Systematic revision of the adeleid haemogregarines, with creation of Bartazoon n. g., reassignment of Hepatozoon argantis Garnham, 1954 to Hemolivia, and molecular data on Hemolivia stellata

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Abstract – Life cycles and molecular data for terrestrial haemogregarines are reviewed in this article. Collection material was re-examined: Hepatozoon argantis Garnham, 1954 in Argas brumpti was reassigned to Hemolivia as Hemolivia argantis (Garnham, 1954) n. comb; parasite DNA was extracted from a tick crush on smear of an archived slide of Hemolivia stellata in Amblyomma rotondatum, then the 18S ssrRNA gene was amplified by PCR. A systematic revision of the group is proposed, based on biological life cycles and phylogenetic reconstruction. Four types of life cycles, based on parasite vector, vertebrate host and the characteristics of their development, are defined. We propose combining species, based on their biology, into four groups (types I, II, III and IV). The characters of each type are defined and associated with a type genus and a type species. The biological characters of each type are associated with a different genus and a type species. The phylogenetic reconstruction with sequences deposited in the databases and our own new sequence of Hemolivia stellata is consistent with this classification. The classification is as follows: Type I, Hepatozoon Miller, 1908, type species H. perniciosum Miller, 1908; Type II, Karyolysus Labbé, 1894, type species K. lacertae (Danilewsky, 1886) Reichenow, 1913; Type III Hemolivia Petit et al., 1990, type species H. stellata, Petit et al., 1990; and Type IV: Bartazoon n. g., type species B. breinli (Mackerras, 1960).

Key words: Hepatozoon, Bartazoon, Hemolivia, Karyolysus, Life cycle, Systematics revision, Molecular data.
Introduction

The haemogregarines form a group of particularly diverse heteroxenous adeleid coccidia parasites which have exploited all environments, terrestrial or aquatic, and become adapted to numerous vertebrate hosts, i.e. chelonians, crocodiles and other reptiles, amphibians, fishes and many mammals.

(i) In the aquatic environment — the transmission of parasites is obligatorily achieved either by predation between vertebrates [38, 70] or through vectors in close contact with the vertebrate hosts.

For example, in the wild, an Elmeria of fish may be transmitted from fish to fish by cannibalism or via a paratenic host such as a shrimp [38, 70] but not, in natural conditions, by shedding oocysts in the water where they would be immediately diluted.

The haemogregarines of aquatic hosts are transmitted by leeches or by arthropods in which the sexual part of the cycle develops. The sporogony of Haemogregarina stepanovi Danilewsky, 1885 [14] develops in the leech which transmits the infection when feeding on the turtle [60]. The oocysts in the leech are asporate and produce free sporozoites which are inoculated to the turtle. In some vectors of the haemogregarines of fish, a further stage develops from the sporogony: a merogony, in the leech for Cyrilina Lainson, 1981 [36], in the isopod for Desseria Siddall, 1995 [65]; the vertebrate host would become infected when ingesting the vector.

(ii) In the terrestrial environment — the life cycle of haemogregarines comprises roughly four stages: merogony and gamogony in the vertebrate host, and fertilisation and sporogony in the invertebrate host. Merogony in the vector is absent.

In addition to the classical cycle in which transmission is achieved by the bite of the vector or its ingestion by the vertebrate host, a second mode of transmission was acquired by some species: transmission by predation between vertebrates [39]. This mode of transmission is shared by all species when the alimentary diet of the host does not include the direct ingestion of the vector by the vertebrate host.

When, for example, the vector is a mosquito and the vertebrate host a snake, it is obvious that a haemogregarine cannot be transmitted regularly by ingestion of the mosquito. There must be a second vertebrate host which eats insects, develops cysts in its tissues and is part of the diet of the snake. This second host may or may not develop, in addition to cysts, the entire cycle of the parasite.

Transmission by predation is characterised for all parasites by (i) the absence of specificity; (ii) a wide re-partition of infective stages (here the cyst) in the organism of the host. These principles, established for nematodes, are valid for the haemogregarines producing cysts which are disseminated in numerous organs [7].

When the vector is a mite or a tick, the sporogonic development may follow two courses: (i) in one step: the sporogony evolves directly from zygote to oocyst, sporoblasts and sporocytes inside the same envelope, like in Hepatozoon perniciosum Miller, 1908 [51] or (ii) in two steps: oocysts undergo the first division to produce motile sporokinetes instead of sporoblasts. Sporokinetes, after the rupture of the oocyst envelop, invade new cells of the host; sporokinetes complete their development into sporoblasts and sporocytes either in the same host, like in Hemolivia stellata Petit et al., 1990 [58] or in the next host generation when they invade the oocytes of the mite, like in Karyolyssus Labbé, 1894 [35]. In both instances, the vertebrate host ingests either directly the invertebrate host or cysts from the tissues of another vertebrate host. It was suggested that sporocysts of Hemolivia mauritania or H. stellata Petit et al., 1990 [58] might also be excreted with the faeces of the tick and be infective to susceptible hosts or transported by paratenic hosts.

As pointed out by Smith (1996) [68], a great many haemogregarines were described on the basis of gametocyte morphology and very often designated as Hepatozoon spp. or Haemogregarina spp. However, only the observation of stages in the vector may indicate the generic position of the parasite [71]. Through the years, the nomenclature has evolved, while new life cycles have been unravelled. For example, Hemogregarina mauritania Sergent and Sergent, 1904 [64] studied by Laveran (1905) [41] and Brumpt (1938) [5] was renamed successively Hepatozoon mauritianum by Michel (1973) [50] and later Hemolivia mauritania by Landau and Paperna (1997) [40].

The genus Hepatozoon, well defined by the morphology and the life cycle of its type species, H. perniciosum Miller, 1908, has over time become a heterogeneous group of species with diverse life cycles and which, according to work by Barta et al. (2012), is paraphyletic (see Discussion) [3].

The genus Hemolivia, which is clearly defined by its morphological and biological features, has recently been investigated by molecular biology and its phylogenetic relationship with Hepatozoon studied [3, 27, 34]. A molecular analysis of two of the three Hemolivia species, Hemolivia mauritania from Testudo graeca and Testudo marginata [27, 34], Hemolivia mariae from Egernia stokesi and Tiliqua rugosa [3, 34], was performed, as well as for Hemolivia sp. from Rhinolchelys Pulcherrima manni [34]. A number of haemogregarines of reptiles and amphibians could probably be assigned to the genus Hemolivia if their life cycle were known.

In this communication, we (i) present molecular data on H. stellata obtained from 25-year-old archived original material; (ii) partially re-describe the haemogregarine Hepatozoon argantis Garnham, 1954 [20] and reassign it to the genus Hemolivia; and (iii) analyse the haemogregarines’ known life cycles and propose Bartazoon n. g.

Materials and methods

Hemolivia argantis

In 1986, PCC Garnham deposited part of his collection of slides at the Welcome Trust, including many types of haemosporidians [21] and the rest of his collection at the Muséum National d’Histoire Naturelle (MNHN) in Paris, France. In the MNHN’s collection, we found part of the original material used to describe Hepatozoon argantis Garnham, 1954 in the tick Argas brumpti Neumann, 1907 [53]. This material consists of sections of the tick with a massive infection by a haemogregarine which we identified as belonging to the genus Hemolivia and not Hepatozoon.
As we are certain that we are dealing with the original material and that only one species of haemogregarine is present, and because of the absence of type designation by the authors, this material should be considered as syntype. It consists of sections of the tick *Argas brumpti* on which the description of the haemogregarine *H. argantis* was based; the tick was identified as belonging to the original description by a photograph (Fig. 18) in Garnham 1954 [20], showing one of the sections of the tick and by drawings of oocysts of different stages. Some sections were stained by Ehrlich’s haematoxylin and eosin, some by haemalun eosin, and smears of caecal contents by Giemsa stain.

*Hemolivia stellata*

Archived Giemsa-stained blood smears or tissue imprints have been used as a source of DNA for PCR amplification for *Plasmodium* [8, 30, 63, 79], *Leishmania* [77] and *Hepatozoon* [9] species. They provide extremely valuable material for retrospective study [30, 77] and molecular characterisation.

A 25-year-old archived smear of crushed *Amblyomma rotundatum* (Koch, 1844) [31] which was part of the material used for the original description of *H. stellata*, containing many immature stellate oocysts (Fig. 1I), was used as a source of DNA for molecular characterisation of this parasite on the basis of 18S ssrRNA.

The coverslip was dismounted; the smear was scraped off the glass slide with a sterile scalpel; the material collected was incubated at 50°C in ATL buffer containing proteinase K until total digestion; DNA was extracted using the QIAamp DNA Mini Kit from Qiagen® following the manufacturer’s recommendations. DNA was eluted in 50 µL of elution buffer and frozen at −30°C. DNA was amplified by a semi-nested PCR assay. The first amplification was performed with one pair of universal 18S rRNA oligonucleotide primers 2867 [5'-AACCTGGTTGATCCTGCCAG-3'/2868 [5'-TGATCCTTTCTGCAGGTTCACCTAC-3'], as described by Mathew et al. (2000) [47]. For the second step, two semi-nested PCRs were carried out with one external oligonucleotide primer of the first reaction paired with one internal

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Figure 1. Stages of development of *Hemolivia argantis* (A–H) and *Hemolivia stellata* (I) in their invertebrate hosts. B, C and F: Nomarski. A and B: Immature oocysts with peripheral nuclei. C: Budding of future sporokinetes at the periphery of the oocyst. D: Oocyst containing sporokinetes in caecal content. E: Sporocyst inside a digestive cell. F: Mature sporocysts inside the gut contents. G and H: Sporocysts in coxal fluid. I: Star-shaped oocyst of *H. stellata* in the haemocoel of *Amblyomma rotondatum*. Scale bars: 20 µm.
Protozoan-specific primer derivate from Vilcins et al. [74] as follow: 2867/Hep900 [5'-CAAATCTAAGAATTTCACCTCT-GAC-3'] and Hep300 [5'-GTTTCTGACCTATCAGCTTTC-GACG-3'] amplifying to overlapping fragments of 939 bp and 1510 bp, respectively. The PCRs were run in a total volume of 20 l, containing 1X High Fidelity PCR Buffer, 3 mM of MgSO4, 0.5 U of Platinum® Taq DNA Polymerase High Fidelity (Invitrogen™), 0.2 mM of each dNTP (Promega), 0.25 μM of each primer and 3 μL of original DNA template in the first reaction; 1 μL of the PCR product was used as a template in the second reaction. All PCRs were run on a Veriti® Thermal Cycler (Applied Biosystems™). The PCR products were resolved by 1.5% agarose gel electrophoresis prior to purification by the QiAquick® PCR Purification Kit (Qiagen™), following the manufacturer’s recommendations. Purified products were eluted in 30 μL of nuclease-free H2O and frozen at −30 °C. PCR products were prepared for sequencing in both directions, using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) and the respective oligonucleotide primers. The BigDye® reaction products were purified using the BigDye® XTerminator™ Purification Kit (Applied Biosystems™), following the manufacturer’s recommendations before being sequenced on a 3500xl Genetic Analyzer (Applied Biosystems™).

Alignment and cross-checking of the sequences were performed with CLC Main Workbench 5.7 software (CLC bio) and a consensus sequence of 1816 bp was obtained by combining the two overlapping fragments. The nearly complete sequence (1816 bp) of the 18S ssrRNA gene recovered from the original archived material of Hemolivia stellata was deposited in GenBank under Accession Number KP881349. Our new sequence of H. stellata and 179 sequences of adeleid...
parasites (Table 2) were aligned using a Muscle algorithm [19]. Molecular phylogeny was performed by the Maximum Likelihood (ML) method with a GTR + Γ + I model, using PhyML 3.0 software [23]. Nodal robustness of the tree was evaluated by non-parametric bootstrapping (1000 replicates).

Results

Life cycles and morphology

*H. argantis* was described in detail by Garnham, 1954 [20] and we will only provide a general outline: (i) in the haemocoel: large immature oocysts with peripheral nuclei (Figs. 1A & 1B); (ii) round, immature oocysts with sporokinetes budding at the periphery of a large cytoplasmic mass (Fig. 1C) or, apparently free in the oocyst (Fig. 1D); and (iii) sporocysts filled with sporozoites in the haemocoelom (Fig. 1F) and the gut contents of the tick.

In the re-examined material, we also found a few intracellular sporocysts beneath the intestinal epithelium and inside desquamated cells of the gut (Fig. 1E), suggesting that the initial site of development of sporokinetes into sporocysts is intracellular.

*Hemolivia stellata* was described by Petit et al., 1990 [58]. Schizons of this species occur in erythrocytes and leucocytes of the cane toad *Rhinella marina* (Linnaeus, 1758) (as *Bufo marinus*) and gametocytes of this species occur in erythrocytes of this amphibian. Oocysts in the tick, *Amblyomma rotondatum*, are star shaped, and release sporokinetes filled with sporozoites in the haemocoelom (Fig. 1F) and the gut contents of the tick.

**Figure 2.** Continued.
migrate to new intestinal cells and mature into sporocysts; many sporocysts are found in the intestinal fluid.

**Parasite sequences and phylogenetic analysis**

The phylogenetic analysis included 166 published sequences of *Hepatozoon* (140), *Karyolysus* (8) and *Hemolivia* (18) parasites isolated from a variety of vertebrates, 13 other published sequences of Adeleidae as outgroups to root the tree and our sequence of *H. stellata* (Table 2).

A robust *Hemolivia* clade was obtained with *H. stellata* at the base (Fig. 2). It comprises all the *Hemolivia* sequences included in the phylogenetic construction but also a few sequences from *Hepatozoon* isolated in Australia from *Varanus panoptes* (Storr, 1980) and from *Lialis fuscus* Peters, 1873.

What was previously considered as the *Hepatozoon* group was paraphyletic. It was clearly divided into two major clades (Fig. 2).

The first clade was subdivided into two robust subclades containing respectively the *Hepatozoon* from carnivores and a mixed group containing some of the *Hepatozoon* of Mediterranean reptiles and the *Karyolysus* of the European lacertids.

The second clade was also subdivided, with the *Hemolivia* clade on the one hand and a group containing sequences of haemogregarines of reptiles and amphibians transmitted by biting insects (Diptera and Siphonaptera) on the other.

A sequence of a haemogregarine from the bird *Oceano-droma melania* (Bonaparte, 1854) was found at the base of this group, as well as a monophyletic group of sequences of parasites from Australian and South American marsupials, a monophyletic group of sequences from amphibian hosts from various geographic areas, a few sequences of haemogregarines from rodents and three sequences of parasites extracted from the liver of the bats *Hipposideros cervinus* (Gould, 1863) [59].

**Taxonomic summaries**

*Hemolivia argantis* (Garnham, 1954) n. comb.

(=*Hepatozoon argantis* Garnham, 1954).

Host: *Argas brumpti*.

Locality: Egypt.

Syntype: Sections of a tick and smears of caecal contents.

Collection number: MNHN PIV 169-173, 179-182, 184-200 (251YY).

*Bartazoon* n. gen.

urn:lsid:zoobank.org:act:55D637B3-A9D9-4C01-92A4-E0B4F0052958

Type species: *Bartazoon breinli* (Mackerras, 1960) n. comb. (=*Hepatozoon breinli* Mackerras, 1960).

Type host of the type species: *Varanus tristis orientalis*.

Type locality of the type species: Innisfail, Queensland, Australia.

Etymology: named after John R. Barta (University of Guelph, Canada), in recognition of his contribution to the biology of haemogregarines.

**Discussion**

**Taxonomic status of *H. argantis***

It appears clearly that the parasite in *Argas* does not belong to the genus *Hepatozoon* in which the sporoblasts mature into sporocysts inside the oocyst envelop. The sporogony evolves in two stages: (i) mature oocysts release motile sporokinetes, and (ii) sporokinetes invade the cells of the tick and develop into sporoblasts and sporocysts.

Garnham in 1954 [20] did point to differences with the classical cycle of *Hepatozoon* and compared the parasite from *Argas* with another genus existing at the time, *Karyolysus* (Danilewskyi, 1886) [15], which also produces sporokinetes in a mite *Lypomyssus*. However, in the latter, sporokinetes invade the oocytes of the mite and mature only in the next generation. The authors choose to assign the haemogregarine in *Argas* to the genus *Hepatozoon* but noted that it might be a new genus. It is now clear that it belongs to the genus *Hemolivia*.

**Host spectrum**

The vectors, definitive hosts of terrestrial haemogregarines, fall into two groups: insects (Diptera and fleas) on the one hand, and haematophagous Acari (ticks and mites) on the other.

Mosquitoes and fleas are considered as hosts for *Hepatozoon* while Acari are vectors of the three existing genera: *Hepatozoon, Karyolysus* and *Hemolivia*.

*Hepatozoon* is at present a large gathering of species classified in this genus on the sole basis of the presence of gametocytes in the blood. Some species, previously considered as *Hepatozoon*, when their life cycles were unravelled, were assigned to the genus *Hemolivia*: *H. mauritanica, H. mariae, H. stellata* and here *H. argantis*. The development in the vector of many haemogregarines follows different courses which will be discussed in the next section.

**Life cycles**

The fundamental life cycle of a Coccidiomorpha consists of (i) the infective stage, i.e. the sporozoite, (ii) male and female gametes, and (iii) the zygote. Many adaptive additions to this simple scheme arose either to multiply the parasite in the vertebrate host (schizogony) or in the vector (sporogony) or to facilitate transmission according to the hosts and their life habits: free resistant stages ingested in the external environment, infective stages ingested by a paratenic host, bite by a vector and predation between vertebrate hosts.

The adeleids’ fertilisation procedures, including syzygy, gametogenesis with maturation of a macrogamete and production of a small number of microgametes inside a common
envelope, followed by the fertilisation of the macrogamete, were reported from several haemogregarines considered as *Hepatozoon* and *Hemolivia*.

Another mode of fertilisation is described in *Hepatozoon* of mammals transmitted by ticks or mites. *Hepatozoon perniciosum* Miller, 1908 [51] of the rat, the type species of the genus *H. canis*, and *H. americanum* from Canidae: syngamy, which is the association of a pair of male and female gametes and their fusion without production of flagellate microgametes.

In the literature, syngamy was described in the gregarine *Coelogregarina episeta* Ghelelovitch, 1948 [22] from *Ephesia kuehniella* (Zeller, 1879). It would be a unique example of syngamy in the Gregarinomorpha or the Coccidiomorpha may respond to *Hemolivia mauritanica* and some of its variants.

### Molecular data of *Hemolivia*

The molecular data deposited under the name *Hemolivia* into the databanks reaches 21 sequences of the 18S ribosomal RNA gene:

(i) The sequence JN211118 deposited by Barta et al. (2012) [3] as *Hemolivia mariae* Smallridge & Paperna, 1997 [67] was isolated from dined blood films containing gamonts from an experimentally infected *Tiligu rugosa* Gray, 1825 from Australia. This sequence is not included into our tree (Fig. 2) because of its shortness and of poor overlap with the rest of the sequences. However, it clusters with the *Hemolivia* sequences that are long enough to overlap (data not shown). In Barta et al. (2012) [3], it also clusters with the sequence “*Hepatozoon*” sp. EU430236 that belongs to the *Hemolivia* clade in Kvičerová et al. (2014) [34] and in our analyses (Fig. 2).

(ii) The sequence HQ224961 also deposited by Barta et al. (2012) [3] as *Hemolivia mariae* Smallridge and Paperna, 1997 [67] is mistakenly referenced in GenBank while it is clearly stated in the text of the article that it is a sequence of *Babesiosoma stableri* Schmittner & McGhee, 1961 [62] obtained from *Rana septentrionalis* Baird, 1854 collected by hand from Lake Sasajewun, Algonquin Provincial Park, Ontario, Canada. As reported [3, 34], this sequence also clusters in our analysis (Fig. 2) with the related *Dactylosoma ranae* Labbé, 1894 [35].

(iii) The sequence KC512766 deposited as *Hemolivia* sp. by Harris et al. (2013) [27] was isolated from *Hyalomma aegyptium* Linnaeus, 1758 collected on *Testudo graeca* Linnaeus, 1758 in Algeria, the original host and locality of *H. mauritanica*.

(iv) The sequence KF270674 deposited as *Hemolivia* sp. by William et al. (2014) [78] was isolated from the blood of *Panthera leo* (Linnaeus, 1758) from Zambia. This sequence is not included in our tree (Fig. 2) because of its shortness (298 bp) and in addition appears more related to sequences of *Adelina* and *Dactylosoma* than to sequences of *Hemolivia* by BLAST [2].

(v) The 13 sequences KF992698 – KF992710 deposited as *Hemolivia mauritanica* by Kvičerová et al. (2014) [34] were isolated from *Hyalomma aegyptium* L., 1758 collected on *T. graeca* from Algeria, Iraq, Syria and Turkey, and on *Testudo marginata* Shoepf, 1789 from Greece. All these sequences cluster with the sequence KC512766 (Fig. 2) and seem to correspond to *Hemolivia mauritanica* and some of its variants.

(vi) The two sequences KF992711 – KF992712 deposited as *Hemolivia mariae* by Kvičerová et al. (2014) [34] were isolated from *Amblyomma* sp. and *Bohriocroton* sp. collected on *Egernia stokesii* (Gray, 1845) from South Australia. They were collected from the original location and the same vertebrate host but in a different vector. As reported by Kvičerová et al. (2014) [34], these two sequences cluster with some of the *Hepatozoon* sp. from the Australian Reptiles.

(vii) The two sequences KF992713 – KF992714 deposited as *Hemolivia* sp. by Kvičerová et al. (2014) [34] were isolated from the blood of *Rhinoclemmys pulcherrima manni* Dunn, 1930 from Nicaragua. These sequences cluster with the *Hemolivia mauritanica* clade.

(viii) The two sequences KR069082 – KR069083 deposited as *Hemolivia parvula* by Cook et al. 2015 were isolated from the blood of *Kinixys zombensis* Hewit, 1931 from South Africa and as shown by the authors and in our analyses, belong to the *Hemolivia* clade and cluster with the *Hemolivia* of Mediterranean turtles.

Finally, *Karyolysus* and *Hemolivia* have a similar life cycle in two stages but they differ in several respects, such as the vector (according to present knowledge): mite vs. tick; the vertebrate host: lizard vs. turtle; trans-ovarian transmission vs. direct transmission. Molecular biology shows the two genera to be separated into two distinct clades with a well bootstrap value for clade A (in which the sequences of *Karyolysus* are found) but not for clade B (in which the sequences of *Hemolivia* are found).

### Phylogenetic analyses

Our analyses show the phylogenetic position of *H. stellata* to be at the base of a robust *Hemolivia* clade. This clade supports the monophyly of the genus *Hemolivia*, as previously reported [3, 34], and includes several parasites assigned to
Table 1. Main characteristics of the four haemogregarine types and the corresponding genera.

|                | Hepatozoon Type I | Karyolysus Type II | Hemolivia Type III | Bartazoon n. g. Type IV |
|----------------|-------------------|--------------------|--------------------|-------------------------|
| Vector         | Ticks or mites    | Mites              | Ticks              | Biting insects          |
| Vertebrate hosts| Mammals           | Reptiles           | Reptiles, Amphibians| Reptiles, Amphibians, Marsupials, Birds and Rodents |
| Fertilisation  | Syngamy           | Syzygy             | Syzygy             | Syzygy                  |
| Sporogony      | One stage         | Two stages         | Two stages         | One stage               |

The majority of sequences in the phylogenetic tree fall into one of the four groups defined below and correspond to a different genus characterised by its cycle, hosts and vectors. However, a few sequences appear in a group which does not correspond to the life history supposed by their authors. The risk of errors, when classifying a haemogregarine according to molecular data only, is that, when indispensable information on their life cycle is lacking, many species with sequences deposited in the databanks are not identified correctly at the generic level.

The nature of the haemogregarine described in the lizard *Varanus panoptes* (Storr, 1980), EU430236 isolated from *Lialis fuscus* Peters, 1873 from Australia [76]. When more information on their life cycle is known, they might be reassigned to *Hemolivia*.

The main sources of errors are: (i) when, in insect eating animals or their predators, sequences derived from tissues or organs where cysts from an undetermined haemogregarine are present. For example, the sequence derived from a *Hepatozoon* in the liver of a Chiroptera [59] should be interpreted with caution. We have observed on several occasions, cysts from a haemogregarine in sections of the liver from *Miniopterus* (Fig. 3) and considered them to be cysts from a *Hepatozoon* developing in a Dipteran ingested by the bat. Haemogregarine gametocytes were never found in the blood of any bat. These cysts are probably a dead end. (ii) When the host is polyparasitised and only one of the species is sequenced. (iii) When a vector from the wild is assumed to transmit the same parasite as the one seen or sequenced from the blood of the vertebrate host. The sequences of *H. fitzimonsi* KJ702453 and KR069084, originating from blood of a turtle, group with the *Hepatozoon* clade (=Bartazoon). The authors show images of sporocysts in the smears of ticks which may belong to ruptured oocysts of a *Hepatozoon* or of an accumulation of sporocysts from *Hemolivia*. The same applies for sequences EU43033 and EU43034 in samples extracted from ticks engorged on reptiles (*Varanus panoptes, Lialis fuscus* and *Dendrolaphis lustulatus*), from Australia. No description or data on the biology is attached to show that the parasite develops in the tick. The sequences extracted from the blood of Marsupials (EU430237 and EU430238) group with the *Hepatozoon* (=Bartazoon) clade. The authors found ticks on the host but did not demonstrate a role of the Acari in the transmission of the parasite.

### Systematics

The type species of the genus *Hepatozoon* is *H. perniciosum*, which raises a real problem since most other species identified as *Hepatozoon* and *Hemolivia* or *Karyolysus* do not
### Table 2. List of the sequences used in the phylogenetic construction.

The different columns give respectively the accession numbers of the sequences, the name of the parasites to which they are assigned, their vertebrate hosts, their isolation source, the country in which they have been found and the number of the associated reference in the references list. Unpub.: unpublished data only deposited in GenBank; a: experimentally fed on a naturally infected host; b: experimentally infected; c: collected from naturally infected wild host; d: *Amblyrhynchus cristatus* DNA detected in the last blood meal; n.a.: not available.

| Accession number | Parasites | Hosts | Isolation sources | Country | References |
|------------------|-----------|-------|-------------------|---------|------------|
| AF130361         | *Hepatozoon catesbianae* | Lithobates catesbeianus | n.a. | Canada | [6] |
| AF176835         | *Hepatozoon canis* | Canis familiaris | *Rhipicephalus sanguineus*<sup>a</sup> | India | [47] |
| AF176836         | *Hepatozoon americana* | Canis familiaris | *Amblyomma maculatum*<sup>ε</sup> | USA | |
| AF176837         | *Hepatozoon catesbianae* | Lithobates catesbeianus | *Culex tertiarius*<sup>ε</sup> | Canada | |
| AF297085         | *Hepatozoon* | Boiga irregularis | n.a. | Australia | Unpub. |
| AF494058         | *Adelina bambarooniae* | Dermoidea albohirtum | Host larvae | Australia | Unpub. |
| AF494059         | *Adelina bambarooniae* | Dermoidea albohirtum | Host larvae | Australia | |
| AY150067         | *Hepatozoon canis* | Vulpes vulpes | Host spleen | Spain | [13] |
| AY461375         | *Hepatozoon canis* | Cerdocyon thous | Host spleen | Brazil | |
| AY461376         | *Hepatozoon canis* | Lycalopex gymnecorous | Host spleen | Brazil | |
| AY461377         | *Hepatozoon sp.* | Cerdocyon thous | Host spleen | Brazil | |
| AY461378         | *Hepatozoon canis* | Canis familiaris | Host blood | Spain | |
| AY471615         | *Hepatozoon sp.* | Lycalopex gymnecorous | Host spleen | Brazil | |
| AY600625         | *Hepatozoon cf. erhardovae* | Clethrionomys glareolus | Host blood | Spain | |
| AY600626         | *Hepatozoon cf. erhardovae* | Clethrionomys glareolus | Host blood | Spain | |
| AY620232         | *Hepatozoon felis* | Felis catus | Host blood | Spain | |
| AY620681         | *Hepatozoon felis* | Felis catus | Host blood | Spain | |
| AT731062         | *Hepatozoon canis* | Vulpes vulpes | Host spleen | Spain | [32] |
| DQ096836         | *Adelina grylli* | Gryllus bimaculatus | Host fat body<sup>b</sup> | | |
| DQ111754         | *Hepatozoon canis* | Canis familiaris | Host blood | Sudan | [55] |
| DQ439540         | *Hepatozoon canis* | Canis familiaris | Host blood | Venezuela | [12] |
| DQ439541         | *Hepatozoon canis* | Vulpes vulpes | Host spleen | Spain | |
| DQ439543         | *Hepatozoon canis* | Canis familiaris | Host blood | Venezuela | |
| EF125058         | Reported to be host’s DNA | Cerastes cerastes | n.a. | Saudi Arabia | Unpub. |
| EF157822         | *Hepatozoon ayorgbor* | Python regius | *Culex quinquefasciatus*<sup>a</sup> | Ghana | [66] |
| EF222257         | *Hepatozoon sp.* | Martes martes | Host blood | Spain | [11] |
| EF222259         | *Hepatozoon sp.* | Scirurus vulgaris | Host blood | Spain | |
| EU172417         | *Hepatozoon ursi* | Ursus thibetanus japonicus | Host lung and blood | Japan | [33] |
| EU172418         | *Hepatozoon ursi* | Ursus thibetanus japonicus | Host lung | Japan | |
| EU289222         | *Hepatozoon canis* | Canis familiaris | n.a. | Taiwan | Unpub. |
| EU430231         | *Hepatozoon sp.* | Varanus panoptes | *Amblyomma fimbriatum*<sup>ε</sup> | Australia | [76] |
| EU430232         | *Hepatozoon sp.* | Varanus panoptes | *Amblyomma fimbriatum*<sup>ε</sup> | Australia | |
| EU430233         | *Hepatozoon sp.* | Liass fuscus | *Amblyomma moreliae*<sup>ε</sup> | Australia | |
| EU430234         | *Hepatozoon sp.* | Varanus panoptes | *Amblyomma fimbriatum*<sup>ε</sup> | Australia | |
| EU430235         | *Hepatozoon sp.* | Varanus panoptes | *Amblyomma fimbriatum*<sup>ε</sup> | Australia | |
| EU430236         | *Hepatozoon sp.* | Liass fuscus | *Amblyomma fimbriatum*<sup>ε</sup> | Australia | |
| EU430237         | *Hepatozoon sp.* | Sarcophilus harrisii | *Ixodes tasmani*<sup>ε</sup> | Australia | [75] |
| EU430238         | *Hepatozoon sp.* | Sarcophilus harrisii | *Ixodes tasmani*<sup>ε</sup> | Australia | |
| FJ198183         | *Hepatozoon sp.* | Dromiciops gliroides | Host blood | Chile | [49] |
| FJ198184         | *Hepatozoon sp.* | Dromiciops gliroides | Host blood | Chile | |
| FJ198185         | *Hepatozoon sp.* | Abrothrix olivaceus | Host blood | Chile | |
| FJ198186         | *Hepatozoon sp.* | Abrothrix sanborni | Host blood | Chile | |
| FJ198187         | *Hepatozoon sp.* | Abrothrix alivaceus | Host blood | Chile | |
| FJ198188         | *Hepatozoon sp.* | Abrothrix sanborni | Host blood | Chile | |
| HJ198189         | *Hepatozoon sp.* | Abrothrix olivaceus | Host blood | Chile | |
| HJ198189         | *Hepatozoon sp.* | Abrothrix sanborni | Host blood | Chile | |
| HM212625         | *Hepatozoon canis* | Vulpes vulpes | Host spleen | Croatia | [17] |
| HM212626         | *Hepatozoon canis* | Vulpes vulpes | Host spleen | Croatia | |
| HQ224954         | *Hepatozoon cf. catesbianae* | Lithobates catesbeianus | Host blood | Canada | [3] |
| HQ224955         | *Klossia helicina* | Cepaea nemoralis | Host tissue | France | |
| HQ224956         | *Klossia helicina* | Cepaea nemoralis | Host tissue | France | |
| HQ224957         | *Dactylosoma ramaram* | Pelophylax kl. esculentus | Host blood | France | |
| HQ224958         | *Dactylosoma ramaram* | Pelophylax kl. esculentus | Host blood | France | |
| HQ224959         | *Huemogregarina balli* | Chelydra serpentina | Host blood | Canada | |
| HQ224960         | *Hepatozoon magn* | Pelophylax kl. esculentus | Host blood | France | |
| HQ224961         | *Babesiosoma stableri* | Lithobates septentrionalis | Host blood | Canada | |

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Table 2. (continued)

| Accession number | Parasites                | Hosts                                 | Isolation sources            | Country          | References |
|------------------|--------------------------|---------------------------------------|------------------------------|------------------|------------|
| HQ224962         | Hepatozoon cf. clamatae  | Lithobates clamitans                  | Host blood                   | Canada           |            |
| HQ224963         | Hepatozoon cf. clamatae  | Lithobates clamitans                  | Host blood                   | Canada           |            |
| HQ292771         | Hepatozoon sp.           | Tachylepis wrightii                    | Host tail tissue and blood   | Seychelles       | [28]       |
| HQ292772         | Hepatozoon sp.           | Tachylepis wrightii                    | Host tail tissue and blood   | Seychelles       |            |
| HQ292773         | Hepatozoon sp.           | Lycochognathophis seychellensis       | Host tail tissue and blood   | Seychelles       |            |
| HQ292774         | Hepatozoon sp.           | Lycochognathophis seychellensis       | Host tail tissue and blood   | Seychelles       |            |
| HQ292775         | Hepatozoon sp.           | Lycochognathophis seychellensis       | Host tail tissue and blood   | Seychelles       |            |
| HQ734787         | Hepatozoon sp.           | Tarentola mauritania                  | Host tail tissue with blood  | Algeria          | [45]       |
| HQ734788         | Hepatozoon sp.           | Tarentola mauritania                  | Host tail tissue with blood  | Algeria          |            |
| HQ734789         | Hepatozoon sp.           | Quedenfeldia moerens                  | Host tail tissue with blood  | Morocco          |            |
| HQ734790         | Hepatozoon sp.           | Ptyodactylus oudrii                   | Host tail tissue with blood  | Algeria          |            |
| HQ734791         | Hepatozoon sp.           | Scelarcis perspicillata               | Host tail tissue with blood  | Morocco          |            |
| HQ734792         | Hepatozoon sp.           | Podarcis vaucheri                     | Host tail tissue with blood  | Morocco          |            |
| HQ734793         | Hepatozoon sp.           | Podarcis vaucheri                     | Host tail tissue with blood  | Morocco          |            |
| HQ734794         | Hepatozoon sp.           | Podarcis vaucheri                     | Host tail tissue with blood  | Morocco          |            |
| HQ734795         | Hepatozoon sp.           | Podarcis vaucheri                     | Host tail tissue with blood  | Morocco          |            |
| HQ734796         | Hepatozoon sp.           | Eumecees algeriensis                  | Host tail tissue with blood  | Morocco          |            |
| HQ734797         | Hepatozoon sp.           | Eumecees algeriensis                  | Host tail tissue with blood  | Morocco          |            |
| HQ734798         | Hepatozoon sp.           | Atlantolacerta andreanskyi            | Host tail tissue with blood  | Morocco          |            |
| HQ734799         | Hepatozoon sp.           | Timon tangitanus                      | Host tail tissue with blood  | Morocco          |            |
| HQ734800         | Hepatozoon sp.           | Timon tangitanus                      | Host tail tissue with blood  | Morocco          |            |
| HQ734801         | Hepatozoon sp.           | Timon tangitanus                      | Host tail tissue with blood  | Morocco          |            |
| HQ734802         | Hepatozoon sp.           | Timon tangitanus                      | Host tail tissue with blood  | Morocco          |            |
| HQ734803         | Hepatozoon sp.           | Podarcis vaucheri                     | Host tail tissue with blood  | Morocco          |            |
| HQ734804         | Hepatozoon sp.           | Podarcis vaucheri                     | Host tail tissue with blood  | Morocco          |            |
| HQ734805         | Hepatozoon sp.           | Chalcides polyplepis                 | Host tail tissue with blood  | Morocco          |            |
| HQ734806         | Hepatozoon sp.           | Tarentola mauritania                  | Host tail tissue with blood  | Morocco          |            |
| HQ734807         | Hepatozoon sp.           | Timon tangitanus                      | Host tail tissue with blood  | Morocco          |            |
| HQ734808         | Hepatozoon sp.           | Ptyodactylus oudrii                   | Host tail tissue with blood  | Morocco          |            |
| HQ734809         | Hepatozoon sp.           | Quedenfeldia moerens                  | Host tail tissue with blood  | Morocco          |            |
| HQ829430         | Hepatozoon ursi          | Melarsus urinus                       | Host blood                   | India            | [56]       |
| HQ829432         | Hepatozoon ursi          | Melarsus urinus                       | Host blood                   | India            |            |
| HQ829434         | Hepatozoon ursi          | Melarsus urinus                       | Host blood                   | India            |            |
| HQ829436         | Hepatozoon ursi          | Melarsus urinus                       | Host blood                   | India            |            |
| HQ829445         | Hepatozoon felis         | Panthera tigris                       | Host blood                   | India            | [57]       |
| HQ829446         | Hepatozoon felis         | Panthera tigris                       | Host blood                   | India            |            |
| HQ829447         | Hepatozoon canis         | Cuon alpinus                          | Host blood                   | India            |            |
| HQ829448         | Hepatozoon canis         | Cuon alpinus                          | Host blood                   | India            |            |
| JN181157         | Hepatozoon sipedon       | Rana spp. & Nerodia sipedon           | Host blood                   | Canada           | [3]        |
| JN584475         | Hepatozoon felis         | Felis catus                           | Host blood                   | India            | [57]       |
| JN584476         | Hepatozoon felis         | Felis catus                           | Host blood                   | India            |            |
| JQ080302         | Hepatozoon sp.           | not confirmedd                        | Aedes taeniorhynchus         | Ecuador          | [4]        |
| JQ080303         | Hepatozoon sp.           | not confirmedd                        | Aedes taeniorhynchus         | Ecuador          |            |
| JQ080304         | Hepatozoon sp.           | unknown                               | Aedes taeniorhynchus         | Ecuador          |            |
| JQ46622          | Hepatozoon garnhami      | Psammophis schokari                   | Host blood                   | Saudi Arabia     | [1]        |
| JX242666         | Hepatozoon sp.           | Malpolon monspessulanus              | Host tail muscle tissue      | Morocco          | [72]       |
| JX242667         | Hepatozoon sp.           | Hemorrhoids hippocrepis               | Host tail muscle tissue      | Spain            |            |
| JX242668         | Hepatozoon sp.           | Hemorrhoids hippocrepis               | Host tail muscle tissue      | Morocco          |            |
| JX242669         | Hepatozoon sp.           | Hemorrhoids hippocrepis               | Host tail muscle tissue      | Morocco          |            |
| JX531910         | Hepatozoon sp.           | Podarcis hispanicus                  | Host tail tissue with blood  | Spain            | [46]       |
| JX531917         | Hepatozoon sp.           | Podarcis hispanicus                  | Host tail tissue with blood  | Spain            |            |
| JX531920         | Hepatozoon sp.           | Podarcis lillfordi                   | Host tail tissue with blood  | Spain            |            |
| JX531921         | Hepatozoon sp.           | Podarcis bocageti                    | Host tail tissue with blood  | Portugal         |            |
| JX531928         | Hepatozoon sp.           | Podarcis bocageti                    | Host tail tissue with blood  | Portugal         |            |
| JX531930         | Hepatozoon sp.           | Podarcis bocageti                    | Host tail tissue with blood  | Portugal         |            |
| JX531931         | Hepatozoon sp.           | Podarcis bocageti                    | Host tail tissue with blood  | Portugal         |            |
| JX531932         | Hepatozoon sp.           | Podarcis bocageti                    | Host tail tissue with blood  | Portugal         |            |
| JX531933         | Hepatozoon sp.           | Algysodes marchi                     | Host tail tissue with blood  | Spain            |            |

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### Table 2. (continued)

| Accession number | Parasites                  | Hosts                              | Isolation sources                  | Country         | References |
|------------------|----------------------------|------------------------------------|------------------------------------|-----------------|------------|
| JX31940          | Hepatozoon sp.             | Algyroides marchi                  | Host tail tissue with blood        | Spain           |            |
| JX31941          | Hepatozoon sp.             | Algyroides marchi                  | Host tail tissue with blood        | Spain           |            |
| JX31953          | Hepatozoon sp.             | Podarcis bocagei                   | Host tail tissue with blood        | Spain           |            |
| KC342524         | Hepatozoon cuestensi       | Crotalus durissus terrificus       | Host blood                         | Brazil          | [54]       |
| KC342525         | Hepatozoon massardi        | Crotalus durissus terrificus       | Host blood                         | Brazil          |            |
| KC342526         | Hepatozoon cayapii         | Crotalus durissus terrificus       | Host blood                         | Brazil          |            |
| KC342527         | Hepatozoon cuestensi       | Crotalus durissus terrificus       | Host blood                         | Brazil          |            |
| KC342528         | Hepatozoon cuestensi       | Crotalus durissus terrificus       | Host blood                         | Brazil          |            |
| KS12766          | Hemolivia sp. mauritanica  | Testudo graeca                     | Hyalomma aegyptium                 | Algeria         | [27]       |
| KC696564         | Hepatozoon sp.             | Psammophys schokari                | Host tail muscle tissue            | Morocco         | [73]       |
| KC696565         | Hepatozoon sp.             | Psammophys schokari                | Host tail muscle tissue            | Morocco         |            |
| KC696566         | Hepatozoon sp.             | Psammophys schokari                | Host tail muscle tissue            | Niger           |            |
| KC696567         | Hepatozoon sp.             | Psammophys sibilans                | Host tail muscle tissue            | Burkina Faso    |            |
| KC696568         | Hepatozoon sp.             | Psammophys elegans                 | Host tail muscle tissue            | Mali            |            |
| KC696569         | Hepatozoon sp.             | Psammophys schokari                | Host tail muscle tissue            | Algeria         |            |
| KC840055         | Hepatozoon sp.             | Hippodiseros cervinus              | Host liver                         | Malaysia        | [59]       |
| KC840056         | Hepatozoon sp.             | Hippodiseros cervinus              | Host liver                         | Malaysia        |            |
| KC840057         | Hepatozoon sp.             | Hippodiseros cervinus              | Host liver                         | Malaysia        |            |
| KP022102         | Hepatozoon peireei         | Hydrobates melanita                | Mexico                             | [48]            |
| KF246565         | Hepatozoon seychellensis   | Grandisonia alternans              | Host blood                         | Seychelles      | [26]       |
| KF246566         | Hepatozoon seychellensis   | Grandisonia alternans              | Host blood                         | Seychelles      |            |
| KF257924         | Haemogregarina sp.         | Pseudos marani                    | Host blood                         | Gabon           | [18]       |
| KF257926         | Haemogregarina steapanowi  | Mauremys caspica                   | Host blood                         | Iran            |            |
| KF939620         | Hepatozoon chinensis       | Elaphe carinata                   | Host blood                         | China           | [24]       |
| KF992697         | Haemogregarina steapanowi  | Mauremys caspica                   | Host blood                         | Turkey          | [34]       |
| KF992698         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Turkey          |            |
| KF992699         | Hemolivia mauritanica      | Testudo margarita                  | Host blood                         | Greece          |            |
| KF992700         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Greece          |            |
| KF992701         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Iraq            |            |
| KF992702         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992703         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992704         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992705         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992706         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992707         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992708         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992709         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Syria           |            |
| KF992710         | Hemolivia mauritanica      | Testudo graeca                     | Host blood                         | Greece          |            |
| KF992711         | Hemolivia mariae           | Egernia stokesii                   | Host blood                         | Australia       |            |
| KF992712         | Hemolivia mariae           | Egernia stokesii                   | Host blood                         | Australia       |            |
| KF992713         | Hemolivia sp. pulcherrima  | Rhinoclemmys pulcherrima           | Host blood                         | Nicaragua       |            |
| KF992714         | Hemolivia sp. pulcherrima  | Rhinoclemmys pulcherrima           | Host blood                         | Nicaragua       |            |
| KJ189390         | Hepatozoon sp.             | Podarcis bocagei & hispanica      | Host blood                         | Portugal        | [44]       |
| KJ189418         | Hepatozoon sp.             | Podarcis bocagei & hispanica      | Host blood                         | Portugal        |            |
| KJ189426         | Hepatozoon sp.             | Podarcis bocagei & hispanica      | Host blood                         | Portugal        |            |
| KJ461939         | Karyolyus sp.              | Podarcis muralis                  | Host blood                         | Slovakia        | [24]       |
| KJ461940         | Karyolyus sp.              | Lacerta agilis                    | Host blood                         | Poland          |            |
| KJ461941         | Karyolyus sp.              | Lacerta viridis                   | Ixodes ricinus                     | Hungary         |            |
| KJ461942         | Karyolyus sp.              | Lacerta trilineata                | Host blood                         | Romania         |            |
| KJ461943         | Karyolyus sp.              | Lacerta viridis                   | Host blood                         | Hungary         |            |
| KJ461944         | Karyolyus sp.              | Lacerta viridis                   | Ophionyssus sp.                    | Hungary         |            |
| KJ461945         | Karyolyus sp.              | Zootoca vivipara                  | Ophionyssus sp.                    | Poland          |            |
| KJ461946         | Karyolyus sp.              | Zootoca vivipara                  | Host blood                         | Poland          |            |
| KJ702453         | Hepatozoon fitzsimonsi     | Chersina angulata                 | Host blood                         | South Africa    | [9]        |
| KM234646         | Hepatozoon domerguei       | Madagascarthrophis colubrinus     | Host tail muscle tissue            | Madagascar      | [43]       |
| KM234647         | Hepatozoon domerguei       | Madagascarthrophis colubrinus     | Host tail muscle tissue            | Madagascar      |            |
| KM234648         | Hepatozoon domerguei       | Ibycaphus oursi                   | Host tail muscle tissue            | Madagascar      |            |
| KM234649         | Hepatozoon domerguei       | Ibycaphus oursi                   | Host tail muscle tissue            | Madagascar      |            |
| KP119770         | Hepatozoon ixoxo           | Amietophrynus garmani             | Host blood                         | South Africa    | [52]       |

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undergo the process of fertilisation by syngamy. We think that the mode of fertilisation is an important character and a fundamental part of the cycle of haemogregarines. It is at present associated with sporogony in a tick or a mite and schizogony and gametocytes in a mammal.

According to current knowledge on life cycles, terrestrial haemogregarines can be classified into four types:

Type 1: Haemogregarines of mammals transmitted by ticks and mites; fertilisation by syngamy, sporogony in one stage, with complete sporocyst development inside the oocyst envelop: genus Hepatozoon Miller, 1908 [51], type species: Hepatozoon perviciosum Miller, 1908 [51].

Type 2: Haemogregarines of reptiles transmitted so far by mites; syzygy of gamonts followed by microgametogenesis with production of a small number of gametes inside a common envelop with the macrogamete; sporogony in two stages: mature oocysts release sporokinetes which penetrate inside new cells in which the sporocysts develop. Transovarian transmission occurs. Genus: Karyolysus Labbé, 1894 [35], type species K. lacertae (Danilewsky, 1886) Reichenow, 1913 [15, 61].

Type 3: Haemogregarines of reptiles and amphibians transmitted by ticks; syzygy of gamonts followed by microgametogenesis with production of a small number of gametes inside a common envelop with the macrogamete; sporogony in two stages: mature oocysts release sporokinetes which penetrate inside new cells in which the sporocysts develop. No transovarian transmission occurs. Genus: Hepatozoon Petit et al., 1990 [58], type species, H. stellata Petit et al., 1990 [58].

Type 4: Haemogregarines of reptiles, amphibians, birds and rodents transmitted by biting insects: syzygy of gamonts followed by microgametogenesis with production of a small number of gametes inside a common envelop with the microgamete. Sporogony in one stage, complete sporocyst development inside the oocyst envelop; genus Bartazoon n. g. Karadjian, Chavatte and Landau, type species: Bartazoon breinit (Mackerras 1960) [42], n. comb. (=Hepatozoon breiniti) of the varanid lizard.

Surprisingly, Hemolivia and Karyolysus which were considered as biologically closely related belong in fact to two different clusters, the first one with the Bartazoon n. g. and the second one with the Hepatozoon of carnivores.

Conclusion

Classification, particularly of species into genera, aims at defining biological and morphological categories common to several species. We believe that creating a taxon for species or genera is much more helpful and less confusing than leaving parasites that are obviously different together. They may be easily synonymised if new elements are produced.

We propose (i) to reassign Hepatozoon argantis to the genus Hemolivia; (ii) the following new classification of terrestrial haemogregarines, consistent with the recent phylogenetic constructions: Haemogregarines of Type I: genus Hepatozoon; Haemogregarines of Type II: genus Karyolysus; Haemogregarines of Type III: genus Hemolivia; Haemogregarines of Type IV: genus Bartazoon.

This classification is consistent with current knowledge on biology and life cycles and with molecular data on species well identified by their life history.

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References

1. Abdel-Baki AA, Al-Quraishy S, Zhang JY. 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 Parasitologica, 59(2), 294–300.
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410.
3. Barta JR, Ogedengbe JD, Martin DS, Smith TG. 2012. Phylogenetic position of the adelorinid coccidia (Myzozoa, Apicomplexa, Coccidia, Eucoecidiorida, Adeleorina) inferred using 18S rDNA sequences. Journal of Eukaryotic Microbiology, 59(2), 171–180.
4. Bataille A, Fournie G, Cruz M, Cedeno V, Parker PG, Cunningham AA, Goodman SJ. 2012. Host selection and parasite infection in Aedes taeniorhynchus, a vector in the Galapagos Islands. Infection, Genetics and Evolution, 12(8), 1831–1841.
5. Brumpt E. 1938. Formes évolutives d’Hemogregarina mauritanica chez la tique Hyalomma syriacum. Annales de Parasitologie, 16, 350–361.
6. Carreno RA, Martin DS, Barta JR. 1999. Cryptosporidium is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicoplast parasitess inferred using small-subunit ribosomal RNA gene sequences. Parasitology Research, 85(11), 899–904.
7. Chabaud AG. 1965. Cycles évoluifs des Nématodes parasites de Vertébrés. P-P Grassé – Traité de Zoologie, 4(2), 437–463.
8. Cnops L, Van Esbroeck M, Botteau E, Jacobs J. 2010. Giemsa-stained thick blood films as a source of DNA for Plasmodium species-specific real-time PCR. Malaria Journal, 9, 370.
9. Cook CA, Lawton SP, Davies AJ, Smit NJ. 2014. 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(12), 141, 1611–1620.

10. Cook CA, Smit NJ, Davies AJ. 2015. First record of an intraleucocytic Haemogregarine (Adeleorina: Haemogregarinidae) from South African Tortoises of the species Stigmochelys pardalis (Cryptodira: Testudinidae). African Zoology, 49(2), 290–294.

11. Criado-Fornelio A, Buling A, Casado N, Gimenez C, Ruas JL, Girao JA, Farias NA, Soares MJ. 2010. Molecular characterisation of arthropod-borne hematozoans in wild mammals from Brazil, Venezuela and Spain. Acta Parasitologica, 54(3), 187–193.

12. Criado-Fornelio A, Buling A, Cunha-Filho NA, Ruas JL, Farias NA, Rey-Valeirón C, Pingret JL, Etievant M, Barba-Carretéro JC. 2007. Development and evaluation of a quantitative PCR assay for detection of Hepatozoon sp. Veterinary Parasitology, 150(4), 352–356.

13. Criado-Fornelio A, Ruas JL, Casado N, Farias NA, Soares MP, Muller G, Brunt JG, Berne ME, Buling-Sarana A, Barba-Carretéro JC. 2006. New molecular data on mammalian Hepatozoon species (Apicomplexa: Adeleorina) from Brazil and Spain. Journal of Parasitology, 92(1), 93–99.

14. Danilewsky B. 1885. Die häematozoën der kaltblüter. Archiv für Mikroskopische Anatomie, 24, 588–598.

15. Danilewsky B. 1886. Recherche sur la parasitologie du sang. Archives de Zoologie Expérimentale et Générale, 85, 155–168.

16. Desser SS. 1978. Morphological, cytochemical, and biochemical observations on the blood of the tuatara, Sphenodon punctatus, New Zealand. Journal of Zoology, 5(3), 503–508.

17. Dezdek D, Voyta L, Curkovic S, Lipej Z, Mihaljevic Z, Cvetnic Z, Beck R. 2010. Molecular detection of Theileria annae and Hepatozoon canis in foxes (Vulpes vulpes) in Croatia. Veterinary Parasitology, 172(3–4), 333–336.

18. Dvorakova N, Kvicera J, Papousek I, Javanbakht H, Tiar G, Kami H, Sirikey P. 2014. Haemogregarines from western Palaearctic freshwater turtles (genera Emys, Mauremys) are conspecific with Haemogregarina stepanowi Danilewsky, 1885. Parasitology, 141(4), 522–530.

19. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5), 1792–1797.

20. Garnham PCC. 1954. A haemogregarine in Argus brumpti. Rivista di Parasitologia, 15, 425–435.

21. Garnham PCC. 1986. Catalogue of the Garnham collection of malaria parasites and other Haemosporidia. Press syndicate of the University of Cambridge: Cambridge. p. 191

22. Ghélélouitch S. 1948. Coelogregarina ephestiae, schizogréarine parasite d’Ephesia kühniella Z. (Lépidoptère). Archives de Zoologie Expérimentale et Générale, 85, 155–168.

23. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology, 59(3), 307–321.

24. Halkova-Ko Ikova BE, Hi Anova A, Majlath J, Ra Ka K, Harriss D, Faldvani G, Tryjanowski P, Koko Ova N, Mal Ekova B, Majlathova V. 2014. Morphological and molecular characterisation of Karyolyssus inverted question mark a neglected but common parasite infecting some European lizards. Parasites & Vectors, 7(1), 555.

25. Han H, Wu Y, Dong H, Zhu S, Li L, Zhao Q, Wu D, Pei E, Wang Y, Huang B. 2015. First report of Hepatozoon (Apicomplexa: Adeleorina) from king ratsnakes (Elaphe carinata) in Shanghai, with description of a new species. Acta Parasitologica, 60(2), 266–274.

26. Harris DJ, Damas-Moreira I, Maia JP, Perera A. 2014. First report of Hepatozoon (Apicomplexa: Adeleorina) in caecilians, with description of a new species. Journal of Parasitology, 100(1), 117–120.

27. Harris DJ, Graciá E, Jorge F, Maia JPMC, Perera A, Carretéro MA, Giménez A. 2013. Molecular detection of Hemolivia (Apicomplexa: Haemogregarinidae) from ticks of North African Testudo graeca (Testudinidae) and an estimation of their phylogenetic relationships using 18S rRNA sequences. Comparative Parasitology, 80, 292–296.

28. Harris DJ, Maia JP, Perera A. 2011. Molecular characterization of Hepatozoon species in reptiles from the Seychelles. Journal of Parasitology, 97(1), 106–110.

29. Herbert JD, Godfrey SS, Bull CM, Menz RI. 2010. Developmental stages and molecular phylogeny of Hepatozoon tuatrae, a parasite infecting the New Zealand tuatara, Sphenodon punctatus and the tick, Amblyomma sphenodonti. International Journal for Parasitology, 40(11), 1311–1315.

30. Kimura M, Kaneko O, Inoue A, Ishii A, Tanabe K. 1995. Amplification by polymerase chain reaction of Plasmodium falciparum DNA from Giemsa-stained thin blood smears. Molecular and Biochemical Parasitology, 70(1–2), 193–197.

31. Koch CL. 1844. Systematische Übersicht Über die Ordnung der Zecken. Archiv für Naturgeschichte, 10, 217–239.

32. Kopecna J, Jirku M, Obornik M, Tokarev YS, Lukes J, Modry D. 2006. Phylogenetic analysis of coccidian parasites from invertebrates: search for missing links. Protist, 157(2), 173–183.

33. Kubo M, Uni S, Agatsuma T, Nagataki M, Panciera RJ, Tsubota T, Nakamura S, Sakai H, Masegi T, Yanai T. 2008. Hepatozoon urini sp. nov. (Apicomplexa: Hepatozoidae) in Japanese black bear (Ursus thibetanus japonicus). Parasitology International, 57(3), 287–294.

34. Kvicera J, Hyspa V, Dvorakova N, Mikuleck J, Pandyk Z, Gardner MG, Javanbakte H, Tiar G, Sirikey P. 2014. Hemolivia and hepatozoan: haemogregarines with tangled evolutionary relationships. Protist, 165(5), 688–700.

35. Labbé A. 1894. Haemogregarines from western Palaearctic freshwater turtles (genera Emys, Mauremys) are conspecific with Haemogregarina stepanowi Danilewsky, 1885. Parasitology, 141(4), 522–530.

36. Lainson R. 1981. On Trypanosoma bourouli (Trypanosoma bourouli Neiva and Pinto, in the fish Synbranchus marmoratus: simultaneous transmission by the leech Haementeria luzi, in Parasitological topics: A presentation volume to P. C. C. Garnham, F. R. S. on the Occasion of his 80th Birthday, special publication No 1, Canning EU, Editor. Society of Protozoologists: Lawrence, Kansas. p. 150–158.

37. Laird M. 1950. Haemogregarina tuatrae sp.n., from the New Zealand Rhynchocephalian Sphenodon punctatus (Gray). Proceedings of the Zoological Society of London, 120(3), 529–533.

38. Landau I, Marteau M, Golvan Y, Chabaud AG, Boulard Y. 1975. Hétéroxénie chez les coccidies intestinales des poissons.
Compte Rendus Hebdomadaires des Séances de l’Académie des Sciences, Paris, Série D, 281, 1721–1723.

39. Landau I, Michel JC, Chabaud AG, Brygoo E. 1972. Cycle biologique d’Hepatozoon domerguei; discussion sur les caractères fondamentaux d’un cycle de Coccidie. Zeitschrift für Parasitenkunde, 38, 250–270.

40. Landau I, Paperna I. 1997. The assignment of Hepatozoon mauritanicum, a tick-transmitted parasite of tortoise, to the genus Hemolivia. Parasite, 4, 365–367.

41. Laveran I, Nègre L. 1905. Sur un protozoaire parasite de l’homme. Compte Rendu des Séances de la Société de Biologie, 57, 964–966.

42. Mackerras MJ. 1960. The Haematozoa of Australian reptiles. Australian Journal of Zoology, 9, 61–122.

43. Maia JP, Crottini A, Harris DJ. 2014. Microscopic and molecular characterization of Hepatozoon domerguei (Apicomplexa) and Foleylla furcata (Nematoda) in wild endemic reptiles from Madagascar. Parasite, 21, 47.

44. Maia JP, Harris DJ, Carranza S, Gomez-Díaz E. 2014. A comparison of multiple methods for estimating parasitemia of haemogregarine hemoparasites (Apicomplexa: Adeleorina) and its application for studying infection in natural populations. PLoS One, 9(4), e95010.

45. Maia JP, Harris DJ, Perera A. 2011. Molecular survey of Hepatozoon species in lizards from North Africa. Journal of Parasitology, 97(3), 513–517.

46. Maia JP, Paperna A, Harris DJ. 2012. Molecular survey and microscopic examination of Hepatozoon Miller, 1908 (Apicomplexa: Adeleorina) in lacertid lizards from the western Mediterranean. Folia Parasitologica, 59(4), 241–248.

47. Mathew JS, Van Den Bussche RA, Ewing SA, Malayer JR, Latha BR, Panciera RJ. 2000. Phylogenetic relationships of Hepatozoon (Apicomplexa: Adeleorina) based on molecular, morphologic, and life-cycle characters. Journal of Parasitology, 86(2), 366–372.

48. Merino S, Martinez J, Masello JF, Bedolla Y, Quillfeldt P. 2014. First molecular characterization of a Hepatozoon species (Apicomplexa: Hepatozoidea) infecting birds and description of a new species infecting Storm Petrels (Aves: Hydrobatidae). Journal of Parasitology, 100, 338–343.

49. Merino S, Vázquez RA, Martínez J, Celis-Diez JL, Gutiérrez-Jiménez L, Silvina I, Sánchez-Monsalvay I, Martínez-De La Puente J. 2009. Molecular characterization of an ancient Hepatozoon species parasitizing the “living fossil” marsupial “Monito del Monte” Dromiciops gliroides from Chile. Biological Journal of the Linnean Society, 98, 568–576.

50. Michel JC. 1973. Hepatozoon mauritanicum (Sergent & Sergent, 1904) n. comb. parasite de Testudo graeca: redescription de la sporogonie chez Hyalomma aegyptium et de la schizogonie tissulaire d’après le matériel d’E. Brumpt. Annales de Parasitologie Humaine et Comparée, 48, 11–21.

51. Miller WW. 1908. Hepatozoon perniciosum (n. g., n. sp.), a haemogregarine pathogenic for white rats; with a brief description of the sexual cycle in the intermediate host, a mite (Laelaps echidninus Berlese). Bulletin of Hygienic Laboratory of Washington, 46, 1–51.

52. Netherlands EC, Cook CA, Smit NJ. 2014. Hepatozoon species (Adeleorina: Hepatozoidea) of African bufonids, with morphological description and molecular diagnosis of Hepatozoon ixoxo sp. nov, parasitizing three Amphibolurus species (Anura: Bufonidae). Parasites & Vectors, 7, 552.

53. Neumann LG. 1907. Notes sur les Ixodidés. Archives de Parasitologie, 11, 215–232.

54. O’Dwyer LH, Moco TC, Paduan Kdos S, Spensassato C, da Silva RJ, Ribolla PE. 2013. Description of three new species of Hepatozoon (Apicomplexa, Hepatozoidea) from Rattlesnakes (Crotalus durissus terrificus) based on molecular, morphometric and morphologic characters. Experimental Parasitology, 132(2), 200–207.

55. Oyamada M, Davoust B, Boni M, Dereure J, Bucheton B, Hammad A, Itamoto K, Okuda M, Inokuma H. 2005. Detection of Babesia canis rossi, B. canis vogeli, and Hepatozoon canis in dogs in a village of eastern Sudan by using a screening PCR and sequencing methodologies. Clinical and Diagnostic Laboratory Immunology, 12(11), 1343–1346.

56. Pawar RM, Poornachandar A, Arun AS, Manikandan S, Shivaji S. 2011. Molecular prevalence and characterization of Hepatozoon ursi infection in Indian sloth bears (Melursus ursinus). Veterinary Parasitology, 182(2–4), 329–332.

57. Pawar RM, Poornachandar A, Srinivas P, Rao KR, Lakshmi-kantan U, Shivaji S. 2012. Molecular characterization of Hepatozoon spp. infection in endangered Indian wild felids and canids. Veterinary Parasitology, 186(3–4), 475–479.

58. Petit G, Landau I, Baccam D, Lainson R. 1990. Description et cycle biologique d’Hemolivia stellata n. g., n. sp., hémogrégarine de crapauds brésiliens. Annales de Parasitologie Humaine et Comparée, 65, 3–15.

59. Pinto CM, Helgen KM, Fleischer RC, Perkins SL. 2013. Hepatozoon parasites (Apicomplexa: Adeleorina) in bats. Journal of Parasitology, 99(4), 722–724.

60. Reichenow E. 1910. Haemogregarina stepanowi, die Entwicklungsgeschichte einer Haemogregarine. Archiv für Protistenkunde, 20, 251–350.

61. Reichenow E. 1913. Karyolysus lacertae, eine wirtwechselnde Coccidium der Eidechse Lacerta muralis und der Milbe Liponyssus sauvarum. Arbeit von klinischen Gesundheit Berlin, 35, 317–363.

62. Schmittner SM, McGhee RB. 1961. The intra-erythrocytic development of Babesiosoma stableri n. sp. in Rana pipiens pipiens. Journal of Protozoology, 8, 381–386.

63. Scolpel KKG, Fontes CJF, Nunes AC, Horta M, Braga EM. 2004. Low sensitivity of nested PCR using Plasmodium DNA extracted from stained thick blood smears: an epidemiological retrospective study among subjects with low parasitemia in an endemic area of the Brazilian Amazon region. Malaria Journal, 3, 8.

64. Sergent E, Sergent E. 1904. Sur une hémogrégarine, parasite de Testudo mauritanica. Compte Rendu des Séances de la Société de Biologie, 56, 130–131.

65. Siddall ME. 1995. Phylology of adelie blood parasites with a partial systematic revision of the haemogregarine complex. Journal of Eukaryotic Microbiology, 42(2), 116–125.

66. Sloboda M, Kamler M, Bulantova J, Voypatka J, Modry D. 2007. A new species of Hepatozoon (Apicomplexa: Adeleorina) from Python regius (Serpentes: Pythonidae) and its experimental transmission by a mosquito vector. Journal of Parasitology, 93(5), 1189–1198.

67. Smallridge C, Paperna I. 1997. The tick-transmitted haemogregarinid of the Australian sleepy lizard Tiliqua rugosa belongs to the genus Hemolivia. Parasite, 4, 359–363.

68. Smith TG. 1996. The genus Hepatozoon (Apicomplexa: Adeleorina). Journal of Parasitology, 82(4), 565–585.
69. Smith TG, Desser SS. 1997. Phylogenetic analysis of the genus *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina). Systematic Parasitology, 36, 213–221.

70. Solangi MA, Overstreet RM. 1980. Biology and pathogenesis of the coccidium *Eimeria funduli* infecting killifishes. Journal of Parasitology, 66(3), 513–526.

71. Telford SR. 2008. Hemoparasites of the Reptilia: Color Atlas and Text. CRC Press: Boca raton, FL. p. 376.

72. Tome B, Maia JP, Harris DJ. 2012. *Hepatozoon* infection prevalence in four snake genera: influence of diet, prey parasitemia levels, or parasite type? Journal of Parasitology, 98(5), 913–917.

73. Tome B, Maia JP, Harris DJ. 2013. Molecular assessment of apicomplexan parasites in the snake *Psammophis* from North Africa: do multiple parasite lineages reflect the final vertebrate host diet? Journal of Parasitology, 99(5), 883–887.

74. Ujvari B, Madsen T, Olsson M. 2004. High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in water pythons (*Liasis fuscus*) from tropical Australia. Journal of Parasitology, 90(3), 670–672.

75. Vilcins IM, Old JM, Deane E. 2009. Detection of a *Hepatozoon* and spotted fever group *Rickettsia* species in the common marsupial tick (*Ixodes tasmani*) collected from wild Tasmanian devils (*Sarcophilus harrisii*), Tasmania. Veterinary Parasitology, 162(1–2), 23–31.

76. Vilcins IM, Ujvari B, Old JM, Deane E. 2009. Molecular and morphological description of a *Hepatozoon* species in reptiles and their ticks in the Northern Territory, Australia. Journal of Parasitology, 95(2), 434–442.

77. Volpini AC, Marques MJ, Lopes dos Santos S, Machado-Coelho GL, Mayrink W, Romanha AJ. 2006. *Leishmania* identification by PCR of Giemsa-stained lesion imprint slides stored for up to 36 years. Clinical Microbiology and Infection, 12(8), 815–818.

78. Williams BM, Berentsen A, Shock BC, Teixiera M, Dunbar MR, Becker MS, Yabsley MJ. 2014. Prevalence and diversity of *Babesia*, *Hepatozoon*, *Ehrlichia*, and *Bartonella* in wild and domestic carnivores from Zambia, Africa. Parasitology Research, 113, 911–918.

79. Xiao FZ, Zhang SY, Xu LS, Huang JH, Xie HG, Ou YR. 2006. DNA amplification of *Plasmodium vivax* parasites from Giemsa-stained blood smears. Chinese Journal of Parasitology and Parasitic Diseases, 24(4), 290–292.

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