

## **Analysis of Plasmodium knowlesi mitochondrial DNA aiming to identify molecular markers useful for epidemiological analysis of the zoonotic malaria in Sabah**

### **ABSTRACT**

**Introduction:** Although no indigenous human malaria cases have been recorded since 2018 in Malaysia, people in the country, especially those in the two states in Borneo, are still at the risk of zoonotic malaria caused by Plasmodium knowlesi. For better control of P. knowlesi malaria, epidemiological understanding of the disease is essential. Molecular markers in the mitochondrial DNA are expected to be useful, but characterisation of the parasite's mitochondrial genome remains limited. **Methods:** From the total DNA extracted from P. knowlesi malaria blood samples collected in various districts in Sabah in 2020 and 2022, two overlapping DNA fragments covering a 2.3 kb region containing the parasite's entire cox3 coding sequence were PCR amplified. Fragments of 12 different parasite strains were sequenced by direct Sanger sequencing and variations in the 2.3 kb region were identified by comparison with the reference sequence in the database. **Results:** Nine single nucleotide polymorphisms (SNPs) were identified in 10 strains investigated in this study. Seven of these SNPs were strain-specific, while remaining 2 were shared by 3 different strains each. In addition, one of the A clusters present in the reference sequence was extended in all the 12 strains investigated. **Conclusion:** The SNPs identified here will probably serve as useful molecular markers in epidemiological study of P. knowlesi in Sabah. The fact that 9 SNPs were identified in the 2.3 kb region of only 12 strains analysed proves that the mitochondrial DNA is a good source of molecular markers useful in P. knowlesi research.