



Molecular Characterization of *Plasmodium relictum* in Four Common Bird Species in India

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ABSTRACT

The increasing emergence of wildlife diseases with the possibility to ecological threats as well as domestic animals and human health has prompted the importance of understanding disease dynamics and associated risks in biological conservation. The present study was undertaken from North Eastern part of India from January, 2017 to March, 2018 to identify and molecularly characterize *Plasmodium relictum* based on *cyt b* gene in various species of wild birds (*Upupa epops*, *Passer domesticus*, *Pycnonotus cafer*, *Bubulcus ibis*). The birds were captured by netting system. After blood was collected from wing veins, birds were released from the cages. Blood samples were examined after staining with Giemsa stain. The positive samples were used for amplification of *cyt b* gene of *Plasmodium relictum*. Cloning and sequencing of the amplified products of each samples was performed separately. Out of 120 birds examined, 25 were found positive for *P. relictum* based on morphology and subsequently confirmed by PCR which selectively amplified 712bp of *P. relictum*. Based on sequences and phylogenetic analysis of *cyt b* gene, 8 isolates of *P. relictum* were identified. The obtained complete nucleotide sequences of *cyt b* gene from *P. relictum* revealed 100% identity among themselves while the other sequences registered in Gen Bank showed 95% to 99% similarity. In the phylogenetic analysis all the isolates of *P. relictum* formed a separate clade with high bootstrap values. It can be inferred from the study that *P. relictum* is fairly common in wild birds and the *cyt b* gene is highly conserved among different isolates. It seems that *cyt b* could provide suitable genetic markers for discrimination and genetic characterization of *P. relictum*. This is the first attempt of genetic characterization of *P. relictum* from India.

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Authors' Contribution

PB, SG, AD and SSA collected the blood samples. Parasite identification and molecular analysis was done by GP and AS. GP and SKB critically supervised the whole experiment and drafted the manuscript.

Key words

Plasmodium relictum, *cyt b* gene, Molecular characterization, India

INTRODUCTION

Plasmodium relictum infects a wide variety of domestic and wild birds including chickens, ducks, partridges, canaries, pigeons etc (Bensch *et al.*, 2009; Marzal *et al.*, 2014; Drovetski *et al.*, 2014). It may be lethal to the species which have not evolved resistance to parasites such as penguins and naïve birds which often suffer from severe disease and mortality during infection (Ilgūnas *et al.*, 2016). The parasites are easily distinguishable in blood films because of its distinct morphological characteristics. Mature stages typically have predominant nuclei and cytoplasm, numerous pigment granules and markedly

influence the position of host cell nuclei causing lateral shifts in their positions. Microscopic examination of blood films was main diagnostic tool in 20th century worldwide (Atkinson, 2008). Microscopy may reveal the presence of trophozoites, merozoites and gametocytes in erythrocytes.

Several molecular methods are used for detecting avian malarial parasites in blood samples. Most are based on amplification of conserved fragments of 18S rRNA or mitochondrial cytochrome-b gene (Jarvi *et al.*, 2002; Tattiyapong *et al.*, 2016; Valkiūnas *et al.*, 2018). Partial sequences of *cyt b* gene have been successfully used for molecular characterization of parasites and it proved to be excellent molecular marker for disease diagnostics (Elsasser *et al.*, 2009).

Despite wide spread distribution of *P. relictum* in birds, no study has been done in India to characterize the *cyt b* gene and also about the number of copies it

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