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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.

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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, Heizmannia, Topomyia,* and *Uranotaenia,* are left to be assigned by their taxonomic names. Interestingly, in accordance with Ilahi 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. Consecutively, 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, Khamis *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 *Atrophaneura varuna* (11.6%) >*A. annularis* (9.92%) >*A. fluviatilis* (8.4%) >*Anopheles nigerrimus* (4.9%) in Angul. Similar observations were reported by Saleeza *et al*. [12], who agreed that *A. subpictus* was widely distributed than *A. culicifacies* and *A. annularis* of 10 anopheline 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. fluviatilis* 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. fluviatilis* series, *A. fluviatilis* 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. fluviatilis, 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 Dhanda *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, Amusan *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 Ikenne 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 perixiguus* 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

A. albopictus was the dominant species in Balai Ringin followed by *Aedes caecus* and *Aedes seatoi* and was widespread in Malaysia also. Furthermore, the correlation among taxa *Aedes* and *Ochlerotatus* was invested by some researchers such as scientists of WHO, 2007 [49] who stated that the genus *Aedes* is the largest group in Culicinae subfamily having 930 species and *Ochlerotatus* is its subgenus with 187 known species that are having worldwide distribution.

3. MOSQUITO BORNE DISEASES

There are various mosquito species that act as vectors of parasites or viruses affecting public health due to their outbreaks, especially in rural areas, where diagnosis and treatment facilities are rarely available. For example, individuals of the genus *Mansonia* are responsible for causing *Brugian filariasis* [50] and also JEV infection by *M. annulifera* in Dibrugarh region of Assam [51] and Madhya Pradesh [36]. This disease was also spread in Taiwan by *A. subalbatus* [52] and Kerala [53]. Moreover, *M. uniformis* spread the infections of filariasis and Japanese Encephalitis (JE) [38]. To add, this genus leads to Chikungunya and Rift Valley fever (RVF) viral diseases [54]. Furthermore, *Brugia malayi* and *Mansonia* species are causative agents of *B. filariasis* in both India and China [55]. Six species of *Mansonioides* are natural carriers of lymphatic filariasis [56] along with *M. uniformis,* which is the carrier of *B. filariasis* in Thailand [57] and also causes the spread of RVFV, Babanki virus, Perinet virus [57], West Nile virus (WNV) [58], *Wuchereria bancrofti* [59], *Setaria* sp., and *Dirofilaria* spp. [60].

In addition, *A. albopictus* are responsible for causing various threatening diseases such as dengue [61-63], Chikungunya [64-68], yellow fever viruses [69], Zika virus (ZIKV) [56], and WNV virus [70] in India. Likewise, *Aedes* spp. with particular emphasis to *A. aegypti* spreads infection of ZIKV (ZIKV; family *Flaviviridae*, genus *Flavivirus*) on a larger scale among various countries [66] and also causes infections of yellow fever virus [71], DENV [1,72], RVFV [73], and Chikungunya in the areas of Indian ocean [74]. Further, the individuals belonging to genus *Anopheles* also have considerable capacity to create a greater risk to public health. For example, members of this species caused malaria in 91 nations threatening world's 40% population and *A. culicifacies sensu lato* has generated 60-70% malarial victims in India [75,76] and 2–3% victims of malaria in every year [77].

Besides, the individuals of mosquitoes belonging to the genus *Culex* are also harmful to humans causing infections, namely, Filariasis and JE [23,78]. What's more, *C. quinquefasciatus* invasion in given area results into the transmission of filarial worms [79-81]. It leads to 120 million deaths in the globe [29]. As well, it is responsible for St. Louis encephalitis [82], WNV [83], Avion malaria [84], protozoan parasites [85], skin allergies [29], and dengue virus [46]. In addition, it is a vital carrier of bancroftial filariasis [86] as well as JE [86] in India and acts as a transporter of L3 parasite [87-89]. In addition, *C. gelidus* species is responsible for the spread of JE [90] and viruses of *Togaviridae* and *Bunyaviridae* families [42]. Similarly, *C. tritaeniorhynchus* is the vector species of JEV virus [42] and is the major vector of this disease in India [91]. In addition, [92] observed that *C. tritaeniorhynchus* species causes JE and is reason of recurrent death scenes in the upper area of Asam and in accord with Chouin-Carneiro *et al*. [66], *Culex* spp. is a causative agent of ZIKV infection.

In contrast, Diagne *et al*. [93] proposed that transmission of ZIKV by *A. aegypti* was extremely low or not at all. This clearly states that the species under study is less harmful than its counterparts regarding with spread of ZIKV among existed human population. However, Chan *et al*. [94] opposed this observation and stated that *A. aegypti* 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 peditaeniatus, Anopheles barbirostris, Anopheles pallidus,* 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. fluviatilis* 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 resulted into 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 outburst 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, powder 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 16SrRNA 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 Thirupuvanam 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] in >98% of 122 species of mosquitoes collected from China [Table 1]. Nevertheless, the reported values for inter-specific genetic distances are concordant with the threshold value set 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 tool 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 mosquitoes [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

by taxonomy based on morphological data. Nevertheless, COI-based identification can be implemented effectively if other supplementary methods are used.

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 for amplification of targeted molecular marker [142]. In addition, Behura *et al*. [143] suggested another solution of this problem by stating that sequencing of the whole genome of mitochondria can detect the presence of numts. Interestingly, Bensasson *et al*. [140] claimed that NJ analysis can be used for their detection since irregular and unanticipated placement of the clade in question is existed in the tree.

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 outburst 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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