

**Phylogenetic analysis of simian Plasmodium spp. infecting Anopheles
balabacensis Baisas in Sabah, Malaysia**

ABSTRACT

Anopheles balabacensis of the Leucospyrus group has been confirmed as the primary knowlesi malaria vector in Sabah, Malaysian Borneo for some time now. Presently, knowlesi malaria is the only zoonotic simian malaria in Malaysia with a high prevalence recorded in the states of Sabah and Sarawak. Anopheles spp. were sampled using human landing catch (HLC) method at Paradason village in Kudat district of Sabah. The collected Anopheles were identified morphologically and then subjected to total DNA extraction and polymerase chain reaction (PCR) to detect Plasmodium parasites in the mosquitoes. Identification of Plasmodium spp. was confirmed by sequencing the SSU rRNA gene with species specific primers. MEGA4 software was then used to analyse the SSU rRNA sequences and build the phylogenetic tree for inferring the relationship between simian malaria parasites in Sabah. PCR results showed that only 1.61% (23/1,425) of the screened An. balabacensis were infected with one or two of the five simian Plasmodium spp. found in Sabah, viz. Plasmodium coatneyi, P. inui, P. fieldi, P. cynomolgi and P. knowlesi. Sequence analysis of SSU rRNA of Plasmodium isolates showed high percentage of identity within the same Plasmodium sp. group. The phylogenetic tree based on the consensus sequences of P. knowlesi showed 99.7%–100.0% nucleotide identity among the isolates from An. balabacensis, human patients and a long-tailed macaque from the same locality. This is the first study showing high molecular identity between the P. knowlesi isolates from An. balabacensis, human patients and a long-tailed macaque in Sabah. The other common simian Plasmodium spp. found in long-tailed macaques and also detected in An. balabacensis were P. coatneyi, P. inui, P. fieldi and P. cynomolgi. The high percentage identity of nucleotide sequences between the P. knowlesi isolates from the long-tailed macaque, An. balabacensis and human patients suggests a close genetic relationship between the parasites from these hosts.