity among sequences without considering their synapomorphy. Consequently, the relationship among the species revealed in NJ tree of Cywinska et al. [3] and Kumar et al. [128] do not match to the conclusions received from cladistic analysis reported by Reinert et al. [127], Reinert et al. [129], and Reinert et al. [130]. However, outsized sequence variance can be investigated by NJ tree in relation to mosquitoe [3]. On the other hand, Samba Shiva [105] used maximum likelihood as a statistical method for investigating the phylogenetic relationship among mosquito specimens collected from Hyderabad city of India, suggesting instability in the use of specific parameters for phylogeny and species grouping analysis.

In addition, Besansky and Fahey [50] proposed in the phylogenetic tree, species belonging to taxon *Ochlerotatus* are positioned between two clusters, namely, *A. aegypti* and *A. albopictus*. It is because, according to Cook et al. [27], it is considered as a subgenus belonging to the genus *Aedes*. More to the point, in the case of some species, enough reference sequences are not found in the NCBI sequence database [46,64] and not all specimens are identified up to the species level and some may be identified up to only family or genera level depending on availability of required reference sequence in DNA databases. Similarly, Kumar et al. [128], Cywinska et al. [3], and Laurito et al. [36] stated that DNA barcode-based analysis is not having a value in all investigated cases owing to the unavailability of reference sequence databases in not only NCBI but also BOLD systems [46,64] and all voucher specimens. Consequently, this raises the point that the comprehensive database with all required information should be developed and used by researchers on a global scale to confront existing problems related to taxonomy and phylogeny analyses.

4.5. Demerit of BOLD Database

It is observed that currently available BOLD database system does not successfully distinguish closely related species or their isomorphic forms or sibling species unfailingly. For example, there are five isomorphic types of species *A. culicifacies* such as *A. culicifacies* A, *A. culicifacies* B [2], *A. culicifacies* C [131], *A. culicifacies* D [4,21,132], and *A. culicifacies* E [133], which are not clearly distinguished by COI gene sequence data in the BOLD data system. As a matter of fact, these sibling species are classified on the basis of various biological features such as a preference for feeding as well as biting habits along with their vulnerability to frequently used insecticides [21,134-136]. Besides, these are not differentiated with COI gene sequence-based DNA database though Singh et al. [137] and Goswami et al. [76] investigated identification strategies of these species by allele-specific polymerase chain reaction (PCR) targeting to D3 domain of 28S ribosomal DNA. This problem of species identification is also supported by Goswami et al. [76] and Singh et al. [137]. The authors affirmed that morphology-based differentiation of the species in question cannot be achieved and there are realistic problems related to their methods of traditional cytotaxonomy.

Thus, the species identification and differentiation problems are still exist in BOLD data systems which are lacking in certain areas. This can be improved by developing the strategic baseline that can dissolve the errors of assigning different names of species belonging to the same gene sequence. Moreover, COI gene sequences of such types of species do not show enough genetic variation for robust taxonomic identification. It explicitly indicates that there is a need to investigate other additional molecular markers that can identify and differentiate sibling species of diverse taxa effectively to avoid possible misidentifications of unknown or cryptic taxa. However, Chan et al. [64] proposed that it is possible to solve such ambiguities...
4.6. Nuclear Mitochondrial Pseudogenes (numts)

At the time of the beginning of DNA barcoding era, there were many problems in identifying species using COI gene sequences in which amplification of non-coding nuclear genes with mitochondrial gene regions was one of the substantial problems [138,139]. The presence of such type of error was predicted by observing ghost bands in PCR analysis, errors related to sequences, mutations in the sequences in the form of frameshift, and existence of stop codons in the gene sequences [140]. Furthermore, such ambiguity was reported by Cywinska et al. [3], who found a single pseudogene while analyzing DNA barcodes of mosquito species belonging to Canada.

Furthermore, this type of gene amplification may hinder the analysis of ancestral base and phylogeny of species [141] and these genes interfere with precise identification of species. Nevertheless, there is a possibility to avoid this situation in the case where extraction of DNA belonging to only mitochondria is preferred along with developing the PCR primers that are taxon specific. Nevertheless, analysis of ancestral base and phylogeny of species can be inhibited by chemical pesticides such as IRS/DDT/synthetic pyrethroids and LLINs. Furthermore, Sahu et al. [149] stated that the spread of this species can be controlled by deltamethrin. More to the point, sensitization of people, hindering import of malaria infection from nearby states or countries, artemisinin-resistance containment operation to prevent the existence of drug-resistant parasites can be implemented for the control of disease-causing species [148]. Besides, according to the report of WHO, Global Strategy for Dengue Prevention and Control (2012-2020) [8], highly advanced identification and precise coordination in relation to not only epidemiological but also entomological assessment has the potential to reduce dengue morbidity. Further, the use of microscope and diagnostic kits for earlier identification, spraying in houses as well as combination therapy based on artemisinin are key factors for programs that deal with the prevention of diseases in vulnerable areas.

Furthermore, Dev et al. [150] proposed that filariasis can be controlled by diethylcarbamazine + albendazole. In accordance with Dev et al. [150], various measures, namely, managing cases of lymphoedema, enhancing hydrocele programs, operations against viruses in association with cleaning methods such as filling of ditch, pit, low lying area, removal of weeds, and salt can be used to control spread of mosquito-borne diseases. Finally, Dhanda et al. [28] proposed that JE spread can be controlled by thermal malathion fogging and mosquito nets. According to Dev et al. [150], it can be achieved by fish predators of mosquito larvae. Too, the administration of the suitable vaccine in human bodies is also an effective measure lowering the potential risk of disease outbreaks. By taking this view into consideration, India has declared vaccination programs for increasing quality and assurance of public health, particularly in the regions where the chance of infection is more [50].

5. DISEASE CONTROL PROGRAMS

The disease control operations are very important to save people from infectious diseases spread by mosquitoes, which are urgently required to be controlled [98] to protect public health from a potential hazard. This goal can be achieved by identifying disease-spreading mosquito species existed in urban, semi-urban, and rural areas by DNA barcoding and putting control programs into operation in infected regions before the outbreak of infections reducing the transmission of diseases and loss to public health.

The diseases in question can be controlled by identifying and controlling vector species such as A. aegypti and A. albopictus [58]. And also, the identified vector species can be controlled by long-lasting insecticidal nets (LLINs) [144], covering tanks that contain stored water [7,145], biological agents [145], general awareness of health among people [31,146,147], removing water tanks that are not in the condition of use [146,147] especially focusing on specific water tank [17] making the water tanks vacant once a week [145] and accurate waste management protocols for places that are related with houses [147]. In addition, there are other methods which are used for control of disease spreading mosquito species such as physical (ultraviolet [UV] radiation), chemical, and biological (sterile insect technique) [7] used for control of dengue virus [15] transmission.

Moreover, the species under study can also be controlled by HCH, dieldrin, benzene hexachloride, as well as pyrethroids and according to the report submitted by World Health Organization 2006 [148] and World Health Organization (WHO) 2013 [125]. A. culicifacies species can be inhibited by chemical pesticides such as IRS/DDT/synthetic pyrethroids and LLINs. Furthermore, Sahu et al. [149] stated that the spread of this species can be controlled by deltamethrin. More to the point, sensitization of people, hindering import of malaria infection from nearby states or countries, artemisinin-resistance containment operation to prevent the existence of drug-resistant parasites can be implemented for the control of disease-causing species [148]. Besides, according to the report of WHO, Global Strategy for Dengue Prevention and Control (2012-2020) [8], highly advanced identification and precise coordination in relation to not only epidemiological but also entomological assessment has the potential to reduce dengue morbidity. Further, the use of microscope and diagnostic kits for earlier identification, spraying in houses as well as combination therapy based on artemisinin are key factors for programs that deal with the prevention of diseases in vulnerable areas.

Furthermore, Dev et al. [150] proposed that filariasis can be controlled by diethylcarbamazine + albendazole. In accordance with Dev et al. [150], various measures, namely, managing cases of lymphoedema, enhancing hydrocele programs, operations against viruses in association with cleaning methods such as filling of ditch, pit, low lying area, removal of weeds, and salt can be used to control spread of mosquito-borne diseases. Finally, Dhanda et al. [28] proposed that JE spread can be controlled by thermal malathion fogging and mosquito nets. According to Dev et al. [150], it can be achieved by fish predators of mosquito larvae. Too, the administration of the suitable vaccine in human bodies is also an effective measure lowering the potential risk of disease outbreaks. By taking this view into consideration, India has declared vaccination programs for increasing quality and assurance of public health, particularly in the regions where the chance of infection is more [50].

5.1. Role of DNA Barcoding in Mosquito Control

Since for controlling or reducing the number of any biological object requires its identification and other biological information, it is necessary for disease-spreading mosquito species to identify them, which can be achieved by DNA barcoding tool before their control. Furthermore, it is recommended to use a target-specific parasite control program to avoid killing of biologically useful insect species. In fact, since COI-based analysis can be used to detect both harmful mosquito species and other useful insects, it becomes easy to launch control programs only in that area, where the majority of disease-causing species are found, resulting into target-oriented operation. This system, if implemented successfully, can avoid a possible outbreak of diseases with less loss to eco-friendly biota. Moreover, this system may also be cost-effective since it may reduce the use of pesticides in the infected area. In this way, DNA barcode-based analysis has a potential role in the effective control of disease spreading mosquito species. Such harmful species may be considered by insecticide industries to develop specific insecticide for the species in question, assisting the approach of the target-specific parasite control program.

6. CONCLUSION

Disease spreading mosquito species have a wide range of diversity and are distributed in many geographical areas of world. Their successful identification and differentiation up to the species level can be achieved by analyzing their mitochondrial COI gene sequences with the help of available DNA sequence databases such as BOLD and
NCBI although they are lacking in certain areas such as unavailability of sufficient reference sequence database. Moreover, a cutoff criterion for species identification and differentiation has contrast. There is a need to expand the taxonomic coverage of mosquito species through DNA barcodes on the global scale to set maximum intra-specific and minimum inter-specific value for genetic distances. Such type of errors can be resolved by a broad range of sampling and analysis by multiple researchers on an extensive scale. In addition, the identified species responsible for disease outbreak can be controlled by various methods including biological, physical, chemical, and implementing preventive measures such as vaccination in mostly vulnerable areas. COI-based identification standpoint may play a chief role in putting target-specific mosquito control program into operation on larger extent. It is recommended to be implemented by all nations to secure health of people from outbreak and infectivity of harmful mosquito species.

7. AUTHORS’ CONTRIBUTIONS
RD: Developed an idea and wrote manuscript, KA: Collected literature, AS and JK: Corrected the data.

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9. CONFLICTS OF INTEREST
Authors declare that no conflicts of interest exist.

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