



## Molecular and morphological characterization of two avian malaria parasites (Haemosporida: Plasmodiidae), with description of *Plasmodium homonucleophilum* n. sp.

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### Abstract

*Plasmodium homonucleophilum* n. sp. was described from the Common Grasshopper Warbler *Locustella naevia* based on the morphology of blood stages and partial sequences of the mitochondrial cytochrome *b* (*cyt b*) gene. This malaria parasite belongs to the subgenus *Novyella*; it can be readily distinguished from all described *Novyella* parasites due to two features, i. e. the strict adherence of its meronts to the nuclei of infected erythrocytes and the lack of such adherence in the case of gametocytes. We also found the lineage pLZFUS01 in Red-Backed Shrike *Lanius collurio*, identified this parasite and conclude that it belongs to *Plasmodium relictum*. Illustrations of blood stages of these two parasites are given. DNA lineages associated with *P. homonucleophilum* (pSW2, GenBank KC342643) and *P. relictum* (pLZFUS01, GenBank KC342644) are reported and can be used for molecular identification of these malarial infections. Phylogenetic analysis determines DNA lineages closely related to both reported parasites and is in accordance with the parasites' morphological identification. This study contributes to barcoding of avian malaria parasites using partial sequences of *cyt b* gene.

**Key words:** avian haemosporidians, *Plasmodium*, new species, microscopy, PCR, mitochondrial DNA

### Introduction

Avian malaria parasites (*Plasmodium*, Plasmodiidae) have been found on all continents except Antarctic (Valkiūnas 2005). Many species of *Plasmodium* are generalists and parasitize birds belonging to various families and even orders (Garnham 1966; Waldenström *et al.* 2002). These avian blood parasites are transmitted exclusively by blood-sucking dipterans of the Culicidae. The majority of *Plasmodium* species are not vector species-specific; they complete sporogony in mosquitoes belonging to different genera (Valkiūnas 2005; Santiago-Alarcon *et al.* 2012). However, vector species of many avian malaria parasites remain unknown.

Over 50 described species of avian malaria parasites have been traditionally classified in five subgenera (i.e. *Haemamoeba*, *Giovannolaia*, *Novyella*, *Huffia* and *Bennettinia*) (Corradetti *et al.* 1963; Garnham 1966; Valkiūnas 2005; Valkiūnas *et al.* 2009). Many *Plasmodium* genetic lineages have been deposited in GenBank, but a few of them are linked with morphospecies (Palinauskas *et al.* 2007; Valkiūnas *et al.* 2009). This is unfortunate because linkage between DNA sequences and identifications based on morphological species can provide important knowledge for better understanding of transmission and basic life history strategies of pathogens. The knowledge obtained by traditional parasitology, combined with genetic information about haemosporidian isolates, brings researchers to higher level of analysis, when data about biology of parasites are supplemented with sequence information, which is essential for phylogenetic research. Additionally, the description of haemosporidian parasites becomes more precise when combined with genetic data.

Recently, several new species of haemosporidian parasites have been described using both morphological features and molecular markers (Valkiūnas *et al.* 2009; Križanauskienė *et al.* 2010; Iezhova *et al.* 2011; Levin *et al.* 2012; Merino *et al.* 2012). In this study, we continue molecular and morphological characterization of avian