

Phylogenetic relationships of *Hepatozoon* (*Haemogregarina*) *boigae*, *Hepatozoon* sp., *Haemogregarina clelandi* and *Haemoproteus chelodina* from Australian reptiles to other Apicomplexa based on cladistic analyses of ultrastructural and life-cycle characters

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SUMMARY

The phylogeny of representative haemozoan species of the phylum Apicomplexa was reconstructed by cladistic analyses of ultrastructural and life-cycle characteristics. The analysis incorporated 4 apicomplexans previously not included in phylogenetic reconstructions: *Haemogregarina clelandi* from the Brisbane River tortoise (*Emydura signata*), *Hepatozoon* sp. from the slaty grey snake (*Stegonotus cucullatus*), *Hepatozoon* (*Haemogregarina*) *boigae* from the brown tree snake (*Boiga irregularis*), and *Haemoproteus chelodina* from the saw-shelled tortoise (*Elseya latisternum*). There was no apparent correlation between parasite phylogeny and that of their vertebrate hosts, but there appeared to be some relationship between parasites and their intermediate hosts, suggestive of parasite/vector co-evolution.

Key words: Apicomplexa, cladistics, haemogregarines, ultrastructure.

INTRODUCTION

Within the phylum Apicomplexa, there are 3 groups of blood-borne protozoan parasites, including some species (8 genera) of the suborder Adeleorina, species of all genera of the suborder Haemosporina, and some species (2 genera, *Lankesterella* and *Schellackia*) of the suborder Eimeriorina. Blood-dwelling adeleorins and haemosporins are transmitted by a variety of haematophagous invertebrate vectors, including leeches in aquatic hosts and blood-sucking arthropods such as ticks, mites, fleas, lice, mosquitoes and sandflies in terrestrial hosts. Species of these 2 groups have complex 2 or 3 host life-cycles involving schizogony and gamogony that take place in the vertebrate intermediate host, and sporogony that occurs in the invertebrate definitive host (Levine, 1988).

The term haemogregarine is used to describe collectively blood parasites belonging to the suborder Adeleorina. Blood-dwelling adeleorin parasites comprise around 400 species belonging to 4 main genera; *Haemogregarina* Danilewsky, 1885, *Karyolysus* Labbe, 1894, *Hepatozoon* Miller, 1908 and *Cyrilia* Lainson, 1981. A fifth genus, *Hemolivia*, was described by Petit *et al.* (1990) and the genus *Desseria*

was erected by Siddall (1995) to accommodate piscine haemogregarines which lacked erythrocytic merogony. Another family of haemogregarines, the Dactylosomatidae, contains the genera *Dactylosoma* and *Babesiosoma*, both of which infect fish and reptiles (Barta & Desser, 1989). Leeches are the only known vectors for these genera and their vertebrate hosts so far have all been aquatic (Barta, 1991).

The Apicomplexan order Haemosporina Danilewsky, 1885 contains a range of blood parasites including *Leucocytozoon* and *Haemoproteus* which are common in avian hosts and *Plasmodium*, the causative agent of malaria in mammals (including humans), birds and lizards worldwide (Carreno, Martin & Barta, 1999). Other species of *Haemoproteus* occur in reptiles and appear to be well tolerated in their natural hosts.

The systematics of Apicomplexan parasites is controversial and subject to frequent revision, as complex life-cycle data are clarified (Siddall, 1995; Desser, Hong & Martin, 1995; Smith, 1996). The present study examined the phylogenetic relationships of representative species of blood-borne apicomplexan parasites based on cladistic analyses of ultrastructural and life-cycle data. The analysis incorporates 20 taxa for which sufficient biological data are available, and includes 4 species from Australian reptiles previously not included in such analyses.

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Table 1. Characters and character states used in the phylogenetic analyses

Character	Character states
1. Conoid	Present (1) absent (0)
2. Crystalloid bodies	Present (1) absent (0)
3. Amylopectin granules	Present (1) absent (0)
4. Mitochondria	Cristate (1) acristate (0)
5. Micropores	Present (1) absent (0)
6. Blood cells infected	Erythrocytes (0) leucocytes and erythrocytes (1)
7. Polar ring complex	Present (1) absent (0)
8. Life cycle	Monoxenous (0) heteroxenous (1)
9. Paranuclear bodies	Absent (0) present (1)
10. Merogony	Intracellular (0) extracellular (1)
11. Erythrocytic merogony	Absent (0) present (1)
12. Endodyogeny	Absent (0) present (1)
13. Gamonts	Crescentic (0) recurved (1) polymorphic (2)
14. Gamonts	Length to width ratio <3 (0) Length to width ratio >4 (1)
15. Definitive host (vector)	Gastropod (0) leech (1) mosquito (2) biting fly (3) flea (4) acarine (5)
16. Intermediate host	Gastropod mollusc (0) anuran (1) fish (2) turtle (3) lizard (4) snake (5) bird (6) mammal (7) variable (8)
17. Route of infection	Ingestion of infected vector (0) injection by infected vector (1)
18. Haemozoin pigment	Present (1) absent (0)

Table 2. Character matrix used in the analysis of apicomplexan organisms

	Character*																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Klossia helicina</i>	1	1	1	1	1	?	1	0	0	0	0	0	0	0	0	0	0	0
<i>Karyolysus lacertae</i>	1	1	1	1	1	0	1	1	0	0	1	?	0	0	5	4	0	0
<i>Desseria myoxocephali</i>	1	0	1	1	1	0	1	1	0	0	0	0	0	1	2	1	0	0
<i>Lankesterella minima</i>	1	1	1	1	1	1	1	1	1	?	0	0	0	0	1	1	1	0
<i>Babesia bigemina</i>	0	0	0	0	1	0	1	1	0	0	1	0	2	0	5	7	1	0
<i>Haemogregarina balli</i>	1	0	1	1	1	0	1	1	0	0	1	0	0	0	1	3	1	0
<i>Hepatozoon aegypti</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	1	2	1	0	0
<i>H. breinli</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	0	2	4	0	0
<i>H. catesbiana</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	1	2	1	0	0
<i>H. erhardovae</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	0	4	7	0	0
<i>H. gracilis</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	1	2	4	0	0
<i>H. griseisciuri</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	0	5	7	0	0
<i>H. lygosomarum</i>	1	1	1	1	1	0	1	1	0	0	0	0	1	0	5	4	0	0
<i>Hemolivia mauritanicum</i>	1	1	1	1	1	0	1	1	0	0	0	0	1	0	5	3	0	0
<i>H. rarefaciens</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	0	2	5	0	0
<i>H. sipedon</i>	1	1	1	1	1	0	1	1	0	0	0	0	0	1	2	5	0	0
<i>Haemoproteus columbae</i>	1	1	0	1	1	0	1	1	0	0	0	0	2	0	3	6	1	1
<i>Leucocytozoon simondi</i>	1	1	0	1	1	1	1	1	0	0	0	0	2	1	3	6	1	0
<i>Schellackia ptyodactylii</i>	1	1	1	1	1	1	1	1	1	0	0	0	0	0	2	4	1	0
<i>Plasmodium vivax</i>	1	1	0	1	1	0	1	1	0	0	1	0	2	0	2	8	1	1
<i>Haemogregarina clelandi</i>	1	0	1	1	1	0	1	1	0	0	1	1	0	0	1	3	1	0
<i>Hepatozoon</i> sp.	1	1	1	1	1	0	1	1	0	0	0	1	0	0	5	5	0	0
<i>Haemoproteus chelodina</i>	1	1	0	1	1	0	1	1	0	0	0	0	2	0	1	3	1	1
<i>Hepatozoon boigae</i>	0	0	1	0	?	0	0	1	0	0	0	0	2	0	5	5	0	0

* ?, Data missing.

METHODS

Phylogeny and character evolution amongst species of apicomplexan parasites was investigated using cladistic analyses of ultrastructural, biological and life-cycle characteristics. Character state information for taxa included in the analysis were obtained

from Bradbury & Gallucci (1971), Barta (1989), Smith & Desser (1997) and Smith *et al.* (2000). *Klossia helicina* Schneider, 1975 was used for out-group comparison, as these monoxenous organisms have been considered to be representative of ancestral adeleorins (Smith *et al.* 2000). Other Apicomplexa included in the analyses were *Karyolysus*

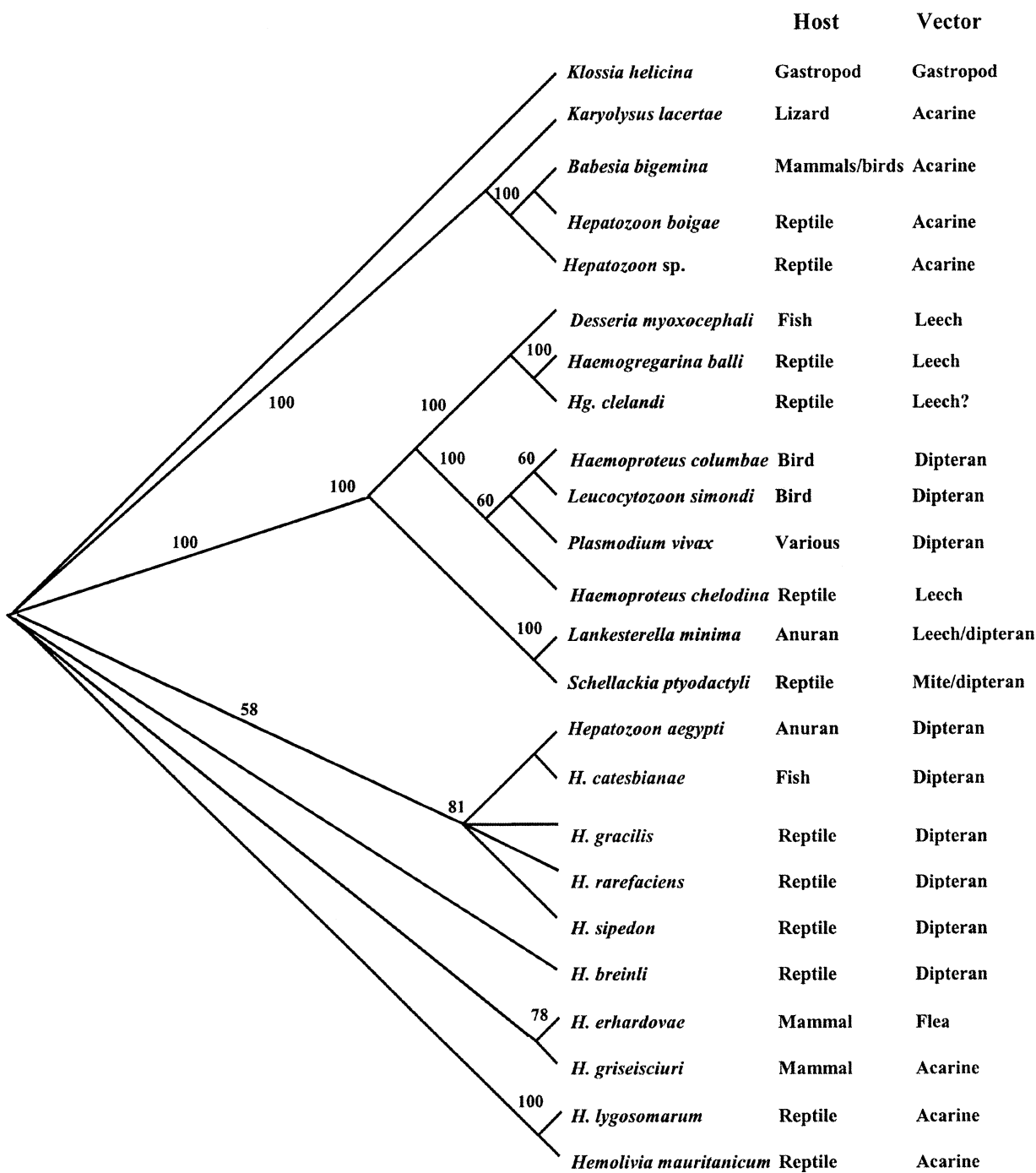


Fig. 1. Phylogenetic relationships between 24 blood-borne apicomplexan parasites based on morphological and life-cycle data. The heuristic search option within PAUP 3.1 was used to generate a majority rule consensus tree from 24 possible tree topologies. The numbers above each node represent the percentage of the 24 equally parsimonious trees supporting each clade. Characters 15 and 16 (definitive and intermediate hosts respectively) were not included in this analysis as they are deemed to be uninheritable characters.

lacertae, *Desseria myoxocephali*, *Lankesterella minima*, *Babesia bigemina*, *Haemogregarina balli*, *Hepatozoon aegypti*, *H. breinli*, *H. catesbiana*, *H. domerguei*, *H. erhardovae*, *H. gracilis*, *H. griseisciuri*, *H. lygosomarum*, *H. mauritanicum*, *H. mocassini*, *H. rarefaciens*, *H. sipedon*, *Haemoproteus columbae*, *Leucocytozoon simondi*, *Schellackia ptyodactyli* and *Plasmodium vivax*. New taxa included in the analyses were *Haemogregarina clelandi* from the Brisbane

River tortoise (*Emydura signata*), *Hepatozoon sp.*, from the slaty grey snake (*Stegonotus cucullatus*), *Haemoproteus chelodina* from the saw-shelled tortoise (*Elseya latisternum*), and *Hepatozoon boigae* from the brown tree snake (*Boiga irregularis*). The organisms isolated from brown tree snakes were considered to belong to the genus *Hepatozoon*, rather than *Haemogregarina* as originally described by Mackerras (1961) on the basis of the presence of merogony in

endothelial cells rather than erythrocytes (*cf.* Siddall, 1995).

The characters and states used in the analyses are shown in Table 1, and the scored matrix in Table 2. Character states were scored directly from transmission electron micrographs or were inferred from biologically valid features.

The heuristic option of PAUP 3.1 was used to search for the most parsimonious tree(s), with polarity of character state changes established using *K. helicina*, a member of the suborder Adeleorina, as the outgroup. All characters introduced to the matrix were unordered, as evolutionary origins of organisms within the group have yet to be determined. Nonetheless, absence was considered more primitive to presence (especially for organelles and developmental cycles), and character states have been coded accordingly. Although characters 15 and 16 (definitive and intermediate hosts respectively) are uninherited characters, they were included in the analysis *a posteriori* to look at potential changes in tree topology.

RESULTS

Parsimony analysis of 24 species of blood-borne apicomplexan parasites yielded 24 equally parsimonious trees consisting of 26 steps. Two of the new species included in this analysis, *Hepatozoon boigae* and *Hepatozoon* sp., associated closely with *Babesia bigemina* and *Karyolysus* respectively to the exclusion of other species of *Hepatozoon*, including those using acarine vectors (Fig. 1). Paraphyly of other species of *Hepatozoon* was evident. The *Haemogregarina* species in the study, *H. clelandi* and *H. balli* formed a clade with *Desseria myoxocephali*, whilst *Haemoproteus chelodina* grouped with the other haemosporidia. The inclusion of definitive hosts (character 15) in the analysis did not change the tree topology, whilst inclusion of the intermediate host (character 16) increased the number of possible trees to 55, with 34 steps.

DISCUSSION

Paraphyly of the biologically and geographically diverse genus *Hepatozoon* has been suspected for more than 30 years (Ball & Oda, 1971), with species regularly being transferred into (Nadler & Miller, 1984; Desser *et al.* 1995; Siddall, 1995; Smith, 1996) and out of (Landau & Paperna, 1997) the genus *Hepatozoon*, following the elucidation of complex life-cycle data.

The phylogenetic placement of the 2 previously undescribed species of *Hepatozoon* in this cladistic analysis is further evidence of paraphyly of the genus *Hepatozoon* as noted by other authors (Smith & Desser, 1997; Mathew *et al.* 2000), and lends support to the proposal by Smith & Desser (1997), that

Hepatozoon could be partitioned into at least 2 genera. The *Hepatozoon* species isolated from the slaty grey snake, *Stegonotus cucullatus*, and the brown tree snake, *Boiga irregularis*, consistently formed a strongly-supported clade with *Babesia bigemina* and *Karyolysus* rather than with other species of *Hepatozoon*. Biological similarities between piroplasms and other adeleorin parasites, particularly *Karyolysus*, have been noted previously (Barta, 1989; Siddall & Desser, 1991). Molecular analyses of rRNA genes have also inferred sister groupings between species of *Babesia* and *Hepatozoon* (Lang-Unnasch *et al.* 1998) and between *Hepatozoon* and species of *Plasmodium* (Carreno *et al.* 1999; Barta *et al.* 2001). However, this latter association was not supported in a recent phylogenetic analysis of small subunit rRNA genes by Perkins & Keller (2001).

The apparent associations between *Hepatozoon* sp. and *H. boigae* from Australian reptiles with *B. bigemina* and *Karyolysus* in this analysis suggest evolutionary relationships between parasites using acarine definitive hosts, rather than between the diverse range of intermediate hosts which are infected by these genera (as found by Barta, 1989).

The haemosporidia in this cladistic analysis formed a well-supported clade, with the tortoise parasite, *Haemoproteus chelodina*, aligning closely with the avian organisms *Leucocytozoon* and *Haemoproteus columbae*, and with the more cosmopolitan organism, *Plasmodium vivax*. The definitive hosts for *H. chelodina* have not been definitively established, although leeches are common on the tortoises despite their long basking periods spent out of water. The sister group to the haemosporidia in this analysis were *Haemogregarina* species. This group included *Haemogregarina clelandi* from the freshwater tortoise *Emydura signata*, *H. balli* from the snapping turtle, *Chelydra serpentina* (Paterson & Desser, 1976) and *Desseria myoxocephali*, parasitic in fish (Siddall, 1995). The definitive hosts for these 3 apicomplexan species are leeches. Again it appears more likely that the evolutionary links are between the vectors of these parasites rather than between their very diverse range of vertebrate hosts, although further morphological and molecular analysis will be helpful in resolving evolutionary associations.

It is becoming increasingly evident that a combination of ultrastructural, life-cycle and molecular analyses involving increased numbers of related taxa are required before the systematics of the Apicomplexa can be satisfactorily resolved.

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