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# Prevalence of haemosporidia in Asian Glossy Starling with discovery of misbinding of *Haemoproteus*-specific primer to *Plasmodium* genera in Sarawak, Malaysian Borneo

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## Abstract

**Background** *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are three mainly studied blood parasites known to cause malarial and pseudomalarial infections in avian worldwide. Although Sarawak is a biodiversity hotspot, molecular data on blood parasite diversity in birds are absent. The objective of the study is to determine the prevalence of blood parasite in Asian Glossy Starlings (AGS), an urban bird with high population density in Sarawak and to elucidate the phylogenetic relationship with other blood parasite.

**Methods** Twenty-nine carcasses of juvenile AGS that were succumbed to death due to window collision were collected around the vicinity of Universiti Malaysia Sarawak. Nested-multiplex and nested PCR targeting the Cytochrome B gene were used to detect *Plasmodium* and *Haemoproteus*, and *Leucocytozoon* respectively. Two primer sets were used for *Haemoproteus* detection to increase detection sensitivity, with one being a genus-specific primer.

**Results** Fourteen samples (prevalence rate: 48.28%) were found positive for avian *Plasmodium*. Phylogenetic analysis divided our sequences into five lineages, pFANTAIL01, pCOLL4, pACCBAD01, pALPSIS01 and pALPSIS02, with two lineages being novel. No *Haemoproteus* and *Leucocytozoon* was found in this study. However, *Haemoproteus*-specific primer used amplified our *Plasmodium* samples, making the primer non-specific to *Haemoproteus* only.

**Conclusion** This is the first blood parasite detection study on AGS using carcasses and blood clot as sample source in Sarawak. Due to the scarcity of longer sequences from regions with high genetic plasticity, usage of genus-specific primers should be validated with sequencing to ensure correct prevalence interpretation.

**Keywords** Misbinding, Avian, Plasmodium, Genera-specific primer, Sarawak

## Introduction

Avian haemosporidia, consisting of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* genera, are blood obligated protozoans which causes malaria and malaria-like diseases in susceptible avian hosts [1–3]. Disease presentations can range from asymptomatic to potentially fatal depending on haemosporidian lineage and bird species. Its importance came to light after the extirpation of numerous endemic Hawaiian wild birds in the 1900s due to the spread of *Plasmodium relictum* lineage,

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