



Molecular characterization of haemosporidian parasites (Haemosporida) in yellow wagtail (*Motacilla flava*), with description of *in vitro* ookinetes of *Haemoproteus motacillae*

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Abstract

In studies of haemosporidian systematics and taxonomy, a combination of microscopic examination and molecular identification has been recommended. The yellow wagtail (*Motacilla flava*) is a frequently used species in studies of host-parasite interactions; blood parasites of this bird have been well studied using microscopic examination of blood films, but data on molecular characterization of the parasites are scattered. Here we present the first study linking several haemosporidian *cyt b* sequences with their morphospecies parasitizing the yellow wagtail, combining light microscopy and nested PCR methods. The lineage hYWT1 of *Haemoproteus motacillae* was the most prevalent parasite; it was recorded in 38% of the birds. The latter lineage and the lineages hYWT2, hYWT3, hYWT5 and hYWT6 form a well-supported clade on the phylogenetic tree and likely represent intraspecific genetic variation of *H. motacillae*, with genetic divergence of 0.3–1.5 % among these lineages. Microscopic examination of smears prepared during an *in vitro* experiment, which was designed for observation of developing ookinetes, showed that *H. motacillae* ookinetes were present 5 hours after exposure of blood to air at 18–20° C. Ookinetes of this parasite belong to a group of large *Haemoproteus* spp. ookinetes (19.9 µm in length on average). Illustrations of the parasites are given. Two of reported *Plasmodium* lineages, pCOLL1 and pYWT4 are phylogenetically closely related to the widespread host generalist parasites *Plasmodium relictum* (*cyt b* lineages pSGS1, pGRW4 and pGRW11), but it was not possible to establish this morphologically. This study contributes to the molecular identification of avian haemosporidian parasites and provides information on morphology of *H. motacillae* ookinetes, which is additional information assisting the microscopic identification of this species.

Key words: avian haemosporidians, microscopic examination, morphospecies, nested PCR, cytochrome *b*

Introduction

Avian haemosporidians (Haemosporida) are obligate heteroxenous blood parasites belonging to the genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*. They are worldwide distributed, except in Antarctica, and undergo sexual process in blood-sucking dipteran insects (Diptera), which are the vectors transmitting the infections among birds (Valkiūnas 2005). On the basis of morphology observed under light microscope, over 210 species of avian haemosporidian parasites have been described up to now (Valkiūnas 2005). Molecular studies consider several-fold greater diversity of morphologically cryptic species (Bensch *et al.* 2000, 2004; Perkins & Schall 2002; Hellgren *et al.* 2004; Waldenström *et al.* 2004). The most generally applicable molecular method for parasite detection and identification is based on a high-sensitive nested PCR protocol, which amplify a part of mitochondrial cytochrome *b* (*cyt b*) gene of these parasites (Hellgren *et al.* 2004; Waldenström *et al.* 2004). It has been confirmed that sequence variation in a rapidly evolving nuclear gene (DHFR-TS) is associated with the variation in mitochondrial DNA (mtDNA), supporting that species status of most of the *cyt b* lineages of avian