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Hemolivia and *Hepatozoon*: Haemogregarines with Tangled Evolutionary Relationships

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The generic name *Hemolivia* has been used for haemogregarines characterized by morphological and biological features. The few molecular studies, focused on other haemogregarine genera but involving *Hemolivia* samples, indicated its close relationship to the genus *Hepatozoon*. Here we analyze molecular data for *Hemolivia* from a broad geographic area and host spectrum and provide detailed

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morphological documentation of the included samples. Based on molecular analyses in context of other haemogregarines, we demonstrate that several sequences deposited in GenBank from isolates described as *Hepatozoon* belong to the *Hemolivia* cluster. This illustrates the overall difficulty with recognizing *Hemolivia* and *Hepatozoon* without sufficient morphological and molecular information. The close proximity of both genera is also reflected in uncertainty about their precise phylogeny when using 18S rDNA. They cluster with almost identical likelihood either as two sister taxa or as monophyletic *Hemolivia* within paraphyletic *Hepatozoon*. However, regardless of these difficulties, the results presented here provide a reliable background for the unequivocal placement of new samples into the *Hemolivia*/ *Hepatozoon* complex.

Key words: Apicomplexa; haemogregarines; *Hemolivia*; *Hepatozoon*; host specificity; phylogeny.

Introduction

Apicomplexa, as an extremely rich and diversified group of protozoan parasites, have traditionally been considered a phylogenetically and evolutionarily challenging taxon. Hypotheses explaining some of the profound questions about their deep origin have been perceived as important landmarks in the research of this group (e.g. position of cryptosporidia; Carreno et al. 1999). However, apart from such fundamental questions, the apicomplexan phylogeny still contains many uncertainties regarding the origin and phylogenetic status of various more recent crown groups. Previous phylogenetic analyses within these groups revealed unexpected relationships conflicting with morphology and/or biology (e.g. *Cyclospora* within *Eimeria*, or *Isospora* vs. *Cystoisospora*; Barta et al. 2005; Pieniazek and Herwaldt 1997; Relman et al. 1996). The genus *Hemolivia* is a representative of such crown groups within apicomplexans with fairly unresolved phylogenetic relationships and evolutionary history. This genus comprises tick-transmitted haemogregarines of ectothermic vertebrates, with only three described species so far; *H. stellata* (the type species) from the Neotropic cane toad *Rhinella marina* (formerly *Bufo marinus*), *H. mariae* from the Australian sleepy lizard *Tiliqua rugosa*, and *H. mauritanica* from the Palaearctic spur-thighed tortoise *Testudo graeca* (Petit et al. 1990; Sergent and Sergent 1904; Smallridge and Paperna 1997). *Hemolivia* differs from the closely related genus *Hepatozoon*, by several characteristics of its biology. First, sporogony is divided into two phases; in the first phase, oocysts with sporokinetes are formed, whereas the formation of sporocysts and sporozoites takes place during the second phase. On the contrary, the genus *Hepatozoon* is characterized by large polysporocystic oocysts. Intraerythrocytic merogony was described in *Hemolivia* (Petit et al. 1990), whereas it has never been observed in *Hepatozoon* (Desser 1993). *Hemolivia* gamonts occurring in the peripheral blood of vertebrate hosts are typical for the presence of a stain-resistant parasitophorous vacuole. These gamonts are morphologically recognizable by their cylindrical or slightly elliptical shape with a straight long axis, differing from the frequently curved structures of related genera, e.g. *Hepatozoon* and *Haemogregarina*. The mature gamonts of *Hemolivia* resemble empty sticks with a blue-stained nucleus at the polar position.

While morphologically and biologically well-defined, *Hemolivia* has been largely neglected in terms of phylogenetic analyses and its position within the haemogregarines remains unclear. The only two molecular studies, which also include a *Hemolivia* species, are a brief study dealing with the molecular detection of *Hemolivia* in ticks from *Testudo graeca* from Algeria (Harris et al. 2013), and a comprehensive analysis focused on the phylogeny of adeleorinids, mainly of the genus *Hepatozoon* (Barta et al. 2012). While the latter analysis is informative with respect to the phylogeny of *Hepatozoon*, it remains uncertain regarding the position of *Hemolivia*. In the phylogenetic tree presented by Barta et al. (2012), *Hemolivia mariae* clusters within *Hepatozoon* as a sister taxon of a sample labeled as *Hepatozoon* sp. (GenBank acc. no. EU430236) from the brown water python *Liasis fuscus*. This arrangement poses serious questions on the monophyly and phylogenetic position of the genus *Hemolivia*. The short distance between *Hemolivia* and the closest *Hepatozoon* indicates a relatively recent split between these two lineages. This implies that *Hemolivia*, with its current diversity and distribution, is either a surprisingly recent group or is a non-monophyletic assemblage of morphologically defined organisms.

There are precedents in the apicomplexan taxonomy of seemingly monophyletic groups, defined morphologically and biologically that turned out to