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Molecular phylogenetics and evolution of mosquitoes

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Abstract

Mosquitoes exert significant influence on human health by acting as carriers of diseases. Gaining insight into the evolutionary process and phylogenetic connections of mosquitoes is essential for effectively managing disease-carrying vectors. In this paper, we provide a thorough examination of the current understanding of mosquito phylogenetics, which is derived from both morphological and molecular data. By analysing nuclear, mitochondrial, and ribosomal DNA sequences, we examine the evolutionary history and classification of mosquitoes, with a specific emphasis on understanding the links between important genera and subgenera. The discrepancies between molecular and morphological phylogenies are emphasised. Additionally, we discuss current discoveries about cryptic species complexes, the introduction of invasive species, and the ways in which phylogenetics can enhance the comprehension of the dynamics of mosquito-borne diseases. Lastly, we will examine the obstacles and potential future paths for study in the field of mosquito phylogenetics.

Keywords: Phylogenetic, COI, Culicidae, cryptic species, homoplasy

Introduction

Mosquitoes, belonging to the order Diptera and the family Culicidae, are a significant group of insects that have adapted to thrive in various aquatic larval habitats and feed on a vast array of vertebrate hosts ^[1]. There are almost 3,500 known species, which are divided into two subfamilies and 112 genera ^[2, 3]. Mosquitoes are well-known for transmitting severe human diseases such as malaria, dengue fever, yellow fever, chikungunya, Zika, West Nile virus, and others. These diseases collectively result in hundreds of millions of infections and over a million fatalities annually ^[4, 5]. Comprehending the evolutionary past and phylogenetic connections of mosquitoes is crucial for efficiently managing them and anticipating occurrences of diseases transmitted by mosquitoes ^[6, 7]. In the past, the classification and evolutionary relationships of mosquitoes were mostly determined by studying the physical characteristics of adult mosquitoes and their immature stages ^[2, 8]. Over the past decades, the use of DNA sequence data in molecular phylogenetics has significantly transformed our comprehension of mosquito evolutionary connections ^[9]. Through the use of molecular phylogenies, scientists have successfully addressed unresolved taxonomic problems, discovered hidden species groups, and gained insights into the geographical origins and diversification of important mosquito lineages ^[10-12].

This article provides a thorough examination of mosquito phylogenetics, combining traditional morphology-based classification with the most recent discoveries from molecular phylogenetic studies. We analyse the connections and classifications of the primary mosquito subfamilies, tribes, genera, and subgenera. The discrepancies between molecular and morphological data are emphasised. Prominence is placed on the significance of precise species identification and the recognition of cryptic species complexes. In addition, we explore the practical uses of mosquito phylogenetics in comprehending the spread of diseases and identifying the responsible vectors.

Results and Discussion

The categorization and evolutionary relationships of mosquitoes have historically been based on the physical characteristics of adult mosquitoes, particularly female genitalia, male genitalia, eggs, larvae, and pupae [13]. Characteristics such as the morphology of the mouthparts, wing veins, scales and bristles, and reproductive organs have been found to be valuable in distinguishing genera and subgenera. Traditional morphological research established the fundamental classification system and proposed several significant branches within the Culicidae family [14, 15]. The groups mentioned consist of two existing subfamilies (Anophelinae and Culicinae), 11 tribes within Culicinae, and several generic groupings. Nevertheless, morphology alone frequently fails to determine more profound phylogenetic connections, and the occurrence of homoplasy in morphological characteristics can hide genuine evolutionary relationships [16, 17]. Mosquitoes possess a restricted set of physical traits that provide valuable information about their evolutionary relationships. There have been other instances of convergent evolution in features associated with blood-feeding and larval habitat [18]. Distinguishing certain lineages based on their physical characteristics can be challenging, particularly when examining their early developmental phases [19]. As a result, the unity and connections of numerous taxonomic taxa have remained unknown or disputed based on their physical characteristics [20-22].

In recent decades, the use of DNA sequences in molecular phylogenetics has significantly enhanced our comprehension of the evolutionary relationships of mosquitoes. Sequence data obtained from nuclear, mitochondrial, and ribosomal genes have been widely utilised in mosquito systematics and phylogeny [23, 24]. Molecular data have been highly valuable in elucidating both profound and superficial connections within the Culicidae family. The nuclear white gene, mitochondrial COI and COII, and ribosomal 18S and 28S are the most often employed molecular markers for studying the evolutionary relationships of mosquitoes [25, 26]. Additional nuclear genes such as CAD, enolase, and arginine kinase have also been found to provide useful information [27, 28]. Research utilising several genetic loci, analysed individually or together as datasets, have produced more accurate and strongly supported evolutionary trees [29-32]. Furthermore, the whole sequences of mitochondrial genomes are currently being employed for the purpose of conducting phylogenetic research on the Culicidae family [33].

Phylogenetic investigations at the molecular level confirm that Culicidae, along with its two subfamilies, Anophelinae and Culicinae, form a monophyletic group. This finding is consistent with previous research that examined the physical characteristics of these organisms. The genus *Anopheles* is consistently found to be monophyletic within the Anophelinae family. The key clades within this genus include subgenera *Cellia*, *Anopheles*, and *Nyssorhynchus*, supported by studies [34, 35]. The genus *Bironella* is considered to be closely related to the genus *Anopheles*, based on genetic analysis [36]. Within the Culicinae subfamily, a majority of tribes and numerous taxa are confirmed to be monophyletic, as indicated by references. Nevertheless, certain conventional taxa such as *Aedes*, *Ochlerotatus*, and *Stegomyia* have been found to be either para- or polyphyletic [37-39]. Phylogenetic investigations have successfully clarified numerous species complexes and discovered previously unknown cryptic species [40, 41]. For

instance, molecular data have uncovered concealed variations within the *Anopheles gambiae* complex [42, 43], the *Culex pipiens* complex [44], and the *Aedes scutellaris* complex [45]. The presence of other undescribed species in groupings such as the *Anopheles punctulatus* complex is indicated by the extensive intraspecific genetic diversity [46].

Molecular phylogenies, along with fossil data and biogeographic studies, are offering valuable insights into the geographical origins and diversification of significant mosquito lineages. Based on the existing evidence, it is believed that Culicidae first appeared during the Jurassic period, more than 145 million years ago [47, 48]. The original mosquitoes likely engaged in pool breeding and lived in tropical woods. The two existing subfamilies, Anophelinae and Culicinae, separated during the early Cretaceous period. The majority of mosquito fossils originate from the Eocene epoch, a period in which numerous contemporary genera and subgenera emerged [49]. The Aedini and Sabethini exhibited widespread radiation during the Cenozoic period [50]. The radiation of these radiations was probably triggered by the diversification of mammals and birds, which offered additional hosts for blood feeds. Currently, the distribution of mosquitoes is primarily found in tropical locations, with the Neotropical realm having a particularly high number of species. Phylogenetic analyses indicate that certain recent speciation events have been influenced by geographic isolation and the process of adapting to new habitats [51]. The Pleistocene climate cycles and geographic variance episodes are believed to have had a significant role in promoting diversity in numerous groupings [52]. A recent study utilised mitogenomes and fossil-calibrated divergence dating to analyse the linkages and biogeographic origins of the subgenera of *Anopheles*.

Evolutionary relationships among organisms and the ecological factors influence the spread and impact of diseases. Studying the evolutionary relationships of mosquitoes can help enhance our comprehension of the ecology and evolution of diseases transmitted by mosquitoes. The phylogenetic links between mosquito species have an impact on their ability to transmit various diseases [53]. *Anopheles* species are the only carriers of malaria parasite *Plasmodium*. However, the capability to transmit *Plasmodium* might differ significantly across different species within the *Anopheles* genus [54]. Furthermore, the transmission of dengue and Zika viruses is particularly facilitated by species of *Aedes* [55]. Phylogenetic investigations conducted within the *Anopheles* genus have found the malaria vectors that exhibit higher efficiency. The subgenera *Cellia* and *Nyssorhynchus* encompass the majority of the primary vectors, whilst *Anopheles* species are primarily considered secondary or minor vectors [56]. These patterns are most likely a result of the long-term coevolution of mosquitoes and parasites. Previous studies have revealed similar results regarding the transmission of *Aedes* mosquitoes and arboviruses. Phylogenetics can enhance vector incrimination efforts by enabling disease control programmes to focus on the most effective vector species. Cryptic species, which frequently exhibit distinct roles in the spread of pathogens, can also be detected. For example, there are two distinct molecular forms of *An. gambiae*, called M and S. That cannot reproduce with each other. These forms have different eating habits and vary in their vulnerability to *Plasmodium* [57].

Although significant advancements have been achieved in the field of mosquito phylogenetics, numerous obstacles still persist. The higher-level relationships within the Culicidae family are not completely resolved, and there are still numerous species that have not been identified or have questionable taxonomic classifications. Additional taxonomic sampling and more reliable molecular databases are required, specifically for less-studied geographical areas and lesser-known groups of species. Phylogenomic methods that utilise a significant number of nuclear loci or even entire genomes show great potential for future research. Another objective is to determine the full scope of cryptic species diversity. Comprehensive investigations of species complexes necessitate the use of several nuclear and mitochondrial markers, crossing experiments, as well as the analysis of ecological and behavioral data. To comprehend phylogeographic trends within species and recreate colonisation paths, it will be necessary to increase the number of population samples taken. Population genomic studies can investigate these concerns and offer understanding into mosquito migration, genetic exchange, and local adaptation. It is crucial to ensure that mosquito phylogenetic and taxonomic information is readily available and practical for vector control programmes and public health practitioners. Online libraries and identification tools can assist in precise species delimitation and direct focused management efforts. An essential objective should be to enhance the connection between mosquito systematics and practical areas such as epidemiology and vector biology ^[58].

Conclusion

The field of mosquito phylogenetics has made significant progress in recent decades, primarily due to the advancements and utilisation of molecular systematics. Phylogenetic analyses have provided clear insights into the relationships across larger groups of organisms, resolved difficult situations involving multiple species, and revealed previously unknown variations in species. The remaining issues encompass the resolution of intricate relationships, the description of novel species, and the comprehension of phylogeographic patterns and gene flow. By integrating phylogenetic information with studies on mosquito behaviour, ecology, and vector competence, it is possible to anticipate and alleviate outbreaks of mosquito-borne diseases. Subsequent investigations should prioritise the integration of these disciplines and enhancing the connection between mosquito phylogenetics and applications in public health.

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