

**Life cycle stages and molecular phylogeny of *Hepatozoon fitzsimonsi* (Dias 1953) (Adeleorina: Hepatozoidae) in tortoises *Stigmochelys pardalis* (Cryptodira: Testudinidae) and ticks of the genus *Amblyomma* (Acari: Ixodidae) from South Africa**

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Haemogregarines (Apicomplexa: Adeleorina) are commonly encountered haemoprotozoan parasites of reptiles, of which, the genus *Hepatozoon* appears to be the most prevalent. Species display a heteroxenous life cycle, requiring a vertebrate and invertebrate host. Based on relationships of haemogregarine genera inferred using the 18S rRNA gene, the genus *Hepatozoon* remains paraphyletic, which lead to a new genus being erected *Bartazoon* Karadjian et al., 2015, with solely haematophagous insects acting as vectors. *Hepatozoon fitzsimonsi* was one of the species proposed to be a member of *Bartazoon*. However, past research done on *H. fitzsimonsi* observed a close association with tortoises and ticks, observing what appeared to be sporogonic stages in ticks collected from tortoises. Recently, two molecular screening studies, identified the presence of *H. fitzsimonsi* in ticks from tortoises in Kenya and South Africa. These findings in mind, the present study aimed to revisit the potential of ticks as vectors for *H. fitzsimonsi* in tortoises, by (i) collecting blood/tissue and ticks from tortoises, (ii) screening both microscopically for the presence of blood, merogonic, and sporogonic stages respectively, and (iii) molecularly characterising these stages using fragments of the 18S rRNA gene to determine if they are that of *H. fitzsimonsi*.

A total of 14 tortoises were collected, including nine individuals of *Kinixys* spp. and five *Stigmochelys pardalis*. Ten of the 14 (71%) tortoises were infested with ticks belonging to three species of *Amblyomma*. As *Kinixys* spp. are known to harbour both *H. fitzsimonsi* and *Hemolivia parvula* concurrently, three of the *S. pardalis* were selected, two of these showing a peripheral blood infection with *H. fitzsimonsi*. Impression slides from ticks showed sporogonic stages within the haemocoel, molecularly comparing to *H. fitzsimonsi*. Furthermore, merogonic stages were observed in the liver of one *S. pardalis* infected with *H. fitzsimonsi*.

The present study thus provides further support for ticks acting as the vectors of *H. fitzsimonsi* based on observation of its developmental stages in tortoises as well as the invertebrate host (*Amblyomma* spp.),

with these stages linked molecularly. This would be the second haemogregarine of tortoises for which ticks are the vector, *Hemolivia mauritanica* being the first, and the first species of *Hepatozoon* infecting chelonians for which ticks have been identified as a definitive host. This will hopefully encourage further research into chelonian *Hepatozoon*, a research area that remains largely neglected at present.