Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2018, 65: 004
doi: 10.14411/fp.2018.004

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Two new species of *Hepatozoon* (Apicomplexa: Hepatozoidae) parasitising species of *Philothamnus* (Ophidia: Colubridae) from South Africa

Courtney Antonia Cook[1,2], **Edward Charles Netherlands**[2,3], **Johann van As**[1] and **Nico Jacobus Smit**[2]

[1] Department of Zoology and Entomology, University of the Free State, Qwaqwa Campus, Phuthaditjhaba, South Africa;

[2] Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa;

[3] Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Leuven, Belgium

Abstract: To date, only a few species of *Hepatozoon* Miller, 1908 have been described from amphibians and reptiles of South Africa, including two species from anuran hosts, three from saurians, one from chelonians, and two from ophidians. *Hepatozoon bitis* (Fantham, 1925) and *Hepatozoon refringens* (Sambon et Seligmann, 1907), parasitising *Bitis arientans* (Merrem) and *Pseudoaspis cana* (Linnaeus), respectively, were described in the early 1900s and since then there have been no further species of *Hepatozoon* described from snakes in South Africa. Blood smears, used in peripheral blood haemogregarine stage morphometrics, and whole blood used in molecular characterisation of haemogregarines were collected from the caudal vein of six snakes of three species, namely *Philothamnus hoplogaster* (Günther), *Philothamnus semivariegatus* (Smith) and *Philothamnus natalensis natalensis* (Smith). For comparison, a comprehensive table summarising available information on species of *Hepatozoon* from African snakes is presented. Haemogregarines found infecting the snakes from the present study were morphologically and molecularly different from any previously described from Africa and are thus here described as *Hepatozoon angeladaviesae* sp. n. and *Hepatozoon cecilhoarei* sp. n. Both haemogregarine species were observed to cause considerable dehaemoglobinisation of the host cell, in case of infection with *H. angeladaviesae* resulting in a characteristic peripheral undulation of the host cell membrane and karyorrhexis. To the authors' knowledge, these are the first haemogregarines parasitising snakes of the genus *Philothamnus* Smith described using both morphological and molecular characteristics in Africa.

Keywords: serpents, snakes, haemogregarines, phylogeny, adeleorid taxonomy, 18S rDNA, haemoparasites

Haemogregarines of the genus *Hepatozoon* Miller, 1908 (Hepatozoidae) are intraerythrocytic or intraleucocytic apicomplexan parasites that are frequently described from amphibian and reptilian hosts (Smith 1996, Cook et al. 2014a). However, as highlighted by Borges-Nojosa et al. (2017), the diversity and systematics of these apicomplexans are still poorly understood. The genus *Hepatozoon* is paraphyletic based on estimated relationships using 18S rRNA gene sequences, the genus *Karyolysus* Labbé, 1894, and in some analyses *Hemolivia* Petit, Landau, Baccam et Lainson, 1990, as well, forming a lineage within *Hepatozoon* (see Barta et al. 2012, Haklová-Kočíková et al. 2014, Kvičerová et al. 2014, Cook et al. 2016).

Recently, a new genus, *Bartazoon* Karadjian, Chavatte et Landau, 2015, was erected as part of a taxonomic revision to try resolve the phylogeny and associated taxonomy of these haemogregarines, the new genus including species from reptiles that were previously included in *Hepatozoon* (see Karadjian et al. 2015). However, the monophyly of *Bartazoon* is not well supported and with all the molecular evidence provided for by the use of a single gene it is at this time premature to consider such taxonomic changes (Maia et al. 2016, Borges-Nojosa et al. 2017). Thus, following Borges-Nojosa et al.'s (2017) recommendation we will conservatively persist in referring to species parasitising reptiles as *Hepatozoon*.

To date, only a handful of species of *Hepatozoon* have been described from amphibians and reptiles of South Africa. From amphibians these include *Hepatozoon theileri* (Laveran, 1905) infecting the common river frog *Amietia delalandii* (Bocage) (Pyxicephalidae), *Hepatozoon ixoxo* Netherlands, Cook et Smit, 2014 infecting typical toads *Sclerophrys pusilla* (Hallowell), *Sclerophrys garmani* (Meek) and *Sclerophrys gutturalis* (Power) (Bufonidae), and recently *Hepatozoon involucrum* Netherlands, Cook et Smit, 2017 infecting *Hyperolius marmoratus* Rapp, *Hepatozoon tenuis* Netherlands, Cook et Smit, 2017 infecting *Afrixalus fornasinii* (Bianconi), *Hyperolius argus* Peters

Address for correspondence: C.A. Cook, Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom Campus, Potchefstroom, 2531, South Africa. Phone: +27 182992493; E-mail: apicomplexan@yahoo.co.za

Zoobank number for article: urn:lsid:zoobank.org:pub:4D0DB4B5-213A-46A0-93EF-C299E266104F

and *Hyp. marmoratus* and *Hepatozoon thori* Netherlands, Cook et Smit, 2017 infecting *Hyp. marmoratus*, *Hyp. argus* and *Hyperolius puncticulatus* (Pfeffer). From reptiles these include *Hepatozoon langii* Van As, Davies et Smit, 2013, and *Hepatozoon vacuolatus* Van As, Davies et Smit, 2013, from crag lizards *Pseudocordylus langi* Loveridge (Cordylidae), *Hepatozoon affluomaloti* Van As, Davies et Smit, 2015 from crag lizards *Pseudocordylus melanotus* Smith, and *Pseudocordylus subviridis* (Smith) (Cordylidae), *Hepatozoon varani* (Laveran, 1905) from monitor lizards *Varanus niloticus* (Linnaeus) (Varanidae), and *Hepatozoon fitzsimonsi* (Dias, 1953) from five species of terrestrial chelonians including *Chersina angulata* (Schweigger), *Kinixys lobatsiana* Power, *Kinixys natalensis* Hewitt, *Kinixys zombensis* Hewitt and *Stigmochelys pardalis* (Bell) (Testudinidae) (see Laveran 1905, Dias 1953, Smith 1996, Cook et al. 2009, 2014a, 2016, Van As et al. 2013, 2015, Netherlands et al. 2014a,b, 2018).

Even though numerous species of *Hepatozoon* have been described from snakes throughout Africa from various families including Colubridae, Elapidae, Lamprophiidae, Natricidae, Pythonidae and Viperidae (Table 1), the only species of *Hepatozoon* described to date from South African snakes are *Hepatozoon bitis* (Fantham, 1925) described from *Bitis arietans* (Merrem) (Viperidae) and *Hepatozoon refringens* (Sambon and Seligmann, 1907) from *Pseudoaspis cana* (Linnaeus) (Lamprophiidae) (Sambon and Seligmann 1907, Fantham 1925).

Within the Colubridae, snakes of the genus *Philothamnus* Smith, frequently formed part of haemoparasite surveys in Africa, these reporting infections of potentially several species of haemogregarines. However, regardless of the sometimes detailed descriptions of these *Hepatozoon* spp., none were ever named (Bouet 1909, Hoare 1920, Schweitz 1931, Garnham and Duke 1953, Ball 1967, Haklová et al. 2014).

As part of a larger project focusing on haemoparasites of South African reptiles and amphibians, two different types of *Hepatozoon* were found in the peripheral blood of three species of *Philothamnus*. Thus, this paper presents the first formal description of species of *Hepatozoon* parasitising species of *Philothamnus* in South Africa based on both morphological description and molecular characterisation.

MATERIALS AND METHODS

Snake collection and blood preparation

A total of six snakes were collected, including two *Philothamnus hoplogaster* (Günther), two *Philothamnus natalensis natalensis* (Smith) and two *Philothamnus semivariegatus* (Smith), in the Ndumo Game Reserve, KwaZulu-Natal (32°18'49"E; 26°54'33"S) (see fig. 1 in Netherlands et al. 2015) from 2014−2016 (Permit no. OP 839/2014). Blood from the caudal vein was aspirated into a sterile 1 ml insulin syringe. Thin blood smears were prepared, air-dried, fixed for 10 min in absolute methanol and stained using a modified solution of Giemsa stain (Sigma-Aldrich, Steinheim, Germany) for 20 min following Cook et al. (2014a, 2016). A small volume (< 0.5 ml) of blood from each specimen was also dropped into molecular grade 70% ethanol (Sigma-Aldrich) for molecular analyses.

Blood screening

Smears were screened using a 100× oil immersion objective, and micrographs and measurements of parasites were taken on a calibrated Nikon Eclipse E800 compound microscope (Nikon, Amsterdam, Netherlands) using the Nikon NIS-Elements microscope imaging software program D3.2 (Nikon). All measurements are in micrometres (µm) unless otherwise indicated. Parasitaemia was calculated per 100 erythrocytes, with ~10^4 erythrocytes examined per blood smear (Cook et al. 2014a, 2016).

DNA extraction, PCR and phylogenetic analysis of 18S rDNA

Whole blood from one *P. hoplogaster*, one *P. semivariegatus* and one *P. n. natalensis*, representing microscopically-identified infections of a single morphotype, along with whole blood from one *P. n. natalensis* representing a microscopically-identified infection of both morphotypes, were used for DNA extraction following the standard protocol method for human or animal tissue and cultured cells as detailed in the NucleoSpin®Tissue Genomic DNA Tissue Kit (Macherey-Nagel, Duren, Germany).

Molecular characterisation of parasites was performed via PCR amplification, amplifying approximately the full 18S rRNA gene in two fragments by using a combination of primer sets. The first fragment, approximately 930 nt in length, was amplified using primer set HAMF 5'-GCCAGTAGTCATATGCTTGTC-3' (Criado-Fornelio et al. 2006) and HepR900 5'-CAAATCTAAGAATTTCACCTCTGAC-3' (Ujvari et al. 2004). The second fragment, approximately 1,400 nt in length, was amplified using primer set HepF300 5'-GTTTCTGACCTATCAGCTTTCGACG-3' (Ujvari et al. 2004) and 2868 5'-TGATCCTTCTGCAGGTTCAC-3' (Medlin et al. 1988, Mathew et al. 2000).

Conditions for PCR of both fragments were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles, entailing a 95 °C denaturation for 30 s, annealing at 61 °C for 30 s with an end extension at 72 °C for 2 min, and following the cycles a final extension of 72 °C for 10 min (Netherlands et al. 2018).

All PCR reactions were performed with volumes of 25 µl, using 12.5 µl Thermo Scientific DreamTaq PCR master mix (2×) (2× DreamTaq buffer, 0.4 mM of each dNTP, and 4 mM $MgCl_2$), 1.25 µl of each primer (10 µM), and at least 25 ng of DNA. PCR grade nuclease free water (Thermo Scientific, Vilnius, Lithuania) was used to make up final reaction volume. Reactions were undertaken in a Bio-Rad C1000 Touch™ Thermal Cycler PCR machine (Bio-Rad, Hemel Hempstead, UK). An agarose gel (1%) stained with gel red was used to visualise resulting amplicons under UV light.

Two PCR products from each sample were sent to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa) for purification and sequencing in both directions. Quality of resultant sequences was assessed using Geneious Ver. 7.1 (http://www.geneious.com, Kearse et al. 2012) before consensus sequences were generated from both forward and reverse sequence reads for both fragments. A consensus sequence was then generated from both fragments, with an overlap of ~600 nt (with 100% identity). Sequences were identified

Table 1. Haemogregarines of the genus *Hepatozoon* Miller, 1908 (Apicomplexa: Hepatozoidae) described from snakes from Africa. Parasite species and authorities, snake host (family), localities (type and other), mature gamont and gamont nucleus description (measurements in μm), and references are provided.

Parasite species and authority	Snake host (family)	Locality	Description (gamont; nucleus)	References
Hepatozoon aegypti Bashtar, Boulos, et Mehlhorn, 1984	*Spalerosophis diadema* (Schlegel) (Colubridae)	Type: Egypt	19–21.5 × 1.7–2.8; -	Bashtar et al. 1984, Morsy et al. 2013
Hepatozoon algiri (Manceaux, 1908)	*Platyceps* sp. (Colubridae)	Type: Algeria	19 × 4; -	Manceaux 1908
Hepatozoon angeladaviesae sp. n.	*Philothamnus semivariegatus* (Smith), *Philothamnus hoplogaster* (Günther), *Philothamnus natalensis natalensis* (Smith) (Colubridae)	Type: South Africa	14.1−17.3 × 4.8−6.5; 3.6−5.7 × 3.7−5.8	Present study
Haemogregarina arabi Ramadan, 1974 (likely *Hepatozoon arabi*)	*Telescopus dhara* (Forskal) (Colubridae)	Type: Egypt	11–18 × 3–5.5	Mohammad et al. 1996
Haemogregarina aswanensis Mohammad, Ramdan, Mohammed et Fawzi, 1996 (likely *Hepatozoon aswanensis*)	*Naja haje* (Linnaeus) (Elapidae)	Type: Egypt	12.5−17.5 × 2.5−6; 3.7 × 2.6	Mohammad et al. 1996
Hepatozoon ayorgbor Sloboda, Kamler, Bulantová, Votýpka et Modrý, 2007	*Python regius* (Shaw) (Pythonidae)	Type: Ghana	11–13 × 2–3.5; 4–6.5 × 1.5–2	Sloboda et al. 2007
Hepatozoon bitis (Fantham, 1925)	*Bitis arietans* Merrem (Viperidae)	Type: South Africa	12.5–14 × 3–4; -	Fantham1925, Hoare 1932, Smith 1996, Sloboda et al. 2007
Hepatozoon boodoni (Phisalix, 1914)	*Boaedon fuliginosus* (Boie) (Lamprophiidae)	Type: Sudan	14–15 × 2–3, 14–15 × 7 ; 5	Phisalix 1914, Smith 1996, Sloboda et al. 2007
Hepatozoon brendae (Sambon et Seligmann, 1907)	*Psammophis sibilans* (Linnaeus) (Lamprophiidae)	Type: Tropical Africa and Egypt	16–17 × 3–4; 5−6 × 3−4	Sambon and Seligmann 1907, Smith 1996, Sloboda et al. 2007
Hepatozoon cecilhoarei sp. n.	*Philothamnus natalensis natalensis*[1], *Philothamnus hoplogaster*[1], *Philothamnus* sp.[2] (Colubridae)	Type: South Africa[1] Other: Uganda[2]	13.1−15.9 × 2.1−2.7; 4.2−5.8 × 1.2−1.6	Present study[1], Hoare 1920[2]
Hepatozoon crotaphopeltis (Hoare, 1932)	*Crotaphopeltis hotamboeia* (Laurenti) (Colubridae)	Type: Uganda	20 × 2; -	Hoare 1932, Smith 1996, Sloboda et al. 2007
Hepatozoon dogieli (Hoare, 1920)	*Bitis gabonica* Duméril, Bibron et Duméril (Viperidae)	Type: Uganda	14 × 6; -	Hoare 1932, Smith 1996, Sloboda et al. 2007
Hepatozoon enswerae (Hoare, 1932)	*Naja melanoleuca* Hallowell (Elapidae)	Type: Uganda	19 × 3, slender vermicular 15 × 3.8, bean-shaped; -	Hoare 1932, Smith 1996, Sloboda et al. 2007
Hepatozoon garnhami (Mohammad, Ramdan, Mohammed et Fawzi, 1996)	*Psammophis aegyptius* Marx (Lamprophiidae)	Type: Egypt	15−20 × 1.5−2.5; 5−9 × 1.5−3	Mohammad et al. 1996, Abdel-Baki et al. 2014
Hepatozoon joannoni (Hagenmuller, 1898)	*Macroprotodon cucullatus* (Geoffroy-St-Hilaire) (Colubridae)	Type: South Europe or North Africa	12–18 in length; -	Sambon and Seligmann 1907, Smith 1996
Hepatozoon malpoloni (Ramadan, 1974)	*Malpolon monspessulanus* (Hermann) (Lamprophiidae)	Type: Egypt	12–25 × 3–5.5; -	Mohammad et al. 1996, Smith 1996
Hepatozoon matruhensis Shazly, Ahmed, Bashtar et Fayed, 1994	*Psammophis schokari* (Lamprophiidae)	Type: Egypt	18–28 × 2.5–6; -	Mohammad et al. 1996, Smith 1996
Hepatozoon mehlhorni Bashtar, Abdel-Ghaffar et Shazly, 1991	*Echis carinatus* (Schneider) (Viperidae)	Type: Egypt	17.2 × 5.4; 6.3 × 5.4	Bashtar et al. 1991, Smith 1996, Morsy et al. 2013
Hepatozoon minchini (Garnham, 1950)	*Crotaphopeltis degeni* (Boulenger) (Colubridae)	Type: Kenya	13−14 × 3−4; -	Garnham 1950, Smith 1996, Sloboda et al. 2007
Hepatozoon musotae (Hoare, 1932)	*Boaedon* sp. Duméril, Bibron et Duméril (Lamprophiidae)	Type: Uganda	17 × 3.8–4.7, bean-shaped 15.2 × 6.6, broad forms; -	Hoare 1932, Smith 1996, Sloboda et al. 2007
Hepatozoon najae (Laveran, 1902)	*Naja naja* (Linnaeus)[1], *Naja nigricollis* Reinhardt[2,4], *Naja haje*[3] (Elapidae)	Type: India[1] Other: Egypt[2], Kenya[3], Tanzania[4]	14 × 3 (folded), 21−22 × 3 (when not folded1); -	Laveran 1902[1], Ball 1967, Bashtar and Abdel-Ghaffar 1987[2], Smith 1996, Telford 2009[4]
Hepatozoon refringens (Sambon et Seligmann, 1907)	*Pseudaspis cana* (Linnaeus) (Lamprophiidae)	Type: South Africa	10–12 × 5–6; -	Sambon and Seligmann 1907, Smith 1996
Hepatozoon robertsonae (Sambon, 1909)	*Python regius*, *Python sebae* (Gmelin) (Pythonidae)	Type: Gambia	12−16 in length; -	Sambon and Seligmann 1907, Sloboda et al. 2007
Hepatozoon sebai (Laveran et Pettit, 1909)	*Python sebae* (Pythonidae)	Type: Senegal	11−13 × 2 (folded), 17−18 × 2 (when not folded); -	Laveran and Pettit 1909, Smith 1996
Hepatozoon seurati (Laveran et Pettit, 1911)	*Cerastes cerastes* Linnaeus (Viperidae)	Type: Algeria Other: Egypt	12−16.5 × 2−3.5; 4.5 × 3.5	Laveran and Pettit 1911, Smith 1996, Morsy et al. 2013
Haemogregarina vaughani Balfour, 1908 (likely *Hepatozoon vaughani*)	*Rhamphiophis rubropunctatus* (Fischer) (Lamprophiidae)	Type: Sudan	15 × 4.5; -	Balfour 1908
Hepatozoon viperini (Billet, 1904)	*Natrix maura* (Linnaeus) (Natricidae)	Type: Algeria	None provided	Billet 1904, Smith 1996, Sambon and Seligmann 1907
Hepatozoon vubirizi (Hoare, 1932)	*Gonionotophis savorgnani* (Mocquard) (Lamprophiidae)	Type: Uganda	15–17 × 3.8–4.7; -	Hoare 1932, Smith 1996, Sloboda et al. 2007

Continued.

Table 1. Continued.

Parasite species and authority	Snake host (family)	Locality	Description (gamont; nucleus)	References
Hepatozoon zambiensis (Peirce, 1984)	*Dispholidus typus* (Smith) (Colubridae)	Type: Zambia	14.9–17.8 × 2.4–5.7; 4.4−8.4 × 2.2−4.1	Peirce 1984, Smith 1996, Sloboda et al. 2007
Hepatozoon zamenis (Laveran, 1902)	*Hemorrhois hippocrepis* (Linnaeus) (Colubridae)	Type: Algeria	18 × 4; -	Laveran 1902, Sambon and Seligmann 1907, Smith 1996
Hepatozoon zumpti (Dias, 1952)	*Dendroaspis polylepis* Günther (Elapidae)	Type: Mozambique	14.3−16.3 × 4.3−5.3; 4.8−5.8 × 3.8−4.5	Dias 1952, Smith 1996

using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/), and deposited in the NCBI GenBank database under the accession numbers: MG519501−MG519504.

For the phylogenetic analysis comparative sequences of species of *Hemolivia*, *Hepatozoon* and *Karyolysus*, with *Haemogregarina balli* Paterson et Desser, 1976 (GenBank: HQ224959) as outgroup, were downloaded from GenBank and aligned to the sequences generated within this study. Sequences were aligned using the MUSCLE alignment tool (Edgar 2004) implemented in Geneious Ver. 7.1. The alignment consisted of 34 sequences and was 945 nt long. To infer phylogenetic relationships of the aligned dataset both Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used.

A model test was performed to determine the most suitable nucleotide substitution model, according to the Akaike information criterion using jModelTest 2.1.7 (Guindon and Gascuel 2003, Darriba et al. 2012). The best model identified was the General Time Reversible model with estimates of invariable sites and a discrete Gamma distribution (GTR + I + Γ). The ML analysis was performed using RAxML Ver. 7.2.8 implemented from within Geneious 7.1. The alphaparameter selected was the GTR GAMMA I model, with support assessed using 500 rapid bootstrap inferences. The BI analysis was implemented from within Geneious 7.1 using MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001).

The analysis was run twice over 10 million generations for the Markov Chains Monte Carlo (MCMC) algorithm. The Markov chain was sampled every 100 cycles, and the MCMC variant contained 4 chains with a temperature of 0.2. The log-likelihood values of the sample point were plotted against the generation time and the first 25% of the trees were discarded as 'burn-in' with no 'burn-in' samples being retained. Results were visualised in Trace (implemented from within Geneious) to assess convergence and the 'burn-in' period. As the topologies for both the ML and BI trees were identical resulting trees were combined in a 50% majority consensus tree.

To compare sequences of isolates of *Hepatozoon* representing fragments of the ~860−1,725 nt region of the 18S rRNA gene from other African snakes, an uncorrected pair-wise distances (p-distance) matrix was used. Comparative sequences (KC800702, KC800703, KC866369, KC866368, KC866370, KC800704, JQ746622) were downloaded from GenBank and aligned to the sequences generated within this study. Sequences were aligned using the MUSCLE alignment tool (Edgar 2004) implemented in Geneious Ver. 7.1. and manually trimmed. The alignment consisted of nine sequences and was 736 nt long when imported into the MEGA7 bioinformatics software program (Kumar et al. 2016) in which the matrix was produced.

Ethics statement. This study received the relevant ethical approval (North-West University ethics approval: NWU-00005-14-S3, NWU-00372-16-A5).

RESULTS

General observations for peripheral blood developmental stages of *Hepatozoon* species Fig. 1

All six individuals, two each, of *Philothamnus hoplogaster*, *P. semivariegatus* and *P. n. natalensis* from Ndumo Game Reserve, KwaZulu-Natal, were found to be parasitised by two morphologically dissimilar species of haemogregarines (Fig. 1). The first species, *Hepatozoon* sp. morphotype A, was found parasitising one *P. hoplogaster* (parasitaemia 0.01%), both *P. semivariegatus* (parasitaemia 0.5% and 5%, respectively) and one *P. n. natalensis* (parasitaemia 3%). This latter infection in *P. n. natalensis* was a co-infection with the second haemogregarine morphotype, *Hepatozoon* sp. morphotype B (parasitaemia 0.01%). Only *Hepatozoon* sp. morphotype B was found parasitising the second specimen of *P. hoplogaster* (parasitaemia 0.2%) and the second *P. n. natalensis* (parasitaemia 1%). Intraerythrocytic mature gamonts were found to be the only stages observed within the peripheral blood from all snake specimens and caused notable host cell alterations. The infection with *Hepatozoon* sp. morphotype A resulted in a cytopathology characterised by an enlarged and dehaemoglobinised host cells with a 'wafer-thin' undulating cell membrane, whilst the infection with *Hepatozoon* sp. morphotype B was characterised by the parasitised host cell's elongation and narrowing.

No potential vectors, such as mosquitoes or ticks, were found feeding on the snakes.

Molecular identification and phylogenetic analysis

Amplicons of between 1,540 nt and 1,713 nt of the 18S rRNA gene were obtained for both species of *Hepatozoon* identified by microscopy. *Hepatozoon* sp. morphotype A was amplified from one *P. hoplogaster* (1,540 nt), one *P. semivariegatus* (1,540 nt) and one *P. n. natalensis* (1,540 nt) (the specimen representing a co-infection with both morphotypes). All three amplicons were identical from which a consensus sequence was made (1,540 nt). *Hepatozoon* sp. morphotype B was amplified from one *P. n. natalensis* (1,713 nt) (the specimen representing an infection with only morphotype B). Both the phylogenetic analysis (Fig. 2) and the evolutionary divergence estimates (Table 2) differentiate *Hepatozoon* sp. morphotype A and

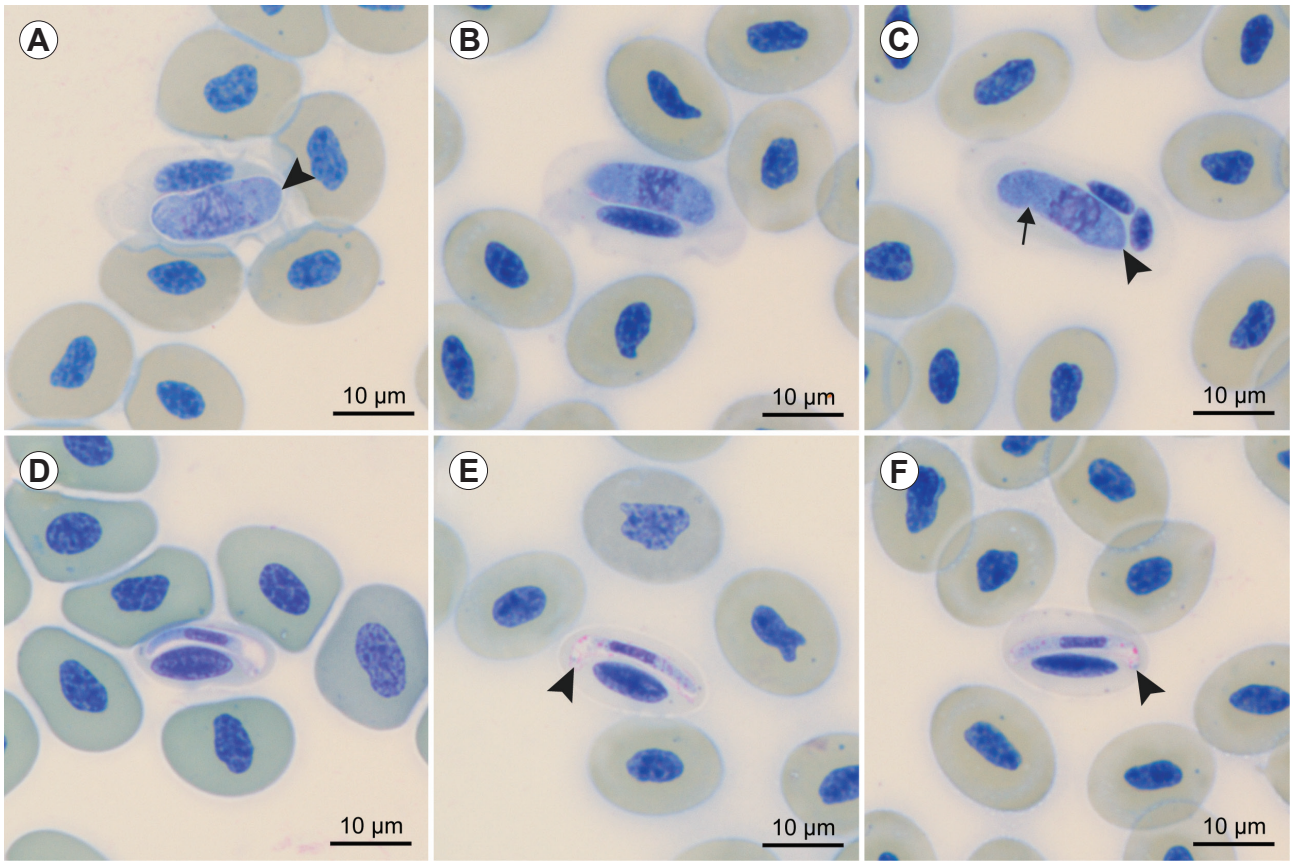

Fig. 1. Peripheral blood stages of two species of *Hepatozoon* Miller, 1908 parasitising species of *Philothamnus* Smith. Mature gamonts, lying singly, within a parasitophorous vacuole, within mature erythrocytes of (A–C) *Hepatozoon angeladaviesae* sp. n. (NMB P 440) parasitising *Philothamnus semivariegatus* (Smith), and (D–F) *Hepatozoon cecilhoarei* sp. n. (NMB P 441) parasitising *Philothamnus natalensis natalensis* (Smith), both from Ndumo Game Reserve, KwaZulu-Natal. **A**–**C** – gamont straighter than curved, tapering to a point at one pole (arrowhead), the other pole rounded, sometimes appearing folded (arrow), the parasitophorous vacuole appearing as a halo-like sheath; **D**–**F** – gamont elongated and curved, parasitophorous vacuole evident, appearing noticeably larger than the gamont with one pole curved into a hook (arrowhead). In both *H. angeladaviesae* sp. n. (A–C) and *H. cecilhoarei* sp. n. (D–F), the host cell nucleus has been displaced and condensed, the gamont of *H. angeladaviesae* sp. n. causing karyolysis of the host cell nucleus (C). Both haemogregarine species cause dehaemoglobinisation, *H. angeladaviesae* sp. n. characteristically causing the 'wafer-thin' undulation of the host cell membrane (A–C).

Hepatozoon sp. morphotype B as separate species, which are described below as *Hepatozoon angeladaviesae* sp. n. and *Hepatozoon cecilhoarei* sp. n., respectively. Both species of *Hepatozoon* are closely related, as can be seen in the phylogenetic analysis and divergence estimates (99.5% identical, p = 0.01, representing the ~860−1,725 nt region, see Table 2; and 99.0% identical, p = 0.01, representing the full 1,540 nt fragment). With high support (73, 0.89) both species form a sister clade to *Hepatozoon sipedon* Smith, Desser et Martin, 1994 from the snake *Nerodia sipedon sipedon* (Linnaeus), this clade in turn clustering within a larger monophyletic clade, sister to a clade containing species of *Hepatozoon* from amphibians.

According to the evolutionary divergence estimates concerning species of *Hepatozoon* from African snakes, which could not be included in the phylogenetic analysis as they represent fragments of the ~860−1,725 nt region of the 18S rRNA, the present material is most closely related to an unnamed species (GenBank: KC800702) isolated from *P. semivariegatus* from Swaziland (96.3−96.6% identity, p = 0.03−0.04), to an unnamed species (GenBank: KC800703) from *Python natalensis* Smith, from Swaziland (96.3−96.6% identity, p = 0.03) and an unnamed species (GenBank: KC866368) from *Dendroaspis polylepis* Günther in Swaziland or Tanzania (96.3−96.6% identity, p = 0.03−0.04) (Haklová et al. 2014).

Description of peripheral blood stages of *Hepatozoon* species

Hepatozoon angeladaviesae sp. n. Fig. 1A–C

ZooBank number for species:
urn:lsid:zoobank.org:act:C04EDDB3-8E91-4AB7-8361-4B1FA25D87CF

Mature gamonts (n = 50): Lying singly within mature erythrocytes, parasitophorous vacuole (PV) rarely evident, but appearing as thin 'halo' when visible (Fig. 1A), 14.1–17.3 (16.0 ± 0.7) long, 4.8–6.5 (5.8 ± 0.5) wide, straighter than curved, one pole faintly pointed (arrowhead) (Fig. 1A,C), opposite pole rounded, sometimes appearing folded (arrow) (Fig. 1C), cytoplasm staining blue.

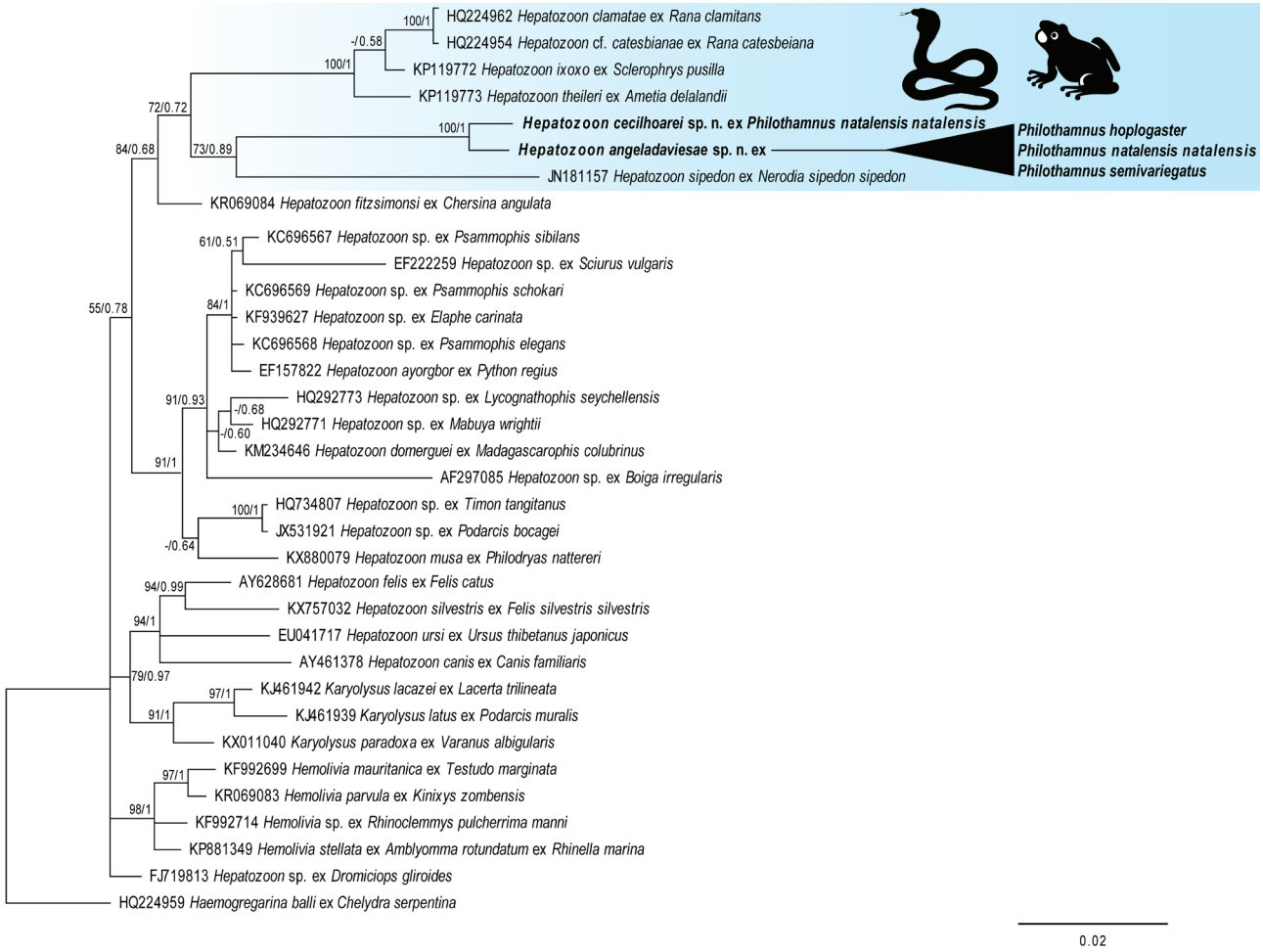

Fig. 2. Maximum Likelihood (ML) and Bayesian Inference (BI) analysis of *Hepatozoon angeladaviesae* sp. n. and *Hepatozoon cecilhoarei* sp. n. from *Philothamnus* spp. and their relationships with other haemogragarins, based on partial 18S rDNA sequences. Tree topologies for both the ML and BI trees were identical; the nodal support values, bootstrap for ML and posterior probability for BI, are represented as ML/BI on the ML tree.

Table 2. Evolutionary differences of species of *Hepatozoon* Miller, 1908 isolated from snakes of Africa not included in the phylogenetic analysis presented in Fig. 2, representing the ~860−1,725 nt region and expressed as percent similarity (bottom left) and pair-wise distance (p-distance) (top right).

	Accession number	*Hepatozoon* species	Host species	1	2	3	4	5	6	7	8	9
1	MG519501–MG519503	***Hepatozoon angeladaviesae*** sp. n.	*Philothamnus hoplogaster* (Günther), *Philothamnus natalensis natalensis* (Smith), *Philothamnus semivariegatus* (Smith)		0.01	0.04	0.03	0.05	0.04	0.03	0.03	0.04
2	MG519504	***Hepatozoon cecilhoarei*** sp. n.	*Philothamnus natalensis natalensis*	99.5		0.03	0.03	0.04	0.03	0.03	0.03	0.04
3	KC800702	*Hepatozoon* sp.	*Philothamnus semivariegatus*	96.3	96.6		0.01	0.02	0.01	0.01	0.01	0.01
4	KC800703	*Hepatozoon* sp.	*Python natalensis* Smith	96.3	96.6	99.2		0.02	0.01	0.00	0.00	0.00
5	KC866369	*Hepatozoon* sp.	*Dendroaspis jamesoni kaimosae* Loveridge	95.1	95.4	97.7	97.4		0.02	0.02	0.02	0.03
6	KC866368	*Hepatozoon* sp.	*Dendroaspis polylepis* Günther	96.3	96.6	99.5	99.2	98.2		0.01	0.01	0.01
7	KC866370	*Hepatozoon* sp.	*Dendroaspis jamesoni jamesoni* (Traill)	96.0	96.3	98.8	99.3	97.4	98.8		0.00	0.00
8	KC800704	*Hepatozoon* sp.	*Gonionotophis capensis capensis* (Smith)	96.2	96.5	99.0	99.9	97.3	99.0	99.2		0.00
9	JQ746622	*Hepatozoon garnhami* (Mohammad, Ramdan, Mohammed et Fawzi, 1996)	*Psammophis schokari* (Forskal)	96.0	96.3	98.9	99.5	97.1	98.9	99.0	99.3	

Nucleus 3.6−5.7 (5.0 ± 0.5) long, 3.7−5.8 (4.7 ± 0.5) wide, staining dark purple-pink with compactly arranged chromatin, situated almost centrally with mid-nucleus to anterior measuring 6.4−8.5 (7.4 ± 0.5), mid-nucleus to posterior 6.5−9.4 (8.5 ± 0.6), square (Fig. 1B) to rounded (Fig. 1C).

Effects on host cell: Normal erythrocytes (n = 50) measure 14.3−19.4 (16.0 ± 0.9) × 9.2−13.4 (11.7 ± 0.7), compared to significantly elongated ($P < 0.01$) parasitised erythrocytes (n = 50) measure 19.6−31.3 (25.4 ± 2.2) × 9.4−16.1 (12.2 ± 1.5). Normal erythrocyte nucleus meas-

ures 5.5−8.1 (6.7 ± 0.6) × 2.7−4.5 (3.7 ± 0.4), compared to significantly elongated (P < 0.01) and condensed (P < 0.01) parasitised host cell nucleus measuring 5.7−13 (10.3 ± 1.2) × 1.7−3.6 (2.7 ± 0.4). Host cell nucleus markedly displaced and condensed, sometimes central and parallel to gamont (Fig. 1B) or to one pole of gamont showing slight degree of karyolysis, but no evidence of vacuolation, in some cases karyorrhexis (Fig. 1C). Dehaemoglobinisation and hypertrophy of host cell evident, resulting in a 'wafer-thin' undulating cell membrane.

Type host: *Philothamnus semivariegatus* (Smith) (Ophidia: Colubridae).

Other hosts: *Philothamnus hoplogaster* (Günther), *Philothamnus natalensis natalensis* (Smith) (Ophidia: Colubridae).

Vector: Unknown.

Type locality: Ndumo Game Reserve (26°52'46"S; 32°15'25"E), KwaZulu-Natal, South Africa.

Type specimens: Hapantotype, deposited in the Protozoan collection of the National Museum, Bloemfontein, South Africa, voucher specimen number: NMB P 440; sequences uploaded onto GenBank, 18S sequence accession numbers: MG519501−MG519503.

Etymology: The species is named after Angela Josephine Davies (1947−2013), to commemorate her contribution to the knowledge of parasitic protozoa in vertebrates, as well as her singular dedication to and enthusiasm in sharing this knowledge with all those she mentored.

Remarks. During the early to mid 1900s, haemogregarines were reported parasitising *Philothamnus* spp. from Equatorial to Saharan Africa. However, most of these reports based solely on peripheral gamont stages that lacked descriptive detail. Furthermore, none of these reports led to a complete description in which the haemogregarines were named. Accounts included a report from a *P. semivariegatus* as well as an unidentified *Philothamnus* sp. in French West Africa, from an unidentified *Philothamnus* sp. in Mabira, Uganda, from a *Philothamnus irregularis* Leach in Stanleyville, Democratic Republic of the Congo and Gambia and lastly from a *P. irregularis* in Nairobi, Kenya (Bouet 1909, Hoare 1920, Schwetz 1931, Garnham and Duke 1953, Ball 1967). Those providing enough detail for a more thorough comparison included the reports of Hoare (1920) and Ball (1967).

Hoare (1920) described a parasite that caused the dehaemoglobinisation of the host cell, the length of which is very similar to that of *H. angeladaviesae* sp. n. described in the present study, but the width was described as considerably narrower (mean width 2.3 µm) compared to the present material (mean width 5.4 µm). The slender form of the parasite and the slight karyolysis of the parasite on the host cell nucleus described by Hoare (1920) led him to suggest its assignment as a species of *Karyolysus*.

The haemogregarine gamont stages described by Ball (1967) were found to be narrower (mean width 3.8 µm) than the gamonts of *H. angeladaviesae* and, in contrast, were not described as causing any dehaemoglobinisation of the host cell. The morphological dissimilarities between the gamont stages described by Hoare (1920), Ball (1967) and those of *H. angeladaviesae* draw us to the conclusion that they do not belong to the same species.

In comparison to described peripheral gamont stages of species of *Hepatozoon* described from other South African snakes, such as those *H. bitis* and *H. refringens* parasitising *Bitis arietans* (Viperidae) and *Pseudoaspis cana* (Lamprophiidae), respectively, the gamonts of *H. angeladaviesae* are much larger, both in length and width (Table 1) with the characteristic dehaemoglobinisation of the host cell, which is not evident in the other two *Hepatozoon* spp. Furthermore, *H. angeladaviesae* does not conform in size, morphology and effects on host cells of other formally described *Hepatozoon* species of snakes from Africa (Table 1).

Hepatozoon cecilhoarei sp. n. Fig. 1D–F

ZooBank number for species:
urn:lsid:zoobank.org:act:91129F0E-3247-4169-B0F2-C8AED06AF255

Mature gamonts (n = 18): Occurr singly, within larger PV (Fig. 1D), within mature erythrocytes, 13.1−15.9 (14.9 ± 0.7) long, 2.1−2.7 (2.3 ± 0.2) wide, gamont slightly curved within curved PV. PV forming hook-like point at one pole (arrowhead) (Fig. 1E,F), other pole rounded, PV showing irregular pink granular deposits; gamont cytoplasm staining blue; nucleus with tightly arranged chromatin, essentially central with mid-nucleus to anterior 5.1−8.0 (6.8 ± 0.7), mid-nucleus to posterior 7.4−10.5 (8.9 ± 0.8), rectangular, measuring 4.2−5.8 (5.1 ± 0.5) long, 1.2−1.6 (1.3 ± 0.1) wide, staining dark purple.

Effects on host cell: Normal erythrocytes (n = 18) measure 14.4−19.4 (16.1 ± 1.2) × 9.2−12.8 (11.4 ± 0.8), compared to significantly elongated and narrowed (P < 0.01) parasitised host cells (n = 18) measuring 16.2−19.3 (17.4 ± 0.8) × 7.6−10.9 (8.8 ± 0.9). Normal erythrocyte nucleus measures 5.7−7.9 (6.9 ± 0.5) × 3.0−4.3 (3.8 ± 0.3), compared to significantly elongated and condensed (P < 0.01) parasitised host cell nucleus measuring 8.8−10.4 (9.6 ± 0.5) × 2.7−3.9 (3.1 ± 0.3). Host cell nucleus displaced to central and parallel region of gamont, host nucleus condensed showing slight degree of karyolysis, but with no vacuoles observed; evident dehaemoglobinisation of host cell cytoplasm.

Type host: *Philothamnus natalensis natalensis* (Smith) (Ophidia: Colubridae).

Other hosts: *Philothamnus hoplogaster* (Günther) (Ophidia: Colubridae).

Vector: Unknown.

Type locality: Ndumo Game Reserve (26°52'46"S; 32°15'25"E), KwaZulu-Natal, South Africa.

Other locality: Mabira (00°23'54"N; 33°00'59"E), Uganda.

Type specimens: Hapantotype, deposited in the Protozoan collection of the National Museum, Bloemfontein, South Africa, voucher specimen number: NMB P 441 sequences uploaded onto GenBank, 18S sequence accession number: MG519504.

Etymology: The species is named after Cecil Arthur Hoare (1892−1984), an eminent parasitologist who did extensive research on the protozoan parasites of African herpetofauna, and whom it is believed by the authors to have first discovered, but not named, this parasite.

Remarks. In comparison to the gamont stages of *H. angeladaviesae* sp. n. (average 16.0 µm × 5.8 µm), those of *H. cecilhoarei* sp. n. are shorter (mean length 14.9 µm) and narrower (mean width 2.3 µm). Furthermore, gamonts of *H. cecilhoarei* do not cause the same enlargement that results in an 'undulating' effect on the host cell, nor do they cause the same karyolysis that leads to the fragmentation of the host cell nucleus. Similarly, as with the gamonts of *H. angeladaviesae*, *H. cecilhoarei* does not conform in size, morphology or effect on the host cell to other species of *Hepatozoon* formally described and named, which parasitise other snake species in Africa.

Hepatozoon cecilhoarei, however, does closely resemble to two haemogregarines described, but not named, from other *Philothamnus* spp. in Africa. As mentioned previously, Hoare (1920) reported a haemogregarine from an unidentified species of *Philothamnus* from Uganda. This parasite was described to be 15.0−16.0 µm × 2.3 µm in size, closely conforming to the length and width of *H. cecilhoarei.* Hoare (1920) also described the gamonts of the haemogregarine he discovered as elongate and slender, with the ends or poles of the gamont slightly bent inwards, but never bent over on the gamont itself. In addition, he described the parasite gamont to usually attain the same length as that of the host cell, or occasionally they could be longer than that of the host cell. As such, in form, the gamont of the haemogregarine described by Hoare (1920) compares closely to that of *H. cecilhoarei* described in the present study.

Considering the effects on the host cell when comparing the description of Hoare (1920) to that of the present study, both haemogregarine gamonts cause host cell alteration, decreasing host cell size and causing dehaemoglobinisation, as well as the host nucleus to become hypertrophied and elongated. In both the present study and that of Hoare (1920), mature gamonts were the only stages identified. Furthermore, the gamont lay parallel to the long axis of the host cell adjacent to the host cell nucleus showing some degree of karyolysis.

However, besides Hoare's (1920) mentioning the slight karyolysis of the host cell nucleus, which caused the chromatin to appear entangled in irregular accumulations and strands, he also mentioned that vacuoles were sometimes present. This was not evident in the present study, but it may be a result of a less mature or developed infection. Hoare (1920) also never observed an evident parasitophorous vacuole or irregular pink granules as was notable in a number of gamonts of *H. cecilhoarei.* Regardless, the overall similarity of the haemogregarine described by Hoare (1920) and that of the present study, suggest strongly that they are the same.

DISCUSSION

The genus *Philothamnus* falls within the family Colubridae, including largely arboreal snakes, which often occupy habitats near to water (Bates et al. 2014). As mentioned previously, the only species of *Hepatozoon* described from South African snakes to date include two from the families Viperidae and Lamprophiidae, respectively, the two species of *Hepatozoon* described in this paper thus representing the first taxa of this genus reported from the family Colubridae in South Africa (Sambon and Seligmann 1907, Fantham 1925, Van As et al. 2013). Since the ecology of snakes has formerly been demonstrated as important with regards to their associated diversity of *Hepatozoon* spp. (Smith et al. 1994, Telford et al. 2001, Telford 2009), it is not surprising that the species infecting *Philothamnus* spp. in this study are different (based on morphology) from those occuring in snakes of the genera of *Bitis* Gray and *Pseudaspis* Fitzinger.

Past studies have shown habitat and the resulting diet of snakes important, particularly where more than one species of intermediate host is involved (Smith et al. 1994, Telford et al. 2001, Telford 2009). *Hepatozoon sipedon*, for example, is known to naturally infect the snake, *Nerodia sipedon sipedon*, through the ingestion of an anuran, *Rana pipiens* (Schreber) (Smith 1994, Netherlands et al. 2014a). Furthermore, these non-ophidian intermediate host associations are becoming evident in the phylogenetic analyses of species of *Hepatozoon* because species infecting snakes with a diet comprising mostly anurans and those with a diet comprising mostly saurians belong to separate lineages (Haklová et al. 2014).

Tomé et al. (2013) demonstrated that diet appears to be a key element for infection of snakes by species of *Hepatozoon*. The authors found that the lineages infecting saurophagous snakes of the genus *Psammophis* Fitzinger clustered together with those from different types of lizards that form a large portion of the diet of these snakes. Both *B. arietans* and *P. cana* fulfill ecologically different roles, being more terrestrial and feeding mostly on rodents, compared to species of *Philothamnus*, which are arboreal, preferring to occupy a habitat nearer to water, feeding on lizards, frogs, fish and nestling birds (Bates et al. 2014). Since all six specimens of *Philothamnus* examined in the present study were found near temporary and permanent pans with a large population of frogs that provide a readily available food source and potential source of infection, and both *H. angeladaviesae* sp. n. and *H. cecilhoarei* sp. n. were found to fall into a clade comprising snake and anuran species of *Hepatozoon* (Fig. 2), it would be beneficial to identify possible frog hosts, but also locate fresh specimens of *H. bitis* and *H. refringens* so that they too may be compared in phylogenetic analyses.

It is unfortunate that the species of *Hepatozoon* parasitising *P. semivariegatus* from Swaziland sequenced by Haklová et al. (2014) could not be compared on a morphological basis to *H. angeladaviesae* and *H. cecilhoarei.* Furthermore, as the fragment used in the evolutionary

divergence estimates represents a relatively conservative section of the 18S rRNA gene, results may demonstrate a closer relatedness than what is truly the case. However, it does emphasise the need to provide descriptions based on a combination of morphology and molecular data, which has been highlighted by numerous authors, e.g. Cook et al. (2016), Tomé et al. (2016) and Borges-Nojosa et al. (2017). Moreover, it is anticipated that a multigene approach will prove useful in resolving the diversity, phylogeny and taxonomy of the paraphyletic *Hepatozoon*, as has been used in the analyses of the haemoproteids and eimeriid coccidia (Pineda-Catalan et al. 2013, Ogedengbe et al. 2015).

Acknowledgements. We would like to thank Ezemvelo KwaZulu-Natal Wildlife for permission to sample reptiles within Ndumo Game Reserve. In addition, we would like to thank Microbiology, Unit for Environmental Sciences and Management, North-West University (NWU), Potchefstroom campus, for use of their facilities. The financial assistance of the South African National Research Foundation (NRF) towards CAC is also hereby acknowledged (project SFP13090332476), and in part to ECN who was supported by the DAAD-NRF doctoral scholarship (Grant UID: 108803), and the VLIR-UOS university scholarship (ID 0620854/Contract 000000076310). JVA was supported by a NRF Thutuka grant (TTK 14042266483) and NJS by a NRF Incentive Funding for Rated Researchers grant (IFR 170210222411). Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to the NRF. This is contribution No. 241 from the NWU-Water Research Group. We are most grateful to Maarten P.M. Vanhove from the Biology Department, Royal Museum for Central Africa (Belgium), for obtaining the work of Schweitz (1931).

REFERENCES

Abdel-Baki A.S., Al-Quraishy S., Zhang J.Y. 2014: Redescription of *Haemogregarina garnhami* (Apicomplexa: Adeleorina) from the blood of *Psammophis schokari* (Serpentes: Colubridae) as *Hepatozoon garnhami* n. comb. based on molecular, morphometric and morphologic characters. Acta Parasitol. 59: 294–300.

Ball G.H. 1967: Blood sporozoans from East African Amphibia. J. Eukaryot. Microbiol. 14: 521–527.

Barta J.R., Ogedengbe J.D., Martin D.S., Smith T.G. 2012: Phylogenetic position of the adeleorinid coccidia (Myzozoa, Apicomplexa, Coccidia, Eucoccidiorida, Adeleorina) inferred using 18S rDNA sequences. J. Eukaryot. Microbiol. 59: 171–180.

Bashtar A.R., Abdel-Ghaffar F.A. 1987: Light microscope study on the life cycle of *Haemogregarina najae* infecting the snake *Naja nigricollis nigricollis* (Elapidae, Proteroglypha, Squamata) from Egypt. Proc. Zool. Soc. A. R. Egypt 14: 33–44.

Bashtar A.R., Abdel-Ghaffar F.A., Shazly M.A. 1991: Life cycle of *Hepatozoon mehlhorni* sp. nov. in the viper *Echis carinatus* and the mosquito *Culex pipiens*. Parasitol. Res. 77: 402–410.

Bashtar A.R., Boulos R., Mehlhorn H. 1984: *Hepatozoon aegypti* nov. sp. Parasitol. Res. 70: 29–41.

Bates M.F., Branch W.R., Bauer A.M., Burger M., Marais J., Alexander G.J., de Villiers M.S. 2014: Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland. Suricata 1. South African National Biodiversity Institute, Pretoria, pp. 485.

Billet M.A. 1904: Sur une hémogrégarine karyolysante de la couleuvre vipérine. C. R. Hebd. Séances Mém. Soc. Biol. 1: 484–485.

Borges-Nojosa D.M., Borges-Leite M.J., Maia J.P., Zanchi-Silva D., da Rocha Braga R., Harris D.J. 2017: A new species of *Hepatozoon* Miller, 1908 (Apicomplexa: Adelerina) from the snake *Philodryas nattereri* Steindachner (Squamata: Dipsadidae) in northeastern Brazil. Syst. Parasitol. 94: 65–72.

Bouet J. 1909: Hemogrégarines de L'Afrique occidentale français. C. R. Séances Soc. biol. 66: 43–45.

Cook C.A., Lawton S.P., Davies A.J., Smit N.J. 2014a: Reassignment of the land tortoise haemogregarine *Haemogregarina fitzsimonsi* Dias 1953, (Adeleorina: Haemogregarinidae) to the genus *Hepatozoon* Miller 1908 (Adeleorina: Hepatozoidae) based on parasite morphology, life cycle and phylogenetic analysis of 18S rDNA sequence fragments. Parasitology 141: 1611–1620.

Cook C.A., Netherlands E.C., Smit N.J. 2016: Redescription, molecular characterization and taxonomic re-evaluation of a unique African monitor lizard haemogregarine *Karyolysus paradoxa* (Dias, 1954) n. comb. (Karyolysidae). Parasit. Vectors 9: 347.

Cook C.A., Smit N.J., Davies A.J. 2009: A redescription of *Haemogregarina fitzsimonsi* Dias, 1953 and some comments on *Haemogregarina parvula* Dias, 1953 (Adeleorina: Haemogregarinidae) from southern African tortoises (Cryptodira: Testudinidae), with new host data and distribution records. Folia Parasitol. 56: 173–179.

Cook C.A., Smit N.J., Davies A.J. 2014b: First record of an intraleucocytic haemogregarine (Adeleorina: Haemogregarinidae) from South African tortoises of the species *Stigmochelys pardalis* (Cryptodira: Testudinidae). Afr. Zool. 49: 290–294.

Criado-Fornelio A., Ruas J.L., Casado N., Farias N.A.R., Soares M.P., Müller G., Brum J.G.W., Berne M.E.A., Buling-Saraña A., Barba-Carretero J.C. 2006: New molecular data on mammalian *Hepatozoon* species (Apicomplexa: Adeleorina) from Brazil and Spain. J. Parasitol. 92: 93–99.

Darriba D., Taboada G.L., Doallo R., Posada D. 2012: jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods. 9: 772.

Dias J.A.T.S. 1952: Resultados de um reconhecimento zoológico no alto Limpopo efectuado pelos Drs. F. Zumpt. Moç. Doc. Trimest. 71: 61–79.

Dias J.A.T.S. 1953: Subsídios para o estudo dos hematozoários dos répteis de Moçambique. Bol. Soc. Est. Moç. 82: 3–19.

Edgar R.C. 2004: MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 5: 1792–1797.

Fantham H.B. 1925: Some parasitic protozoa found in South Africa. – VIII. S. Afr. J. Sci. 22: 346–354.

Garnham P.C.C. 1950: Blood parasites of East African vertebrates, with a brief description of exo-erythrocytic schizogony in *Plasmodium pitmani*. Parasitology 40: 328–337.

Garnham P.C.C., Duke B.O.L. 1953: Certain parasitic Protozoa from the Gambia. Trans. Roy. Soc. Trop. Med. H. 47: 7–8.

Guindon S., Gascuel O. 2003: A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52: 696–704.

Haklová B., Majláthová V., Majláth I., Harris D.J., Petrilla V., Litschka-Koen T., Oros M., Peťko B. 2014: Phylogenetic relationship of *Hepatozoon* blood parasites found in snakes from Africa, America and Asia. Parasitology 141: 389–398.

Haklová-Kočíková B., Hižňanová A., Majláth I., Račka K., Harris D.J., Földvári G., Tryjanowski P., Kokošová

N., Malčeková B., Majláthová V. 2014: Morphological and molecular characterization of *Karyolysus* – a neglected but common parasite infecting some European lizards. Parasit. Vectors 7: 555.

Hoare C.A. 1920: On some new haemogregarines from British East Africa. Parasitology 12: 315–327.

Hoare C.A. 1932: On protozoal blood parasites collected in Uganda. Parasitology 24: 210–224.

Huelsenbeck J.P., Ronquist F. 2001: MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Karadjian G., Chavatte J., Landau I. 2015: Systematic revision of the adeleid haemogregarines, with creation of *Bartazoon* n. g., reassignment of *Hepatozoon argantis*, 1954 to *Hemolivia*, and molecular data on *Hemolivia stellata*. Parasite 22: 31.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Mentjies P., Drummond A. 2012: Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647−1649.

Kumar S., Stecher G., Tamura K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33: 1870–1874.

Kvičerová J., Hypša V., Dvořáková N., Mikulíček P., Jandzik D., George Gardner M., Javanbakht H., Tiar G., Široký P. 2014: *Hemolivia* and *Hepatozoon*: haemogregarines with tangled evolutionary relationships. Protist 165: 688–700.

Laveran A. 1902: Sur quelques haemogregarines des ophidiens. C. R. Hebd. Séances Acad. Sci. 135: 1036−1040.

Laveran A. 1905: Contribution a l'étude des grandes haemogregarines des grenouilles. C. R. Soc. Séances Soc. Biol. 59: 172–175.

Laveran A., Pettit A. 1909: Sur une hémogrégarine du *Python sebai*. C. R. Hebd. Séances Acad. Sci. 148: 1142−1146.

Laveran A., Pettit A. 1911: Sur une hémogrégarine de la vipère à cornes. C. R. Hebd. Séances Acad. Sci. Biol. 63: 95−96.

Maia J.P., Carranza S., Harris D.J. 2016: Comments on the systematic revision of adeleid haemogregarines: is more data needed? J. Parasitol. 102: 549−552.

Manceaux L. 1908: Hemogregarines de *Zamensis hippocrepis* et de *Zamensis algirus*. Arch. Inst. Pasteur, Tunis 4: 186−189.

Mathew J.S., Van Den Bussche R.A., Ewing S.A., Malayer J.R., Latha B.R., Panciera R.J. 2000: Phylogenetic relationships of *Hepatozoon* (Apicomplexa: Adeleorina) based on molecular, morphologic, and life-cycle characters. J. Parasitol. 86: 366−372.

Medlin L., Elwood H.J., Stickel S., Sogin M.L. 1988: The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71: 491−499.

Mohammad F.A.S., Ramdan N.F., Mohammed S.H., Fawzi S.M. 1996: On two haemogregarines (Protozoa: Apicomplexa) from colubrid and Elapidae snakes in Egypt. Qatar Univ. Sci. J. 16: 127−139.

Morsy K., Bashtar A.R., Abdel-Ghaffar F., Al-Quraishy S., Al-Hashimi S., Al-Ghamdi A., Shazly M. 2013: Development stages of *Hepatozoon seurati* (Laveran and Pettit 1911) comb. nov., a parasite of the corned viper *Cerastes cerastes* and the mosquito *Culex pipiens* from Egypt. Parasitol. Res. 112: 2533−2542.

Netherlands E.C., Cook C.A., Du Preez L.H., Vanhove M.P.M, Brendonck L., Smit N.J. 2018: Monophyly of the species of *Hepatozoon* (Adeleorina: Hepatozoidae) parasitizing (African) anurans, with the description of three new species from hyperoliid frogs in South Africa. Parasitology: (in press).

Netherlands E.C., Cook C.A., Kruger D.J.D, Du Preez L.H., Smit N.J. 2015: Biodiversity of frog haemoparasites from sub-tropical northern KwaZulu-Natal, South Africa. Int. J. Parasitol. Parasites. Wildl. 4: 135–141.

Netherlands E.C., Cook C.A., Smit N.J. 2014b: *Hepatozoon* species (Adeleorina: Hepatozoidae) of African bufonids, with morphological description and molecular diagnosis of *Hepatozoon ixoxo* sp. nov. parasitising three *Amietophrynus* species (Anura: Bufonidae). Parasit. Vectors 7: 552.

Netherlands E.C., Cook C.A., Smit N.J., Du Preez L.H. 2014a: Redescription and molecular diagnosis of *Hepatozoon theileri* (Laveran, 1905) (Apicomplexa: Adeleorina: Hepatozoidae), infecting *Amietia quecketti* (Anura: Pyxicephalidae). Folia Parasitol. 61: 293−300.

Ogedengbe J.D., Ogedengbe M.E., Hafeez M.A., Barta J.R. 2015: Molecular phylogenetics of eimeriid coccidia (Eimeriidae, Eimeriorina, Apicomplexa, Alveolata): a preliminary multi-gene and multi-genome approach. Parasitol. Res. 114: 4149–4160.

Peirce M.A. 1984: Some parasites of reptiles from Zambia and Indian Ocean islands with a description of *Haemogregarina zambiensis* sp. n. from *Dispholidus typus* (Colubridae). J. Nat. Hist. 18: 211–217.

Phisalix M. 1914: Sur une hémogregarine nouvelle, parasite de *Boodon fuliginosus* Boie, et ses formes de multiplication endogene. Bull. Soc. Pathol. Exot. 7: 575–577.

Pineda-Catalan O., Perkins S.L., Peirce M.A., Engstrand R., Garcia-Davila C., Pinedo-Vasquez M., Alonso Aguirre A. 2013: Revision of hemoproteid genera and description and redescription of two species of chelonian hemoproteid parasites. J. Parasitol. 99: 1089–1098.

Sambon L.W., Seligmann C.G. 1907: Descriptions of five new species of haemogregarines from snakes. Proc. General Meetings for Scientific Business of the Zoological Society of London, London, March 1907. Messrs. Longmans, Green, and Co., London, pp. 283−284.

Sambon L.W., Seligmann C.G. 1909: The haemogregarines of snakes. T. Pathol. Soc. Lond. 58: 310–370.

Schweitz J. 1931: Les hématozoaires des serpents de Stanleyville (Congo Belge). Ann. Parasitol. 9: 303–310.

Sloboda M., Kamler M., Bulantová J., Votýpka J., Modrý D. 2007: A new species of *Hepatozoon* (Apicomplexa: Adeleorina) from *Python regius* (Serpentes: Boidae) and its experimental transmission by a mosquito vector. J. Parasitol. 93: 1189−1198.

Smith T.G. 1996: The genus *Hepatozoon* (Apicomplexa: Adeleina). J. Parasitol. 82: 565–585.

Smith T.G., Desser S.S., Martin D.S. 1994: The development of *Hepatozoon sipedon* sp. nov. (Apicomplexa: Adeleina: Hepatozoidae) in its natural host, the northern water snake (*Nerodia sipedon sipedon*), in the culicine vectors *Culex pipiens* and *C. territans*, and in an intermediate host, the northern leopard frog (*Rana pipiens*). Parasitol. Res. 80: 559–568.

Telford S.R. 2009: Hemoparasites of the Reptilia: Color Atlas and Text. CRC Press, New York, 376 pp.

Telford S.R., Wozniak E.J., Butler J.F. 2001: Haemogregarine specificity in two communities of Florida snakes, with descriptions of six new species of *Hepatozoon* (Apicomplexa: Hepatozoidae) and a possible species of *Haemogregarina* (Apicomplexa: Haemogregarinidae). J. Parasitol. 87: 890−905.

Tomé B., Maia J.P.M.C, Harris D.J. 2013. Molecular assessment of apicomplexan parasites in the snake *Psammophis* from North Africa: do multiple parasite lineages reflect the final vertebrate host diet? J. Parasitol. 99: 883–887.

Tomé B., Rato C., Harris D.J., Perera A. 2016: High diversity of *Hepatozoon* spp. in geckos of the genus *Tarentola*. J. Parasitol. 102: 476−480.

Ujvari B., Madsen T., Olsson M. 2004: High prevalence of *Hepatozoon* spp. (Apicomplexa: Hepatozoidae) infection in water pythons (*Liasis fuscus*) from tropical Australia. J. Parasitol. 90: 670−672.

Van As J., Davies A.J., Smit N.J. 2013: *Hepatozoon langii* n. sp. and *Hepatozoon vacuolatus* n. sp. (Apicomplexa: Adeleorina: Hepatozoidae) from the crag lizard (Sauria: Cordylidae) *Pseu-*

docordylus langi from the North Eastern Drakensberg escarpment, Eastern Free State, South Africa. Zootaxa 3608: 345–356.
Van As J., Davies A.J., Smit N.J. 2015: Life cycle of *Hepatozoon affluomaloti* sp. n. (Apicomplexa: Haemogregarinidae) in crag lizards (Sauria: Cordylidae) and in culicine mosquitoes from South Africa. Folia Parasitol. 62: 008.

Received 23 June 2017 Accepted 10 January 2018 Published online 3 April 2018

Cite this article as: Cook C.A., Netherlands E.C., van As J., Smit N.J. 2018: Two new species of *Hepatozoon* (Apicomplexa: Hepatozoidae) parasitising species of *Philothamnus* (Ophidia: Colubridae) from South Africa. Folia Parasitol. 65: 004.