



Comparison of mitochondrial cytochrome *b* lineages and morphospecies of two avian malaria parasites of the subgenera *Haemamoeba* and *Giovannolaia* (Haemosporida: Plasmodiidae)

VAIDAS PALINAUSKAS¹, VLAD KOSAREV², ANATOLY SHAPOVAL², STAFFAN BENSCH³ & GEDIMINAS VALKIŪNAS¹

¹ Institute of Ecology, Vilnius University, Akademijos 2, Vilnius 2100, LT-08412, Lithuania. E-mail: gedvalk@eko.lt (author for correspondence: palinauskas@gmail.com) ² Biological Station of the Zoological Institute, Russian Academy of Sciences, Rybachy, 238535 Kaliningrad District, Russia. E-mail: kosarev@bioryb.koenig.ru, shapoval@bioryb.koenig.ru

³ Department of Animal Ecology, Ecology Building, Lund University, SE-22362 Lund, Sweden. E-mail: staffan.bensch@zoekol.lu.se

Abstract

PCR-based methods have been increasingly used in diagnosis of parasitic diseases. Over 40 morphospecies of avian malaria parasites of the genus *Plasmodium* have been described. However, only nine of them have been identified on the level of their mitochondrial cytochrome *b* (*cyt b*) gene lineages, which are frequently used in molecular biology studies of avian blood haemosporidian parasites. In this study, we linked two common mitochondrial *cyt b* lineages P-SGS1 and P-TURDUS1 with their morphospecies. Light infections with two species of malaria parasites of the subgenera *Haemamoeba* and *Giovannolaia* were isolated from naturally infected adult Hawfinches, *Coccothraustes coccothraustes* Linnaeus, on the Curonian Spit in the Baltic Sea. These parasites were inoculated to naive juveniles of the Common Crossbill, *Loxia curvirostra* Linnaeus, and House Sparrow, *Passer domesticus* Linnaeus. Heavy parasitemia of *Plasmodium* (*Haemamoeba*) *relictum* Grassi & Feletti, 1891 (lineage P-SGS1) and *Plasmodium* (*Giovannolaia*) *circumflexum* Kikuth, 1931 (P-TURDUS1) developed in the subinoculated Common Crossbills and House Sparrows, respectively, which enabled the detailed illustration of all main blood stages of these parasites and the deposition of their voucher specimens. The parasites of both lineages are actively transmitted in Europe and inhabit a broad range of avian hosts. Lineages closely related to *P. relictum* and *P. circumflexum* were identified. This study contributes to establishing of combined PCR-based and morphological identification of avian malaria parasites.

Key words: avian malaria, *Plasmodium relictum*, *Plasmodium circumflexum*, microscopy, PCR, phylogeny

Introduction

After Grassi and Feletti (1891) described the first two species of avian malaria parasites, the “similarity species concept”, which is based on morphological and some other biological characters, has traditionally been used in taxonomy of species of *Plasmodium* and other related blood haemosporidian parasites (order Haemosporida). In systematics of avian malaria parasites, it is generally accepted that any new species should only be established if supported (at least) by the full range of blood stages Valkiūnas. As a result, this provides opportunities for detailed comparison of morphology of similar parasites and identification of their species.

Five subgenera of avian *Plasmodium* have been described using traditional methods. These are: *Haemamoeba*, *Giovannolaia*, *Novyella*, *Huffia*, and *Bennettinia* (Corradetti *et al.* 1963; Garnham 1966; Valkiūnas 2005). Phylogenetic relationships between species of these subgenera remain insufficiently investigated. A recent molecular study by Martinsen *et al.* (2007) supported validity of the subgenera *Haemamoeba*, *Huffia*, and *Bennettinia*, but questioned the validity of the *Giovannolaia* and *Novyella*. This required clarification by