Cryptic Diversity and Demographic Expansion of *Plasmodium knowlesi* Malaria Vectors in Malaysia

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Abstract: Although Malaysia is considered free of human malaria, there has been a growing number of *Plasmodium knowlesi* cases. This alarming trend highlighted the need for our understanding of this parasite and its associated vectors, especially considering the role of genetic diversity in the adaptation and evolution among vectors in endemic areas, which is currently a significant knowledge gap in their fundamental biology. Thus, this study aimed to investigate the genetic diversity of *Anopheles balabacensis*, *Anopheles cracens*, *Anopheles introlatus*, and *Anopheles latens*—the vectors for *P. knowlesi* malaria in Malaysia. Based on cytochrome c oxidase 1 (*CO1*) and internal transcribed spacer 2 (*ITS2*) markers, the genealogic networks of *An. latens* showed a separation of the haplotypes between Peninsular Malaysia and Malaysia Borneo, forming two distinct clusters. Additionally, the genetic distances between these clusters were high (2.3–5.2% for *CO1*) and (2.3–4.7% for *ITS2*), indicating the likely presence of two distinct species or cryptic species within *An. latens*. In contrast, no distinct clusters were observed in *An. cracens*, *An. balabacensis*, or *An. introlatus*, implying a lack of pronounced genetic differentiation among their populations. It is worth noting that there were varying levels of polymorphism observed across the different subpopulations, highlighting some levels of genetic variation within these mosquito species. Nevertheless, further analyses revealed that all four species have undergone demographic expansion, suggesting population growth and potential range expansion for these vectors in this region.

Keywords: *Plasmodium knowlesi*; Malaysia; *Anopheles*; mosquitoes; genetic diversity

1. Introduction

Malaria remains a persistent global public health challenge, and countries in Southeast Asia have been assigned the goal of malaria elimination by 2030. This ambitious target highlights the urgency and importance of concerted efforts to combat malaria and reduce its burden in the region. Malaysia has been free of human malaria since 2018 [1], but *P. knowlesi*, a simian malaria parasite, is the predominant species currently occurring in the country [2]. All countries in SEA have reported the occurrence of *P. knowlesi*, with the exception of Timor-Leste [3]. It is crucial to consider the WHO [4] recommendation to postpone the certification of a malaria-free status for countries reporting significant *P. knowlesi* cases in the region. This highlights the importance of ongoing surveillance, monitoring, and control efforts to effectively address the persistence of malaria and prevent the potential reintroduction of human malaria in Malaysia and neighboring countries.