

Fish haemogregarines (Adeleorina: Apicomplexa) – a neglected group of blood parasites with some phylogenetic surprises

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INTRODUCTION

Apicomplexan haemogregarines are obligate parasites of many species of vertebrates¹. While many of these from amphibians, reptiles and mammals have been characterised molecularly using the 18S rRNA gene, those infecting fishes have relied solely on morphological identification by microscopy². Recent research by Hayes and Smit (2019) on *Haemogregarina* (s.l.) *bigemina* (UK) represents the first molecular characterisation for any of the named haemogregarines of fishes. Their findings suggest *H. bigemina* to fall outside of the “*Haemogregarina*” clade and altogether out of the Adeleorina, the group to which all haemogregarines of fishes currently belong (genera: *Babesiasoma*, *Cyrtilia*, *Dactylosoma*, *Desseria* and *Haemogregarina*), this species rather falling in a separate apicomplexan marine clade³. Furthermore, since *H. bigemina*'s description in 1901, it has been reported infecting >90 species of fishes of different families and genera across the world. This cosmopolitan distribution has been questioned for over a decade⁴.

MATERIALS & METHODS

Thin blood smears and whole blood (fixed in 70% ethanol) were collected from fishes from off the coasts of Australia (AUS), the Caribbean (CAR), the Philippines and South Africa (SA). Smears were screened determining prevalence and morphology, whilst DNA was extracted from whole blood. PCR was carried out using tried and tested primer sets to amplify parasite DNA^{2,5,6}. Amplicons of the present material were compared to each other and to comparative sequences of apicomplexans retrieved from GenBank in a ML analysis implemented from within Geneious ver. 7.1.9 using PhyML (500 replicates).

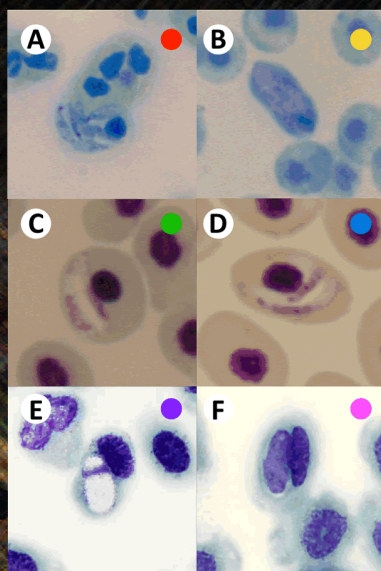


Fig. 1. Peripheral blood intraerythrocytic stages in fishes. A. merozoites in Porcupine fish sp. (AUS); B. merozoites in Puffer fish sp. (AUS); C-D. paired gamonts in Blenny fish sp. (CAR) and Klipfish sp. (SA) respectively. E-F. mature gamonts in catshark and shyshark sp. (SA). Scale bar = 10µm. Colour codes correlate with those in Fig. 2.

RESULTS

A total of 1,618 fishes of 186 species of 115 genera were collected. Thus far, 13 species of eight families have been found infected with haemogregarines of known and unknown identity, most of these falling within an unknown marine apicomplexan clade (Fig. 1 A-D, Fig. 2, purple) along with *H. bigemina* from the UK, except for those from the Scyliorhinidae (cat- and shysharks), which fell within the *Dactylosoma* (Fig. 1 E-F, Fig. 2, green).

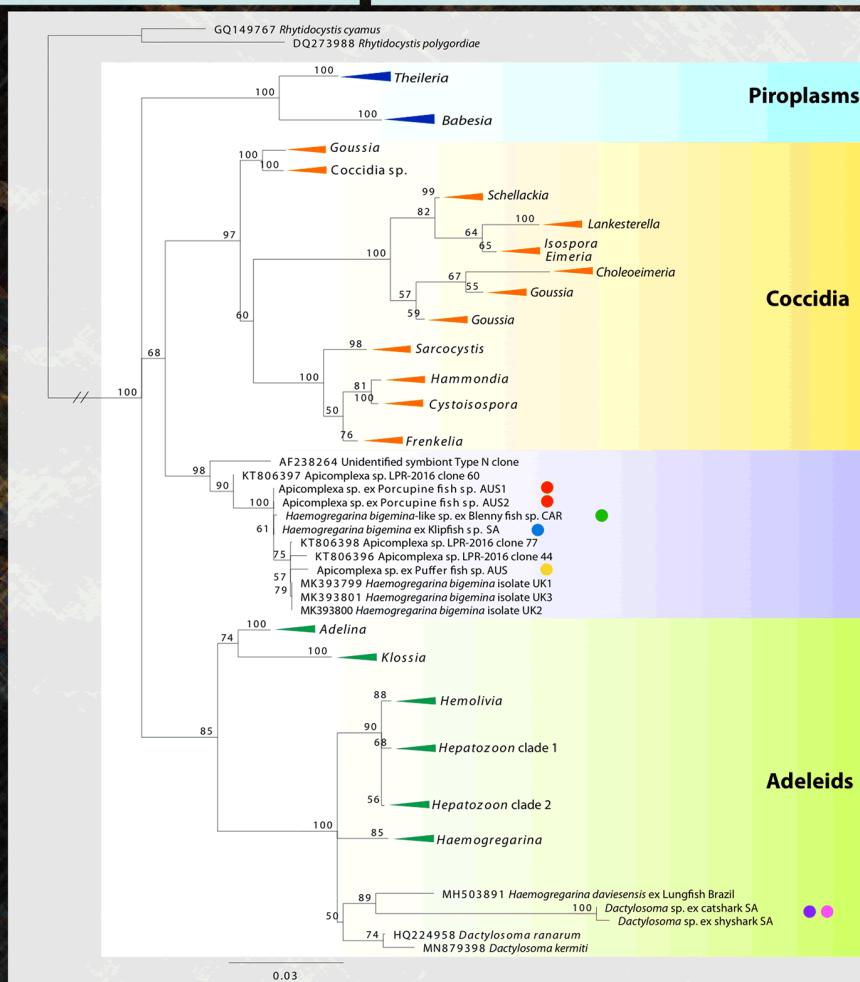


Fig. 2. Phylogenetic analysis of haemogregarines of fishes in comparison to other apicomplexans based on 18S rDNA sequences. Maximum Likelihood (ML) analysis. All comparative sequences downloaded from GenBank. Nodal support values >50% are shown. Colour codes correlate to those seen in Fig. 1.

DISCUSSION

Ever since life cycle stages of *H. bigemina* were identified in gnathiid isopods, instead of leeches (considered to be the vectors of this genus), the taxonomy of this parasite has been questioned, suggesting a potential new genus^{4,7}. The current phylogeny suggests this to be the case, but with the inclusion of other taxa, further suggests a potential new family. The perception of this species being cosmopolitan is also questioned, as according to the phylogenetic analysis and divergence values, the *H. bigemina*-like parasite from fishes of the Caribbean and *H. bigemina* from fishes from South Africa, suggest these three to be separate species, in turn questioning other reports of this species around the world. Unexpectedly, the haemogregarines from the Scyliorhinidae fell within the *Dactylosoma*. This thus represents the first sequence data for this genus from fishes and the first from sharks. It is apparent from the current research that further survey efforts are needed, increasing the robustness of the analyses, particularly for some genera. With these, a new family and potentially new genera are anticipated for parasites which at present are in a state of taxonomic limbo.

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ACKNOWLEDGEMENTS

National Research Foundation (NRF) of South Africa (NRF project IFR170210222411, NJ Smit, PI); NRF project RA161107208698, CA Cook, PI), the US National Science Foundation (grant number NSF OCE-121615 and OCE-1536794, PC Sikkell, PI), Puerto Rico Sea Grant (grant number R-31-1-14, PC Sikkell, PI), and the Falconwood Corporation. T. Cribb (ABRS), provision of samples from Australia. K. Hadfield-Malherbe (NWU) collection of samples. Opinions expressed, and conclusions arrived at, are those of the authors and are not necessarily those of the funding bodies.