*Parasitology*

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# Diversity of *Karyolysus* and *Schellackia* from the Iberian lizard *Lacerta schreiberi* with sequence data from engorged ticks

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**Research Article**

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## Abstract

Apicomplexan haemoparasites of the genera *Schellackia* Reichenow, 1919, and *Karyolysus* Labbé, 1894, seem to be common in lizards and widespread across the world. For decades, their identification has been based on morphological descriptions and life cycle patterns, with molecular characterizations, applied only recently. We used molecular characterization to confirm the identification of haemoparasites detected by microscopy in blood smears of *Lacerta schreiberi* Bedriaga, 1878, a lizard of the Iberian Peninsula. Since blood samples other than blood smears were not available from the studied lizards, 264 engorged ticks *Ixodes ricinus* (Linneaus, 1758) collected from them were used as an alternative non-invasive source of haemoparasite DNA for molecular genetic analyses. Of the 48 blood smears microscopically examined, 31 were positive for blood parasites (64.6% prevalence). We identified trophozoites and gamonts similar to *Karyolysus lacazei* (Labbé, 1894) (24/48; 50%) and *Schellackia*-like sporozoites (20/48; 41.7%). Mixed infections with both species occurred in 13 blood smears (27.1%). Sequence data were obtained for both parasites from engorged ticks. Phylogenetic analyses placed our unique haemogregarine sequence within the *Karyolysus* clade, nevertheless, within substantial polytomy. Thus, according to its morphology and effect on the host cell, we refer to this haemogregarine as *Karyolysus* cf. *lacazei*. Besides the *Schellackia* sequences being identical to a previously identified haplotype, we also obtained sequences of three new closely related haplotypes.

## Introduction

The apicomplexan genera *Karyolysus* Labbé, 1894 (Adeleorina) and *Schellackia* Reichenow, 1919 (Eimeriorina) are parasites with heteroxenous life cycles that parasitize saurian hosts, both genera being transmitted by the ingestion of infected invertebrate vectors. In the life cycle of *Karyolysus*, the mite *Ophionyssus* Mégnin, 1884, is considered as the main invertebrate host (Reichenow, 1913; Svahn, 1975; Haklová-Kočíková *et al*., 2014). After blood feeding on the infected lizard, gamogony and sporogony occur in the associated mites. Merogony takes place in the endothelial cells of the liver, lungs, spleen and heart of the vertebrate host (Reichenow, 1913; Svahn, 1975; Telford, 2009). *Schellackia* parasites use a variety of invertebrates (leeches and arthropods) as paratenic hosts for the transmission of sporozoites, whereas all development takes place in the gut of vertebrates. In the peripheral blood of vertebrate hosts, the intraerythrocytic stages most frequently found are trophozoites or gamonts for *Karyolysus* and sporozoites for *Schellackia*.

Altogether, 18 species of the genus *Karyolysus* have been described and named so far (Table 1). They have been considered to be parasites of Palearctic lizards, mainly reported from European countries such as Poland, Hungary, Romania, Slovakia and Spain (Álvarez-Calvo, 1975; Haklová-Kočíková *et al*., 2014), but also from Scandinavia (Svahn, 1974, 1975) and Russia (Beyer and Sidorenko, 1984). Newly, seven species have been found in the Canary Islands (Tomé *et al*., 2019). Nevertheless, reports from India (de Mello and de Meyrelles, 1937), Kenya (Mutinga and Dipeolu, 1989) and recently also from South Africa (Cook *et al*., 2016) are changing this view. The prevailing vertebrate hosts are lacertid lizards of the genera *Gallotia*, *Lacerta*, *Podarcis* and *Zootoca*. However, records of *Karyolysus* from varanid, scincid and geckonid lizards (Mutinga and Dipeolu, 1989; Cook *et al*., 2016; Tomé *et al*., 2019) have broadened its known host range. Furthermore, based on molecular surveys, there are many probably incorrectly assigned *Hepatozoon* isolates from lizards and snakes, which might resemble species of *Karyolysus* (Maia *et al*., 2011; Tomé *et al*., 2012, 2013; Karadjian *et al*., 2015). For the genus *Schellackia*, there are 11 described and named species parasitizing lizards worldwide. Eight species have been reported from the Old World, two from North America and one in Brazil (Table 1). Additionally, two species from South America are reported infecting frogs: *Schellackia balli* Le Bail and Landau, 1974 from the Cane toad (*Rhinella marina*) and an unnamed *Schellackia* from a Brazilian tree-frog (*Phrynohyas venulosa*) (Le Bail and Landau, 1974; Paperna and Lainson, 1995).



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**Table 1.** List of described and named *Karyolysus* and *Schellackia* species

| |
|---|
| *Karyolysus* species |
| *K. atlanticus* (Tomé *et al.*, 2019) |
| *K. biretortus* (Nicolle, 1904) |
| *K. canariensis* (Tomé *et al.*, 2019) |
| *K. galloti* (Tomé *et al.*, 2019) |
| *K. gomerensis* (Tomé *et al.*, 2019) |
| *K. jorgei* (de Mello and de Meyrelles, 1937) |
| *K. lacazei* (Labbé, 1894) |
| *K. lacertae* (Danilewsky, 1886) |
| *K. latus* (Svahn, 1975) |
| *K. makariogeckonis* (Tomé *et al.*, 2019) |
| *K. minor* (Svahn, 1975) |
| *K. octocromosomi* (Álvarez-Calvo, 1975) |
| *K. paradoxa* (Dias, 1954) |
| *K. poleensis* (Mutinga and Dipeolu, 1989) |
| *K. stehlini* (Tomé *et al.*, 2019) |
| *K. subtilis* (Ricci, 1954) |
| *K. tinerfensis* (Tomé *et al.*, 2019) |
| *K. zuluetai* (Reichenow, 1920) |
| *Schellackia* species (including *Lainsonia* spp. transferred to the genus *Schellackia*) |
| *S. agamae* (Laveran and Pettit, 1909) |
| *S. balli* (Le Bail and Landau, 1974) |
| *S. bocagei* (Álvarez-Calvo, 1975) |
| *S. bolivari* (Reichenow, 1919) |
| *S. brygooi* (Landau, 1973) |
| *S. calotesi* (Ray and Sarkar, 1969) |
| *S. golvani* (Rogier and Landau, 1975) |
| *S. iguanae* (Landau, 1973) syn. *L. iguanae* (Landau 1973) |
| *S. landaue* (Lainson *et al.*, 1976) |
| *S. mabuyai* (Mutinga and Dipeolu, 1989) |
| *S. occidentalis* (Bonorris and Ball, 1955) |
| *S. orientalis* (Telford, 1993) |
| *S. ptyodactyli* (Paperna and Finkelman, 1996) |
| *S. weinbergi* (Leger and Mouzels, 1917) syn. *L. legeri* (Landau *et al.*, 1974) |

Furthermore, two *Lainsonia* Landau, 1973 species (*Lainsonia iguana* Landau, 1973 and *Lainsonia legeri* Landau *et al.*, 1974), described also from Brazil (Landau, 1973; Landau *et al.*, 1974) are considered by some authors to be *Schellackia* (Levine, 1980; Upton, 2000). Thus, according to their distribution data, the two genera, *Karyolysus* and *Schellackia*, potentially overlap over a large geographic area. Specifically, from the Iberian Peninsula, *Schellackia bolivari* Reichenow, 1919, *Schellackia bocagei* Álvarez-Calvo, 1975, *Karyolysus lacertae* (Danilewsky, 1886) and *Karyolysus octocromosomi* Álvarez-Calvo, 1975, have been reported (Reichenow, 1919; Álvarez-Calvo, 1975).

Former descriptions of these parasites are based on the morphology of intraerythrocytic stages, often supplied with detailed life history (Reichenow, 1919; Landau, 1973; Álvarez-Calvo, 1975; Svahn, 1975; Mutinga and Dipeolu, 1989; Bristovetzky and Paperna, 1990; Finkelman and Paperna, 1998). Increasingly, traditional morphological approaches are being supported by molecular genetic tools, which clarify the phylogenetic position and correct taxonomic placement, for example, the polyphyly of the genus *Hepatozoon* Miller, 1908, and family Lankesterellidae, or the change of generic allocation of *Karyolysus paradoxa* (Dias, 1954) from *Hepatozoon* to *Karyolysus* (Megía-Palma *et al.*, 2013, 2014, 2018; Haklová-Kočíková *et al.*, 2014; Karadjian *et al.*, 2015; Cook *et al.*, 2016).

In a previous study, Stuart-Fox *et al.* (2009) detected unspecified haemogregarines from blood smears of *Lacerta schreiberi* Bedriaga, 1878, from the Iberian Peninsula. Here, we work with material from that study to identify these haemoparasites by combining both microscopy and PCR-based methods. Since no blood other than the blood smears was available, we use engorged ticks collected from the lizards as an alternative source of host blood and also of haemoparasite DNA. Hence, a secondary aim of this study was to test the possibility of using this alternative non-invasive source of blood to genetically characterize the haemoparasites of these lizards.

## Materials and methods

### Sampling

Ticks were collected from *L. schreiberi* lizards in several localities in the Central System mountains of the Iberian Peninsula around the Portuguese–Spanish border during April and May 2006 in the framework of a previous study, and during an additional sampling season in May 2007 (see Stuart-Fox *et al.*, 2009 and Table S1). A subset of the ticks found on the lizards was collected and preserved in 70% ethanol (see Kubelová *et al.*, 2015). After clipping the tail tip, blood smears were made of a drop of blood (Stuart-Fox *et al.*, 2009). Tick species and life-stage determination was done using available keys (Nosek and Sixl, 1972; Estrada-Peña *et al.*, 2004). The ticks and blood smears used in the present study are from 48 lizards (see details in Table S1).

### Morphological diagnosis of blood protists

All 48 blood smears were fixed in absolute methanol for 5 min, stained with Giemsa for 15 min and examined with an Olympus BX53 microscope using the 100× magnification lens equipped with immersion oil (Široký *et al.*, 2004). The intensity of parasitaemia was estimated for each infected lizard as the percentage of infected red blood cells found in approximately $10^4$ cells (Široký *et al.*, 2007). Images of parasites were captured using an Olympus DP 73 digital camera and QuickPhoto Micro 3.0 software. Basic morphometric characteristics were measured: maximum length and width of distinguished developmental stages of the parasite, and maximum length and width of nuclei for gamonts where possible; LW (length × width) and L/W (length/width) values were calculated. All measurements are given in micrometres ($\mu$m) as the mean followed by standard deviation and range in parentheses. Morphological identification of blood parasites was based on data from the literature (Álvarez-Calvo, 1975; Svahn, 1975; Telford, 2009; Megía-Palma *et al.*, 2013, 2014; Haklová-Kočíková *et al.*, 2014).

### DNA extraction, amplification and sequencing

A total of 264 DNA samples of *Ixodes ricinus* (Linneaus, 1758) ticks were used. Each DNA sample represents a single nymph of *I. ricinus*. Nymphs were collected from 48 lizards with 1–23 (5.5 on average) nymphs per lizard. These samples correspond to a subset of the nymph DNA samples used in Kubelová *et al.* (2015) and obtained by alkaline hydrolysis (Rijpkema *et al.*,

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**Table 2.** *p*-distances (lower-left) and number of nucleotide differences (upper-right), between our *Karyolysus* cf. *lacazei* sequence and haemogregarine sequences in three groups composed according to phylogenetic tree in Fig. 2

| | | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 1 | *Karyolysus* cf. *lacazei* MK497254 | | 1 | 9 | 13 | 26 |
| 2 | *Karyolysus* clade | 0.002 | | 6 | 10 | 23 |
| 3 | Intraleucocytic *Hepatozoon* clade | 0.020 | 0.014 | | 7 | 20 |
| 4 | Other *Hepatozoon* clade | 0.030 | 0.024 | 0.016 | | 20 |
| 5 | Outgroup | 0.058 | 0.052 | 0.045 | 0.045 | |

The number of base differences per site from estimation of net average between groups of sequences in the 455 bp long fragment are shown. All positions containing gaps and missing data were eliminated.

1996), following the modified protocol as described in Kubelová *et al.* (2011).

For PCR detection of apicomplexan DNA, we used five pairs of primers targeting part of the 18S rDNA (for the primer sequences and PCR conditions, see Table S2). Primers EF/ER were designed for *Eimeria* spp. and they also amplify both haemogregarines and haemococcidia (Kvičerová *et al.* 2008). To distinguish between haemogregarines and haemococcidia, and for diagnostic purposes, the other primers specific for haemococcidia were also used, which amplify shorter portions of the same gene (BT-F1/EimRodR, Hep600F1/Hep1600R, Sbol2F/EimRodR and BT-F1/SbolR) (Megía-Palma *et al.* 2013, 2014). All PCR reactions were carried out in 25 μl volumes, including 1 μl each of 10 μM primer, 12.5 μl of Combi PPP Master Mix (Top-Bio, Czech Republic), 9.5 μl of water and 1 μl of extracted DNA. Morphologic determination of ticks was completed by molecular analysis because of the similar morphologies of preimaginal stages of ixodid ticks, especially between *I. ricinus* and the newly described species from the Mediterranean – *Ixodes inopinatus* (Estrada-Peña *et al.*, 2014). This molecular determination was based on the part of the 16S rRNA gene (Table S2, Mangold *et al.* 1998). PCR reactions for species diagnosis of ticks were carried out in a final volume of 23 μl including 0.25 μl each of 10 μM primer, 12.5 μl of Combi PPP Master Mix, 9.5 μl of water and 0.5 μl of extracted DNA. Products were separated by electrophoresis on a 1.5% agarose gel with nucleic acid staining solution Midori Green (Elisabeth Pharmacon, Brno, Czech Republic) and visualized using a UV transilluminator. All parasite positive PCR products were cut off as gel slices and purified using a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, New Taipei City, Taiwan) and Sanger sequenced on an automatic 3730XL DNA analyser (Macrogen Inc., Amsterdam, the Netherlands).

### Phylogenetic analyses

The sequences generated in this study were edited in Geneious 9.1.5 (Kearse *et al.*, 2012) and compared with the GenBank database (www.ncbi.nlm.nih.gov/genbank/) using the BLAST algorithm (blast.ncbi.nlm.nih.gov/Blast.cgi). The following analyses were carried out for haemogregarines and *Schellackia*-like parasites separately. Our sequences together with selected sequences available from GenBank (see Tables S3 and S4 for accession numbers) were aligned using the MUSCLE algorithm (Edgar, 2004). The final alignment of haemogregarines with a length of 1850 bp consisted of 45 sequences. The final alignment for haemococcidians with 1618 bp consisted of 24 sequences. A substitution model GTR + G + I was selected by the Smart model selection software (Lefort *et al.*, 2017) as the best model. Bayesian inference (BI) analysis was performed in Geneious 9.1.5 (Ronquist and Huelsenbeck, 2003) using Mr. Bayes 3.2.6 plugin with the GTR + G + I model for $10^7$ generations, with trees and parameters sampled every 200 generations. The trees were summarized after a 'burn-in' of 25%. Maximum likelihood (ML) phylogenetic analysis was performed by PhyML 2.2.0 plugin in Geneious 9.1.5 (Guindon and Gascuel, 2003) with the GTR + G + I model and parameters estimated from the data. Nodal supports were generated with 1000 bootstrap replicates. *Haemogregarina stepanowi* Danilewsky, 1885, was set as an outgroup for the haemogregarines and *Acroeimeria tropidura* (Aquino-Shuster, Duszynski and Snell, 1990) for the haemococcidia. Trees were visualized using Tree-Graph 2.12.0 (Stöver and Müller, 2010) and graphically edited in Adobe Illustrator CS6.

Genetic distances as *p*-distances and number of nucleotide differences were calculated in Mega 5.0 (Tamura *et al.*, 2011). Our haemogregarine sequence was compared with the groups corresponding to clades obtained in the phylogenetic analyses (Table 2) and then to groups created within the *Karyolysus* clade on the base of the phylogeny (Table 3) and their geographical origin (Table 4). Pairwise distances between our haemogregarine sequence and other sequences from *Karyolysus* clade were also calculated (Table S5).

## Results

### Parasite identification

Altogether, 31 out of the 48 examined lizards (prevalence 64.6%) were diagnosed to be infected by blood protists. Two types of parasites were observed: trophozoites and gamonts similar to those previously described as *Karyolysus lacazei* (Labbé, 1894) in 24 smears (50%; Fig. 1A–D); and *Schellackia*-like sporozoites in 20 blood smears (41.7%; Fig. 1E–H). Double infection by both parasites occurred in 13 cases (27.1%). Parasitaemia of infected individuals ranged between 0.02 and 4.24% (mean 0.62%) for *Karyolysus* cf. *lacazei*; and between 0.01 and 0.12% (mean 0.04%) for *Schellackia*. Parasites were localized in red blood cells or occasionally seen freely in plasma. We also found objects similar to the sporozoites of *Schellackia*, rarely found in leucocytes (Fig. 1H).

Two developmental stages of *Karyolysus* were distinguished. Trophozoites showed a variety of shapes, from reniform (Fig. 1A) to oval or slender (Fig. 1B). Pale blue or pinkish stained cytoplasm was markedly vacuolated. A granular or reticular nucleus spread across the whole width of the parasite was placed centrally. Trophozoites measured 14.6 ± 1.6 (12–18) × 3.9 ± 1.0 (3–6) ($n$ = 35). Gamonts (Fig. 1C, D) appeared uniform with an elongate slender shape, and one end recurved as described by Reichenow (1919), or bent according to Svahn (1975); usually, they were placed parallel to the host cell nucleus. Cytoplasm stained pale blue and no vacuoles were present. The nuclei of gamonts were elongated and filled the entire width of the parasite. Although sometimes clearly demarcated, they were usually diffuse making their exact measurements impossible. Nuclei were located in the centre, or more often shifted closer to the bend. Macro- and micro-gamonts were indistinguishable. Gamonts measured: 20.3 ± 1.6 (17–26) × 3.1 ± 0.4 (2–4)

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**Table 3.** *p*-distances (lower-left) and number of nucleotide differences (upper-right) between our *Karyolysus* cf. *lacazei* sequence and clades I–V in the *Karyolysus* clade

| | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 1 | *Karyolysus* cf. *lacazei* MK497254 | | 1 | 5 | 6 | 9 | 27 |
| 2 | Clade V | 0.001 | | 6 | 6 | 9 | 26 |
| 3 | Clade IV | 0.011 | 0.012 | | 3 | 6 | 26 |
| 4 | Clade III | 0.014 | 0.013 | 0.007 | | 7 | 25 |
| 5 | Clade II | 0.020 | 0.019 | 0.014 | 0.016 | | 31 |
| 6 | Clade I | 0.059 | 0.057 | 0.058 | 0.055 | 0.068 | |

The number of base differences per site from estimation of net average between groups of sequences in the 455 bp long fragment are shown. All positions containing gaps and missing data were eliminated.

**Table 4.** *p*-distances (lower-left) and number of nucleotide differences (upper-right) between our *Karyolysus* cf. *lacazei* sequence and four geographic groups from *Karyolysus* clade sequences

| | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 1 | *Karyolysus* cf. *lacazei* MK497254 | | 1 | 1 | 3 | 9 | 27 |
| 2 | *Karyolysus* spp. of Central Europe | 0.002 | | 2 | 4 | 9 | 28 |
| 3 | *Karyolysus* spp. of Canary Islands[a] | 0.002 | 0.004 | | 2 | 8 | 24 |
| 4 | *Hepatozoon* spp. (*Karyolysus*) of North Africa | 0.006 | 0.008 | 0.003 | | 6 | 24 |
| 5 | *K. paradoxa* from South Africa | 0.020 | 0.019 | 0.018 | 0.013 | | 31 |
| 6 | *Karyolysus makariogeckonis* from Canary Islands | 0.059 | 0.061 | 0.053 | 0.052 | 0.068 | |

*K. makariogeckonis* is separated from Canary Island group due to its significant genetic difference. The number of base differences per site from estimation of net average between groups of sequences in the 455 bp long fragment are shown. All positions containing gaps and missing data were eliminated.
[a]Without *K. makariogeckonis* MG787251.

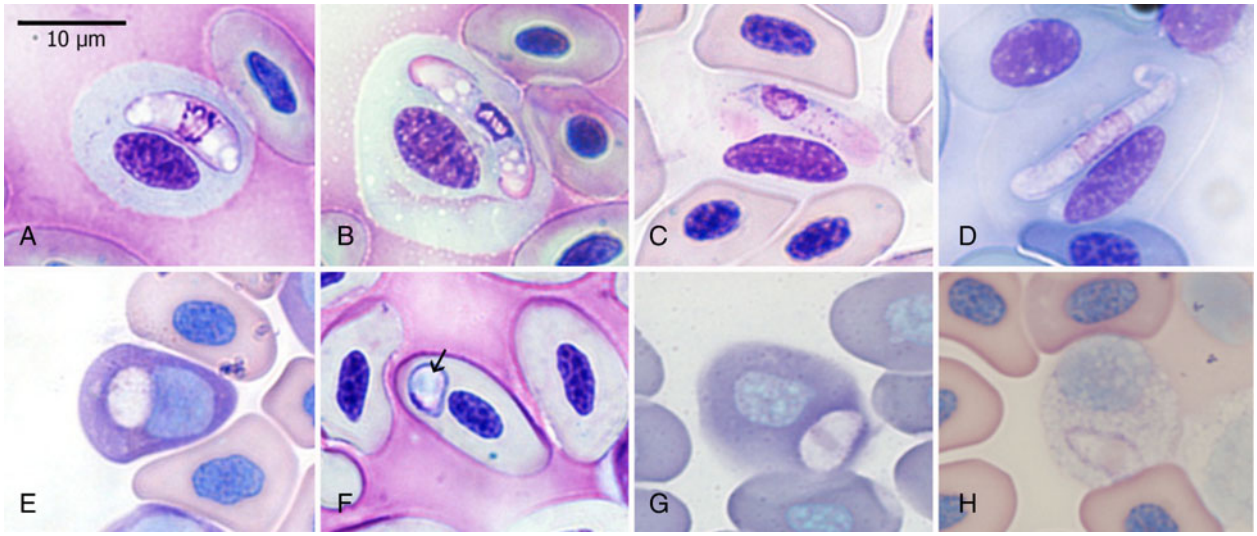


**Fig. 1.** Haemoparasites of *L. schreiberi*. *K.* cf. *lacazei*: trophozoites (A, B) and gamonts (C, D). Sporozoites of *Schellackia* sp. (E–F). The arrow indicates refractile body.

($n = 118$), LW = 63.3 ± 9.2 (38–96) $\mu m^2$, L/W = 6.6 ± 0.9 (4.3–10.5). Nuclei measured: 5.8 ± 1.0 (4–8) × 3.3 ± 0.3 (3–4) ($n = 32$), LW = 17.9 ± 3.2 (12–24) $\mu m^2$, L/W = 1.9 ± 0.3 (1.3–2.7).

Gamonts and trophozoites had an evident impact on the host cell (Fig. 1A-D). Hypertrophy and loss of the staining properties of cytoplasm and nucleus appeared in all infected cells. In some cases, erythrocyte cytoplasm was completely absent. The host cell nucleus stained paler in infected than uninfected cells, and was swollen and displaced laterally.

The shape of *Schellackia* sporozoites ranged from round (in 11% of cases) and oval (23%) (Fig. 1E) to broadly pyriform (66%) (Fig. 1F–H). Cytoplasm stained light pinkish. A single round refractile body with diameter 2 $\mu m$ located in the centre of the sporozoite was clearly seen in only one case (Fig. 1F). The nuclei showed variable forms and placements: narrow bands touching the parasite membrane (Fig. 1F), a band-like nucleus usually situated centrally (Fig. 1G, H); a couple of chromatin granules, or diffuse areas of chromatin (Fig. 1E). Sporozoites were situated in a polar or lateropolar position within erythrocytes and under light microscopy generated no visible changes to the infected cells. Sporozoites measured 5.89 ± 0.70 (5.0–7.0) × 3.49 ± 0.52 (3.0–5.0) ($n = 81$).

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**Fig. 2.** Phylogenetic tree of *Karyolysus* and related haemogregarines based on Bayesian inference (BI) analyses of 18S rDNA. Numbers at the nodes show posterior probabilities under BI/bootstrap values for maximum likelihood (ML) higher than 0.50 or 50%, respectively. Nodal supports (BI/ML) lower than 0.50 or 50% are marked with an asterisk (*). Branch with *K. makariogeckonis* is shortened in half. New sequence obtained in this study is marked in bold.

All ticks were identified by microscopic examination as nymphs of *I. ricinus*. All analysed ticks showed 100% molecular identity with *I. ricinus*.

## Phylogenetic analyses

We obtained 24 18S rDNA sequences: four sequences (929–1442 bp), all identical, belonged to haemogregarines, and 20 sequences corresponding to four haplotypes belonging to haemococcidia (926–1497 bp). For the phylogenetic analysis, our longest haemogregarine sequence (1442 bp) and three new haplotypes of haemococcidia (1025 bp for Ls-E, 1006 bp for Ls-D and 1497 bp for Ls-C) were used (see Tables S3 and S4 for accession numbers). Both BI and ML analyses provided phylogenies with identical topologies. The phylogenetic tree for haemogregarines is shown in Fig. 2 and the one for *Schellackia*-like sequences in Fig. 3.

Our haemogregarine sequence (MK497254) clustered together with known *Karyolysus* species from lacertid, geckonid and varanid hosts and *Hepatozoon* species from snakes (Colubridae, Lamprophiidae), skinks (Scincidae), lizards (Lacertidae) and geckos (Phyllodactylidae) (Fig. 2). Genetic distances are shown in Tables 2, 3, 4 and Table S5. From three groups of sequences according to the clades in the haemogregarine phylogenetic tree (*Karyolysus* clade, intraleucocytic *Hepatozoon* clade and *Hepatozoon* clade) all of our haemogregarine sequences were most closely related to the *Karyolysus* clade with a *p*-distance of 0.002, whereas the genetic distance to the intraleucocytic *Hepatozoon* species was 0.020 and to other *Hepatozoon* species 0.030 (Table 2). Five main clades (clades I–V in Fig. 2) are in the *Karyolysus* clade: I. *K. makariogeckonis*; II. *K. paradoxa*; III. *K. canariensis* and haemogregarines from other lacertids; IV. haemogregarines from North African snakes and skinks; V. haemogregarines from lizards including the rest of the Canarian *Karyolysus* species and the sequence identified in this study (for their *p*-distances see Table 3). Three species in the fifth clade, *K. lacazei*, *K. galloti* and *K. gomerensis*, formed separated clusters while other sequences remained in the polytomy. Within the *Karyolysus* clade, our sequence was most closely related to the *Karyolysus* species of Central Europe and Canary Islands (*p*-distance 0.002 for both) (Table 4). According to the pairwise distances, our haemogregarine was most similar to *Karyolysus atlanticus* (MG787249) from the Canary Islands and *Karyolysus* sp. (KJ461944) from Central Europe (Hungary) (Table S5).

Three out of 20 obtained sequences of *Schellackia* were identical to haplotype Ls-A (JX984674) previously found in *L. schreiberi* (Megía-Palma *et al.*, 2013). Three other new haplotypes, Ls-C (MK512382), Ls-D (MK512383) and Ls-E (MK512384), formed a monophyletic cluster with this *Schellackia* sp. haplotype Ls-A (Fig. 3). All *Schellackia* sequences form a monophyletic clade but with low support with *Eimeria arnyi* (AY613853) from the ringneck snake and *E. ranae* (EU717219) from terrestrial frogs at basal positions. Our new haplotypes came from 17 ticks collected from 9 individual lizards. Haplotype Ls-E was the most common (9/17), followed by haplotype Ls-C (7/17), while haplotype Ls-D occurred only in one tick. One lizard was infested by ticks containing all four haplotypes; two lizards were infested by ticks containing two haplotypes, Ls-C and Ls-E, and another one with Ls-A and Ls-E. Each tick contained only one haplotype.

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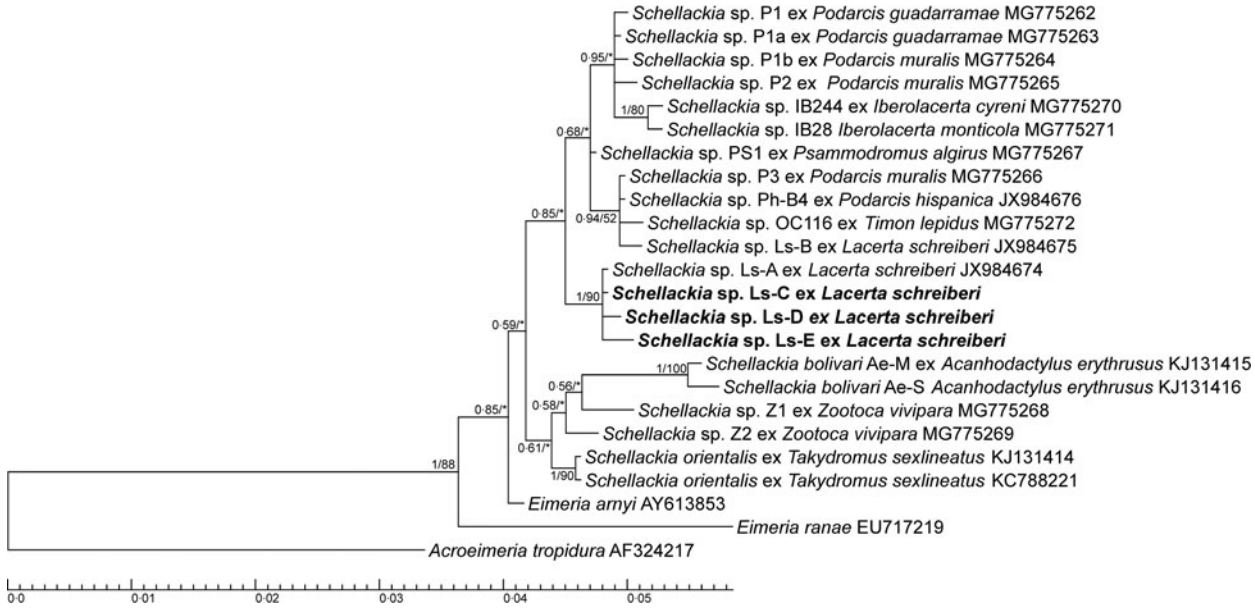


**Fig. 3.** Phylogenetic tree of *Schellackia* sp. based on Bayesian inference (BI) analyses of 18S rDNA. Numbers at the nodes show posterior probabilities under BI/bootstrap values for maximum likelihood (ML) higher than 0.50 or 50%, respectively. Nodal supports (BI/ML) lower than 0.50 or 50% are marked with an asterisk (*). The three new *Schellackia* haplotypes (Ls-C, Ls-D, Ls-E) are marked in bold.

### *Usability of engorged ticks as an alternative source of haemoparasite DNA*

Primer pair EF/ER confirms positive microscopic findings in 71% of cases, but notably, the primer pair also diagnoses infection in 41% of microscopically negative samples. Primers Sbol2F/EimRodR confirm microscopic findings of haemococcidia in 61% of cases; another 15% were discovered in specimens microscopically haemococcidia negative. Hence, primers EF/ER had a sensitivity of 71%, a specificity of 59% and an accuracy of 67%, whereas primers Sbol2F/EimRodR had a sensitivity of 61%, a specificity of 85% and an accuracy of 71% (Fig. S1).

The correlation between PCR detection of haemoparasites from engorged nymphs and microscopic findings is high for lizards with six and more collected nymphs (6–23 nymphs). Three exceptions included lizards that were PCR positive for *Schellackia*, but negative for haemococcidia in microscopy. Results were markedly contrasted in lizards carrying 1–5 nymphs. Among 35 lizards, for 18 lizards their PCR and microscopy findings correlated fully (microscopy and PCR for both haemogregarine and haemococcidia), for four lizards only partially, whereas for the remaining 13 lizards they did not correlate. Negative microscopy with positive PCR results accounted for most of the miscorrelations (8/13 lizards). The remaining five lizards were microscopically positive with negative results from PCR analyses. For a comparison of the microscopy and PCR based analyses for each particular lizard see Fig. 4.

## Discussion

We detected two types of apicomplexan parasites, the genera *Karyolysus* and *Schellackia*, infecting the Iberian lizard *L. schreiberi* using a combination of microscopy and PCR based methods. The genetic diversity of *Schellackia* was higher than that of *Karyolysus*, with four haplotypes detected in our tick samples *vs* a single haplotype, respectively, although haemogregarines were more prevalent.

Morphological uniformity of reptilian blood apicomplexans makes their microscopic differentiation on the generic level difficult. Recently, the combination of microscopy with the use of molecular genetic markers has become standard in species diagnosis and taxonomy of apicomplexans. Nevertheless, even such an approach cannot avoid all risk of error, such as mismatches between the results of microscopy and molecular genetic data from mixed infections. Such mismatch can occur, for example, when one of the co-infecting parasitic species dominates on blood smears, whereas PCR based diagnosis can be more sensitive to the other one, e.g. due to the use of better matching primers. Also, the use of engorged nymphs itself as the source of host blood brings uncertainty into the results. The use of *I. ricinus*, a species of three-host tick, introduces the possibility that blood parasites from a previous host become included in the analysis. Thus far, ticks have not been reported as part of the *Karyolysus* life cycle. Gamonts and sporokinetes have been found in mites (Svahn, 1975; Haklová-Kočíková *et al.*, 2014), while they have not been observed in tick squashes (Haklová-Kočíková *et al.*, 2014; Cook *et al.*, 2016). For haemococcidia, haematophagous invertebrates including ticks are merely passive vectors. Sporozoites accumulate in the wall of the digestive tract of invertebrates but there is no development (Telford, 2009). We are not aware of transstadial or transovarial transmission of these haemoparasites in *I. ricinus* having been recorded. Based on the available information, the infection of the ectoparasites before blood feeding seems to be unlikely. Thus, we assume that the examined ticks were merely acting as paratenic or temporary transport hosts for *Schellackia* and *Karyolysus* respectively by feeding on the sampled lizards. Nevertheless, haematophagous ectoparasites can be used as a valuable non-invasive source of host and/or haemoparasite genetic information (Mathew *et al.*, 2000; Sloboda *et al.*, 2007; Vilcins *et al.*, 2009; Bataille *et al.*, 2012; Harris *et al.*, 2013; Haklová-Kočíková *et al.*, 2014; Hamšíková *et al.*, 2016). This method may be suitable especially for small-sized hosts where manipulation and blood collection can be difficult, particularly in threatened or endangered species.

The application of engorged nymphs as the source of host/parasite DNA is possible, but its reliability remains uncertain. Our dataset was unbalanced regarding nymph collection (1–23 nymphs per lizard, mean 5.5, S.D. 5.4). The used primers possess various specificity and sensitivity, and amplicons of different length. Individual lizards were set as PCR positive if carrying at

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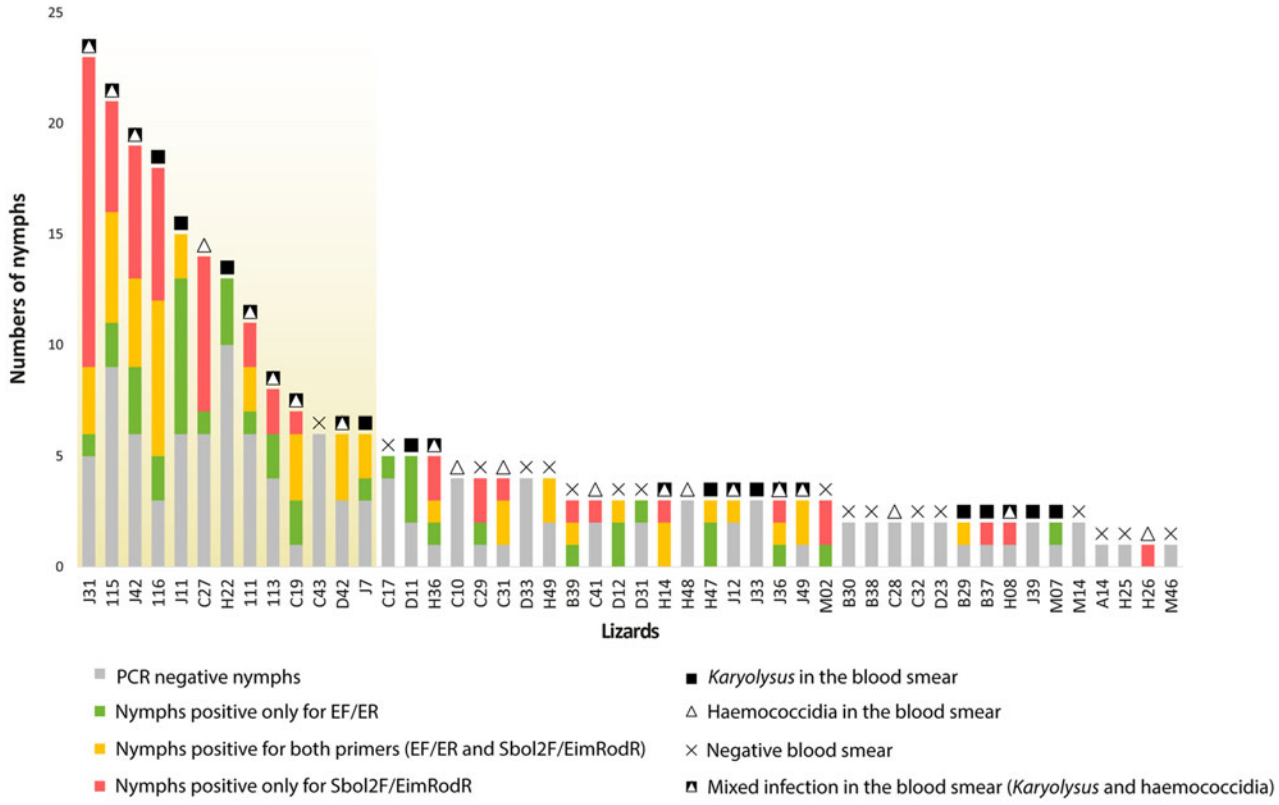


**Fig. 4.** Summary view on PCR and microscopic results of individual lizards. Lizards are ordered in descending number of collected nymphs. Symbols above columns refer to microscopic findings. Correlating results between PCR detection of haemoparasites from engorged nymphs and microscopic findings on blood smears in the lizards with six and more collected nymphs are marked with a yellowish background.

least one positive nymph. These factors may all have distorted the results provided in Fig. 4, and Fig. S1, where correlations between lizards, PCR results, and microscopy are shown. Nevertheless, the values of sensitivity, specificity and accuracy according to Fig. S1 indicate our method to be suitable. According to this study, six and more collected nymphs per lizard could provide reliable PCR analysis comparing with microscopic findings. PCR detection is generally more sensitive than microscopy. Nevertheless, the volumes of blood meal of nymphs are small and depend on feeding time. Thus the rate of nymphs' engorgement also influences the results and should be adjusted for. This might be so in the case of microscopically positive, but PCR negative lizards in our study.

The observed morphology of available life stages of haemogregarines was similar or identical to those of *K. lacazei* (Svahn, 1975; Haklová-Kočíková *et al.*, 2014). Both species reported from Spain, *K. octocromosomi* and *K. lacertae*, differ from our haemogregarine in morphology (see Table S6 for comparison of *Karyolysus* species). The phylogenetic analysis places our sequence within a clade containing ten morphologically described and named species of *Karyolysus* (see Fig. 2) along with *Hepatozoon* isolates lacking morphological descriptions. These *Hepatozoon* isolates originate mostly from lacertid hosts. However, a few sequences are from other squamates from the Mediterranean region and North Africa (snakes and skinks), and are clearly separated from the clade of intraerythrocytic *Hepatozoon* from amphibians and reptiles. *Hepatozoon* isolates in the *Karyolysus* clade are obviously wrongly assigned members of the genus *Karyolysus* (Fig. 2). This statement is in concordance with generic transfers of *K. paradoxa* and *K. canariensis* on the basis of both molecular and morphological traits. According to phylogeny and nomenclature development on the generic level, including generic allocations of haemogregarines discussed thoroughly during the last decade (Maia *et al.*, 2012; Haklová-Kočíková *et al.*, 2014; Kvičerová *et al.*, 2014; Karadjian *et al.*, 2015; Cook *et al.*, 2016), we consider the placement of our isolate into the genus *Karyolysus* to be correct. The alignment of 18S rDNA did not solve the phylogeny of our *Karyolysus* isolate, which together with other available sequences of different species of *Karyolysus* form a poorly resolved clade with considerable polytomy. Genetic distances revealed that *Karyolysus* species from Central Europe (*Karyolysus* sp. from Hungary) and *K. atlanticus* from the Canary Islands are the most closely related to our isolate, having almost equal *p*-distance values. Overall, genetic distances based on available data for *Karyolysus* clade are low and these organisms are genetically very similar regardless of their geographic origin.

Along with molecular data, the morphology and biology of the parasites may help their diagnosis. *Karyolysus* gamonts cause typical destruction of the host cell with some exceptions (Telford, 2009); e.g. *Karyolysus minor* Svahn, 1975, does not seem to have any effects on host cells (Svahn, 1975). Haklová-Kočíková *et al.* (2014) considered that the *Karyolysus* clade includes both forms – with and without an effect on the host cell. Maia *et al.* (2012) did not provide morphological descriptions; however, in the presented figures typical changes of host cells are visible. Other studies (Maia *et al.*, 2011; Tomé *et al.*, 2012, 2013, 2016; Damas-Moreira *et al.*, 2014) provided no information about morphology. However, morphological changes of host cells analogous to those induced by *Karyolysus* have been reported in *Hepatozoon theileri* (Laveran, 1905) from frog hosts that are grouped within the *Hepatozoon* clade (Fig. 2) (Netherlands *et al.*, 2014; Conradie *et al.*, 2017). *Hepatozoon curvirostris* (Billet, 1904) from the lacertid host *Timon pater* (Lataste, 1880) also causes karyolysis, but an early study of this by Billet (1904) naturally lacks molecular data. The effect on host cells is not

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always uniform among species. For example, various levels of erythrocyte distortion were observed between seven Canarian species of *Karyolysus* (Tomé *et al*., 2019). The recently redescribed *K. paradoxa* (Cook *et al*., 2016) shows specific effects on host cells such as the shrinkage of erythrocytes and vacuolization of its nucleus, different from the effects of other species of *Karyolysus*. Additionally, the hypertrophy of the host cell observed with light microscopy may look different under electron microscopy. While for *Hepatozoon ixoxo* Netherlands *et al*., 2014, hypertrophy was confirmed, *H. theileri* can be observed causing extensive flattening of the host cell, with small excrescences covering the surface of the infected erythrocyte; this is not observable at the light microscopy level (Conradie *et al*., 2017). According to the morphology of available blood stages, phylogenetic analysis and the distortion effect on host cells, we refer to this haemogregarine as *Karyolysus* cf. *lacazei*. Our finding also represents the first detection of *Karyolysus* in *L. schreiberi*.

It seems that the *Schellackia*-like parasites show some genetic variability within the 18S rRNA. A recent study by Megía-Palma *et al*. (2018) found a high haplotype diversity for *Schellackia* in lacertid lizards with 11 new haplotypes, which mostly showed high host-specificity. To date, two *Schellackia* haplotypes have been found in *L. schreiberi* and have been shown to be host specific (Megía-Palma *et al*., 2013, 2018). We obtained four *Schellackia* haplotypes, one identical to haplotype Ls-A, the first genetically characterized *Schellackia* species (Megía-Palma *et al*., 2013), and three new haplotypes closely related to haplotype Ls-A. Haplotype Ls-A was previously found in the same host species in Segovia (Spain) 300 km away from our sampling area. The morphology of our isolates is very similar to the description of *Schellackia* haplotype Ls-A (Megía-Palma *et al*., 2013). Additionally, another *Schellackia* sp. with similar morphology but genetically different, haplotype Ls-B, was found in *L. schreiberi* in the same locality as haplotype Ls-A (Megía-Palma *et al*., 2013). Within named *Schellackia* species, two were described from the Iberian Peninsula – *S. bocagei* and *S. bolivari* (the type species of the genus). However, both differ from our isolates in morphological traits, and *S. bolivari* also by the available sequence data (Álvarez-Calvo, 1975; Reichenow, 1919; Megía-Palma *et al*., 2014). We observed no correlation between the shape of sporozoites and the haplotypes. The variety of shapes of sporozoites was seen in each of the positive blood smears. Also, various haplotypes were found in several ticks collected from one lizard. Phylogenetic analysis placed our isolates among a group of unnamed *Schellackia* (Fig. 3), and available morphological traits obtained from blood smears were insufficient to make thorough comparisons with already described species and/or a proper description of new species, respectively.

Proper identification of the haemoprotozoa requires a complex approach. Molecular tools, parasite morphology and its effect on the host cell, life cycle patterns, host associations and geographic distribution should be used together to reach the most accurate result. Even applied molecular genetic methods are not always specific enough for species determination. The 18S rDNA is handy for comparison with data from public databases but offers insufficient information for studies on the phylogeny of lower taxa, namely species. This situation may improve with the application of a multigene approach.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0031182019001112.

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**Conflict of interest.** None.

**Ethical standards.** Not applicable.

## References

**Álvarez-Calvo JA** (1975) Nuevas especies de hemococcidios en lacértidos españoles. *Cuadernos de Ciencias Biológicas* **4**, 207–222.

**Bataille A, Fournié G, Cruz M, Cedeño V, Parker PG, Cunningham AA and Goodman SJ** (2012) Host selection and parasite infection in *Aedes taeniorhynchus*, endemic disease vector in the Galápagos Islands. *Infection, Genetics and Evolution* **12**, 1831–1841.

**Beyer TV and Sidorenko NV** (1984) *Karyolysus* sp. (Haemogregarinidae, Adeleida, Apicomplexa): host–parasite relationships of persisting stages. *Journal of Protozoology* **31**, 513–517.

**Billet A** (1904) Sur l'hémogrégarine du lézard ocellé d'Algérie. *Comptes Rendus des Séances de la Société de Biologie* **16**, 186.

**Bristovetzky M and Paperna I** (1990) Life cycle and transmission of *Schellackia* cf. *agamae*, a parasite of the starred lizard *Agama stellio*. *International Journal for Parasitology* **20**, 883–892.

**Conradie R, Cook CA, du Preez LH, Jordaan A and Netherlands EC** (2017) Ultrastructural comparison of *Hepatozoon ixoxo* and *Hepatozoon theileri* (Adeleorina: Hepatozoidae), parasitising South African anurans. *Journal of Eukaryotic Microbiology* **64**, 193–203.

**Cook CA, Netherlands EC and Smit NJ** (2016) Redescription, molecular characterisation and taxonomic re-evaluation of a unique African monitor lizard haemogregarine *Karyolysus paradoxa* (Dias, 1954) n. comb. (Karyolysidae). *Parasites & Vectors* **9**, 347.

**Damas-Moreira I, Harris DJ, Rosado D, Tavares I, Maia JP, Salvi D and Perera A** (2014) Consequences of haemogregarine infection on the escape distance in the lacertid lizard, *Podarcis vaucheri*. *Acta Herpetologica* **9**, 119–123.

**de Mello F and de Meyrelles CC** (1937) On the classification and schizogonic cycle of a blood parasite of the Indian lizard Calotes versicolor Daud, subspecies major Blyth. *Proceedings of the Indian Academy of Sciences - Section B* **5**, 119–141.

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

**Estrada-Peña A, Bouattour A, Camicas JL and Walker AR** (2004) *Ticks of Domestic Animals in the Mediterranean Region. A Guide to Identification of Species*. Zaragoza, Spain: University of Zaragoza.

**Estrada-Peña A, Nava S and Petney T** (2014) Description of all the stages of *Ixodes inopinatus* n. sp. (Acari: Ixodidae). *Ticks and Tick-borne Diseases*. **5**, 734–743.

**Finkelman S and Paperna I** (1998) *Schellackia calotesi* n. sp. from agamid lizards of the genus *Calotes* in Thailand. *Parasite* **5**, 23–26.

**Guindon S and Gascuel O** (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704.

**Haklová-Kočíková B, Hižňanová A, Majláth I, Račka K, Harris DJ, Földvári G, Tryjanowski P, Kokošová N, Malčeková B and Majláthová V** (2014) Morphological and molecular characterization of *Karyolysus* – a neglected but common parasite infecting some European lizards. *Parasites & Vectors* **7**, 555.

**Hamšíková Z, Silaghi C, Rudolf I, Venclíková K, Mahríková L, Slovák M, Mendel J, Blažejová H, Berthová L, Kocianová E, Hubálek Z, Schnittger L and Kazimírová M** (2016) Molecular detection and phylogenetic analysis of *Hepatozoon* spp. in questing *Ixodes ricinus* ticks and rodents from Slovakia and Czech Republic. *Parasitology Research* **115**, 3897–3904.

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

**Karadjian G, Chavatte JM and Landau I** (2015) 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*. *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, Meintjes P and Drummond A** (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* **28**, 1647–1649.

**Kubelová M, Tkadlec E, Bednář M, Roubalová E and Široký P** (2011) West-to-east differences of *Babesia canis* canis prevalence in *Dermacentor reticulatus* ticks in Slovakia. *Veterinary Parasitology* **180**, 191–196.

**Kubelová M, Papoušek I, Bělohlávek T, De Bellocq JG, Baird SJE and Široký P** (2015) Spotted fever group rickettsiae detected in immature stages of ticks parasitizing on Iberian endemic lizard *Lacerta schreiberi* Bedriaga, 1878. *Ticks and Tick-borne Diseases* **6**, 711–714.

**Kvičerová J, Pakandl M and Hypša V** (2008) Phylogenetic relationships among *Eimeria* spp. (Apicomplexa, Eimeriidae) infecting rabbits: evolutionary significance of biological and morphological features. *Parasitology* **135**, 443–452.

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

**Landau I** (1973) Diversité des mécanismes assurant la pérennité de l'infection chez lês sporozoaires coccidiomorphes. *Memoirs du Museum national d'histoire naturelle* (*France*). *Nouvelle Serie. Serie A. Zoologie* **77**, 1–62.

**Landau I, Lainson R, Boulard Y and Shaw JJ** (1974) Transmission au laboratoire et description de l'Hémogrégarine *Lainsonia legeri* n. sp. (Lankesterellidae) parasite de Lézards brésiliens. *Annales de Parasitologie Humaine et Comparée* **49**, 254–263.

**Le Bail O and Landau I** (1974) Laboratoire Description et cycle biologique expérimental de Schellackia balli n. sp. (Lankesterellidae) parasite de Crapauds de Guyane. *Annales de Parasitologie Humaine et Comparée* **49**, 663–668.

**Lefort V, Longueville JE and Gascuel O** (2017) SMS: Smart Model Selection in PhyML. *Molecular Biology and Evolution* **34**, 2422–2424.

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

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

**Mangold AJ, Bargues MD and Mas-Coma S** (1998) Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitology Research* **84**, 478–484.

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

**Megía-Palma R, Martínez J and Merino S** (2013) Phylogenetic analysis based on 18S rRNA gene sequences of *Schellackia* parasites (Apicomplexa: Lankesterellidae) reveals their close relationship to the genus *Eimeria*. *Parasitology* **140**, 1149–1157.

**Megía-Palma R, Martínez J and Merino S** (2014) Molecular characterization of haemococcidia genus *Schellackia* (apicomplexa) reveals the polyphyletic origin of the family Lankesterellidae. *Zoologica Scripta* **43**, 304–312.

**Megía-Palma R, Martínez J, Cuervo JJ, Belliure J, Jiménez-Robles O, Gomes V, Cabido C, Pausas JG, Fitze PS, Martín J and Merino S** (2018) Molecular evidence for host–parasite co-speciation between lizards and *Schellackia* parasites. *International Journal for Parasitology* **48**, 709–718.

**Mutinga MJ and Dipeolu OO** (1989) Saurian malaria in Kenya: description of new species of haemoproteid and haemogregarine parasites, *Anaplasma*-like and *Pirhemocyton*-like organisms in the blood of lizards in West Pokot District. *International Journal of Tropical Insect Science* **10**, 401–412.

**Netherlands EC, Cook CA, Smit NJ and du Preez LH** (2014) Redescription and molecular diagnosis of *Hepatozoon theileri* (Laveran, 1905) (Apicomplexa: Adeleorina: Hepatozoidae), infecting *Amietia quecketti* (Anura: Pyxicephalidae). *Folia Parasitologica* **61**, 293–300.

**Nosek J and Sixl W** (1972) Central European ticks (Ixodoidea): key for determination. *Mitteilungen der Abteilung fuer Zoologie am Landesmuseum Joanneum* **1**, 61–92.

**Paperna I and Lainson R** (1995) *Schellackia* (Apicomplexa: Eimeriidae) of the Brazilian Tree-Frog, *Phrynohyas venulosa* (Amphibia: Anura) from Amazonian Brazil. *Memorias do Instituto Oswaldo Cruz* **90**, 589–592.

**Reichenow E** (1913) Karyolysus lacertae, ein wirtwechselndes Coccidium der Eidechse Lacerta muralis und der Milbe Liponyssys saurarum. *Arbeiten aus dem Gesundheitsamte Berlin* **45**, 317–363.

**Reichenow E** (1919) Der Entwicklungsgang der Hämococcidien *Karyolysus* und *Schellackia* nov. gen. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin* **10**, 440–447.

**Rijpkema S, Golubić D, Molkenboer M, Verbeek-De Kruif N and Schellekens J** (1996) Identification of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. *Experimental and Applied Acarology* **20**, 23–30.

**Ronquist F and Huelsenbeck JP** (2003) Mrbayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* **19**, 1572–1574.

**Široký P, Kamler M and Modrý D** (2004) Long-term occurrence of *Hemolivia* cf. *mauritanica* (apicomplexa: Adeleina: Haemogregarinidae) in captive *Testudo marginata* (Reptilia: Testudinidae): evidence for cyclic merogony? *Journal of Parasitology* **90**, 1391–1393.

**Široký P, Kamler M, Frye FL, Fictum P and Modrý D** (2007) Endogenous development of *Hemolivia mauritanica* (Apicomplexa: Adeleina: Haemogregarinidae) in the marginated tortoise *Testudo marginata* (Reptilia: Testudinidae): evidence from experimental infection. *Folia Parasitologica* **54**, 13–18.

**Sloboda M, Kamler M, Bulantová J, Votýpka J and Modrý 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**, 1189–1198.

**Stöver BC and Müller KF** (2010) Treegraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**, 7.

**Stuart-Fox D, Godinho R, de Bellocq JG, Irwin NR, Brito JC, Moussalli A, Široký P, Hugall AF and Baird SJE** (2009) Variation in phenotype, parasite load and male competitive ability across a cryptic hybrid zone. *PLoS ONE* **4**, e5677.

**Svahn K** (1974) Incidence of blood parasites of the genus *Karyolysus* (coccidia) in Scandinavian lizards. *Oikos* **25**, 43–53.

**Svahn K** (1975) Blood parasites of the genus *Karyolysus* (coccidia, Adeleidae) in Scandinavian lizards. Description and life cycle. *Norwegian Journal of Zoology* **23**, 277–295.

**Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S** (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

**Telford Jr SR** (2009) *Hemoparasites of the Reptilia: Color Atlas and Text*. Boca Raton, Florida, USA: CRC Press, Taylor & Francis Group.

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

**Tomé B, Maia JPMC and 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**, 883–887.

**Tomé B, Rato C, Perera A and Harris DJ** (2016) High diversity of *Hepatozoon* spp. in geckos of the genus *Tarentola*. *Journal of Parasitology* **102**, 476–480.

**Tomé B, Pereira A, Harris DJ, Carretero MA and Perera A** (2019) A paradise for parasites? Seven new haemogregarine species infecting lizards from the Canary Islands. *Parasitology* **146**, 728–739.

**Upton SJ** (2000) *The Illustrated Guide to the Protozoa*, vol. **1**. Lawrence, Kansas, USA: Allen Press.

**Vilcins IME, Ujvari B, Old JM and 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**, 434–442.

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