Species Identification of *Mansonia dives* (Diptera: Culicidae) in Thailand using DNA barcoding

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Abstract

Accurate mosquito species identification is essential for effective vector surveillance and disease control. *Mansonia dives* (Diptera: Culicidae) is a medically important mosquito species implicated in the transmission of filarial parasites such as *Brugia malayi* and *Wuchereria bancrofti*. Morphological identification of *Mansonia* species is often challenging due to overlapping diagnostic characteristics and intraspecific variation. This study aimed to confirm the species identity of *Ma. dives* in Thailand using DNA barcoding based on the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene. Ten mosquito specimens morphologically identified as *Ma. dives* were collected from Koh Kood District, Trat Province, and subjected to DNA extraction, PCR amplification, sequencing, and phylogenetic analysis. Sequence comparisons with two international databases, including GenBank and the Barcode of Life Data Systems (BOLD), revealed 99.69–100 % similarity with reference *Ma. dives* sequences. Phylogenetic analysis based on the neighbor-joining method showed all specimens clustering within the *Ma. dives* clade, closely related to *Ma. bonneae*, *Ma. uniformis*, and *Ma. annulifera*. These results confirm that DNA barcoding provides a reliable, precise, and reproducible molecular tool for identifying *Ma. dives* and differentiating it from closely related species. The findings support the integration of DNA barcoding into routine mosquito surveillance to enhance taxonomic accuracy and strengthen vector monitoring programs in Thailand.

Keywords: DNA barcoding, *Mansonia dives*, phylogenetic analysis, mosquito identification

1. Introduction

Mosquitoes are among the most widespread and diverse groups of insects globally, playing significant roles in public health as vectors of numerous pathogens (Damapong et al., 2016; Promprao et al., 2025). To date, approximately 3,618 mosquito species have been recorded worldwide, of which about 412 have been reported in Thailand. Accurate species identification is a fundamental component of vector surveillance and control programs, as mosquito species vary considerably in their capacity to transmit pathogens. The genus *Mansonia* (Diptera: Culicidae) comprises several medically important species known to transmit filarial parasites such as *Brugia malayi* and *Wuchereria bancrofti*, the causative agents of lymphatic filariasis in humans (Rattanarithikul et al., 2005). Among them, *Mansonia dives* is a notable species commonly found in freshwater swamps, ponds, and habitats rich in aquatic vegetation. It has been implicated as a potential vector of filarial parasites and arboviruses in several regions of Southeast Asia, including Thailand (Rueanghiran et al., 2017).

Traditional morphological identification of *Mansonia* species is often challenging due to overlapping diagnostic characteristics, intraspecific variation, and frequent damage to key morphological features during field collection. These limitations underscore the need for molecular approaches that provide more precise, reliable, and reproducible species identification, thereby complementing or, when necessary, replacing morphology-based methods.

DNA barcoding, based on a standardized fragment of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene, has emerged as a powerful and widely adopted molecular tool for species identification and biodiversity assessment in insects (Bušić et al., 2024). This technique enables the accurate discrimination of morphologically similar or cryptic species through genetic divergence analysis and comparison with reference sequences in international databases such as GenBank and the Barcode of Life Data Systems (BOLD). In mosquitoes, *COI* barcoding has proven to be an effective and cost-efficient method for confirming species identity, uncovering cryptic diversity, and providing valuable insights for vector surveillance and disease control.

The present study aims to verify the species identity of *Ma. dives* collected from different regions of Thailand using DNA barcoding. The *COI* sequences obtained will be compared with reference sequences from global databases to assess genetic similarity and phylogenetic relationships. The findings are expected to strengthen taxonomic accuracy, enhance the effectiveness of entomological surveillance, and contribute to a better understanding of the distribution and genetic diversity of *Mansonia* species in Thailand.

2. Research Objective

This study aims to identify *Ma. dives* in Thailand using DNA barcoding.

3. Methodology

Collection of Mosquitoes in the Field

Mosquitoes were collected in Koh Kood Subdistrict, Koh Kood District, Trat Province, Thailand (11°36'30.9"N, 102°32'46.8"E) in 2022 using a BG-Pro CDC-style trap (BioGents, Regensburg, Germany). This trap was selected for its proven efficiency in field mosquito collection and its durable, water-resistant design, which makes it suitable for outdoor use under varying environmental conditions. The device operated continuously throughout the trapping period using a portable power bank as its power source, eliminating the need for a direct electrical connection. The BG-Pro trap utilizes multiple attractant cues, including light, chemical lures, and dry ice (CO_2). Subsequently, *Ma. dives* specimens were identified based on morphological characteristics.

DNA Extraction, Polymerase Chain Reaction (PCR), and DNA Sequencing

DNA extraction was conducted using the FavorPrep Mini Kit (Favorgen Biotech, PingTung, Taiwan) in accordance with the manufacturer's instructions. Approximately three to six legs were removed from each mosquito specimen and used as the source material for extraction. The extracted DNA was eluted into microcentrifuge tubes and stored at −20°C until subsequent molecular analyses were performed. DNA barcoding was employed to identify *Ma. dives* by amplifying the *COI* gene. Two universal primers were used for standard amplification: the forward primer 5'-GGA TTT GGA AAT TGA TTA GTT CCT T-3' and the reverse primer 5'-AAA AAT TTT AAT TCC AGT TGG AAC AGC-3'. Each polymerase chain reaction (PCR) had a final volume of 25 µl, consisting of distilled water (14.85 µl), 10× PCR buffer (2.5 µl), 10 mM dNTP (0.5 µl), 10 µM forward primer (0.5 µl), 10 µM reverse primer (0.5 µl), 5 U Taq polymerase (0.15 µl), 50 mM $MgCl_2$ (0.75 µl), dimethyl sulfoxide (DMSO, 1.25 µl), and template DNA (4 µl).

PCR amplification was performed under the following thermocycling conditions: an initial denaturation at 95°C for 5 minutes, followed by five cycles of denaturation at 94°C for 40 seconds, annealing at 45°C for 60 seconds, and extension at 72°C for 60 seconds. This was followed by 35 additional cycles consisting of denaturation at 94°C for 40 seconds, annealing at 54°C for 60 seconds, and extension at 72°C for 60 seconds. A final extension was carried out at 72°C for 10 minutes, after which the reaction was held at 4°C to terminate the process. The PCR products were examined using agarose gel electrophoresis, and DNA bands were visualized with Midori Green Advance DNA Staining Solution (Nippon Gene, Tokyo, Japan). Gel images were captured using an ImageQuant LAS 500 imager (GE Healthcare Japan Corp., Tokyo, Japan). Samples that produced clear and distinct DNA bands were subsequently sent for sequencing to SolGent Co., Ltd. (Daejeon, South Korea).

Analysis of *COI* Gene Nucleotide Sequences

The nucleotide sequence data of the *COI* gene in mosquitoes were obtained as trace files representing nucleotide chromatograms. Low-quality regions containing background noise or ambiguous base calls were trimmed to ensure sequence accuracy. The forward and reverse sequences were then aligned and assembled to generate consensus sequences using BioEdit software (Hall, 1999). The resulting consensus sequences for each specimen were compared with reference sequences of *Mansonia* species available in the Barcode of Life Data Systems (BOLD) database to confirm species identification. Finally, phylogenetic relationships among the *Mansonia* species in Thailand were inferred using MEGA 11 software (Tamura et al., 2021), with tree construction based on the neighbor-joining (NJ) method and 1,000 bootstrap replicates.

4. Results

DNA Barcoding

Based on the *COI* gene sequences of *Ma. dives* identified through morphological examination, 10 specimens were analyzed using two genetic databases, including GenBank and the BOLD, to confirm species identity. The comparative DNA analysis verified that all specimens were correctly identified as *Ma. dives*. The results of this comparison are summarized in Table 1. Sequences obtained from both databases corresponded to the same species, showing a high degree of nucleotide similarity. The percentage similarity values derived from GenBank and BOLD were nearly identical, confirming the accuracy and reliability of DNA barcoding for species-level identification.

Phylogenetic Tree

The phylogenetic analysis demonstrated that all 10 *Ma. dives* specimens identified morphologically in this study (indicated in the red box) clustered within the Ma. dives clade (indicated in the orange box). This clade showed the closest genetic relationship to *Ma. bonneae* (indicated in the green box), followed by *Ma. uniformis* and *Ma. annulifera* (both indicated in green), as shown in Figure 1.

Table 1. Information on *Ma. dives* specimens analyzed using the DNA barcoding and percentage similarity with reference sequences in DNA databases.

ID in this study	Species	Species match	Percentage similarity with the Top Match mosquito species	
			GenBank	BOLD
001	*Ma. dives*	*Ma. dives*	100 %	99.84 %
002	*Ma. dives*	*Ma. dives*	99.85 %	99.84 %
003	*Ma. dives*	*Ma. dives*	99.86 %	99.69 %
004	*Ma. dives*	*Ma. dives*	100 %	99.84 %
005	*Ma. dives*	*Ma. dives*	100 %	99.84 %
006	*Ma. dives*	*Ma. dives*	100 %	99.84 %
007	*Ma. dives*	*Ma. dives*	99.86 %	99.84 %
008	*Ma. dives*	*Ma. dives*	100 %	99.84 %
009	*Ma. dives*	*Ma. dives*	100 %	99.84 %
010	*Ma. dives*	*Ma. dives*	99.86 %	99.69 %

5. Discussions

In this study, DNA barcoding was employed to identify *Ma. dives*. The *COI* gene, widely recognized as a standard genetic marker for species identification, was amplified, sequenced, and analyzed (Bušić et al., 2024). The resulting nucleotide sequences were compared with reference sequences available in two international databases, including GenBank and BOLD, to verify species identity. Comparative analysis revealed that all 10 *Ma. dives* specimens examined in this study were consistently identified as *Ma. dives* in both databases. The percentage similarity values were exceptionally high and nearly identical between GenBank and BOLD, confirming the reliability, precision, and reproducibility of the DNA barcoding approach. Furthermore, phylogenetic analysis supported these findings, showing that all specimens clustered within the *Ma. dives* clade, thereby validating the molecular identification results.

DNA barcoding has emerged as one of the most powerful and reliable molecular tools for species identification across a wide range of taxa, including insects of medical and veterinary importance (Noureldin et al., 2022). The effectiveness of this technique is largely attributed to the *COI* gene's high interspecific variability coupled with low intraspecific divergence, which allows for the clear discrimination of closely related species. The findings of this study are consistent with previous research and further demonstrate the utility of DNA barcoding in mosquito taxonomy and vector surveillance. For example, Ruangsittichai et al. (2011) successfully applied DNA barcoding to differentiate *Ma. dives* from *Ma. bonneae*, highlighting the method's accuracy and robustness in resolving morphologically similar *Mansonia* species. Likewise, Chaiphongpachara et al. (2022) confirmed the reliability of DNA barcoding for mosquito identification in Thailand and emphasized its significant contribution to improving vector monitoring, surveillance, and control programs through precise molecular species identification. Beyond its value for taxonomic identification, DNA barcoding offers several additional advantages. It enables rapid confirmation of vector species, facilitates biodiversity assessments, and assists in detecting invasive or cryptic taxa that may otherwise remain unrecognized.

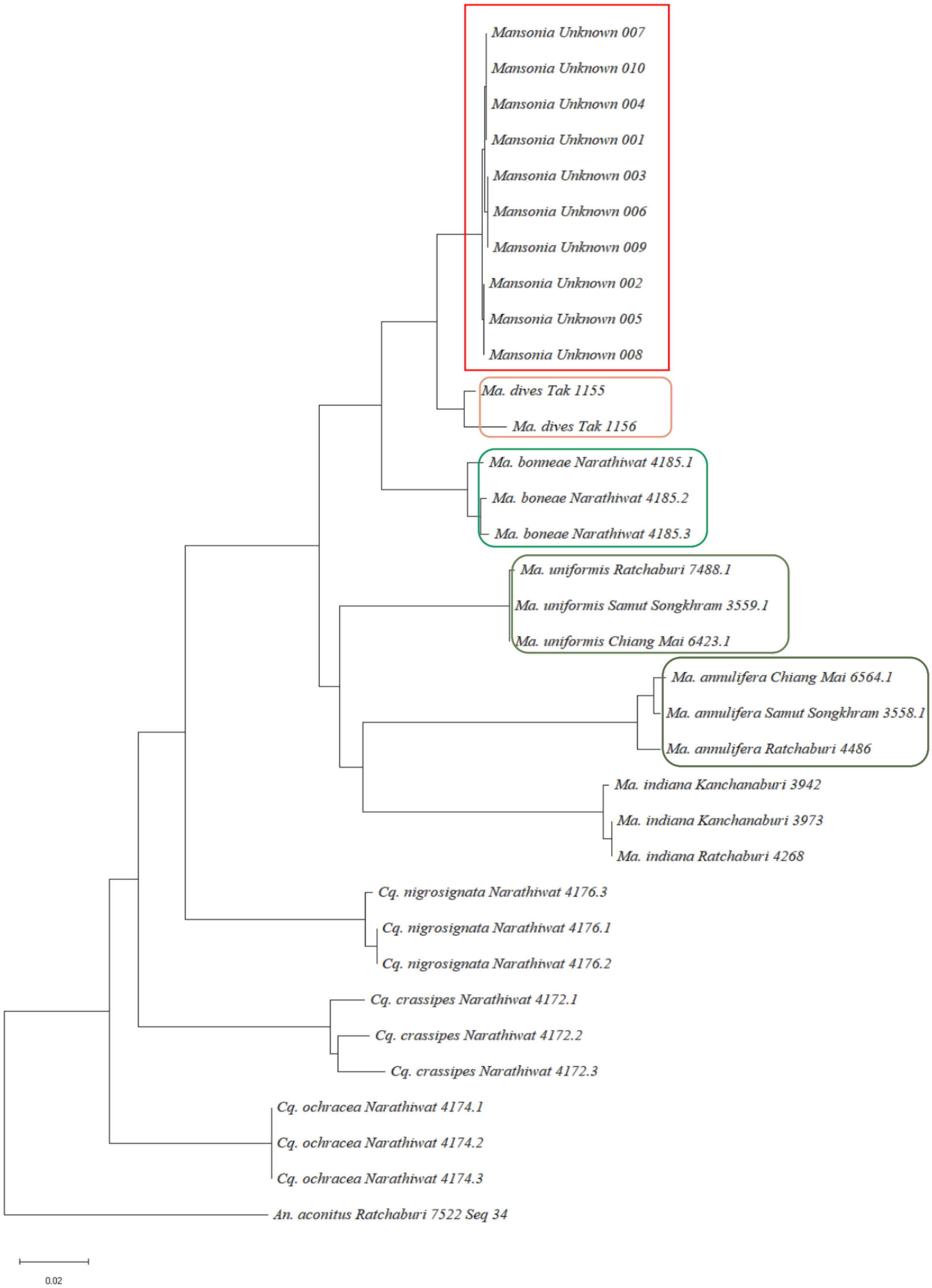

Figure 1. Neighbor-joining phylogenetic tree (1,000 bootstrap replicates) of nine mosquito species, showing that all ten *Ma. dives* specimens from this study clustered within the *Ma. dives* group.

In regions such as Thailand, where mosquito diversity is remarkably high, with more than 400 species recorded, DNA barcoding serves as an indispensable complement to traditional morphological taxonomy, particularly when specimens are damaged, incomplete, or morphologically ambiguous. However, despite its proven effectiveness, DNA barcoding also has limitations (Cheng et al., 2023). The method may be less effective in distinguishing recently diverged or cryptic species that share highly similar *COI* sequences. Moreover, inaccuracies in public reference databases, including misidentified or incomplete entries, can occasionally lead to erroneous species assignments.

6. Conclusion

The findings confirm that DNA barcoding is a reliable and reproducible molecular tool for distinguishing *Ma. dives* and can effectively complement traditional morphological methods for mosquito identification. Its effectiveness lies in the *COI* gene's ability to discriminate closely related species, making it particularly valuable in regions with high mosquito diversity, such as Thailand.

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