RESEARCH

Molecular characterization of haemosporidian and haemogregarine diversity in southwestern Iberian amphibians and reptiles

Daniel Parejo-Pulido[1] · Carlos Mora-Rubio[2] · Alfonso Marzal[2,3] · Sergio Magallanes[2,4]

Abstract
The knowledge of the diversity and geographic distribution of parasite species is the first step towards understanding processes of global epidemiology and species conservation. Despite recent increases in research on haemosporidian and haemogregarine parasites of reptiles and amphibians, we still know little about their diversity and parasite-host interactions, especially in the Iberian Peninsula, where a few studies have been conducted. In this study, the haemosporidian and haemogregarine diversity and phylogenetic relationships of the parasites in southwestern Iberian amphibians and reptiles were assessed using PCR approaches on blood samples of 145 individuals from five amphibian and 13 reptile species. The amphibians did not present any of both groups of parasites studied. Regarding reptiles, five *Hepatozoon*, one *Haemogregarina*, and one *Haemocystidum* haplotypes were found infecting four different species, revealing new host records for these parasites. Among them, we found one new *Haemocystidium* haplotype and three new and a previously reported *Hepatozoon* haplotype from a north African snake. The latter finding suggests that some *Hepatozoon* parasites may not be host-specific and have large geographic ranges even crossing geographical barriers. These results increased the knowledge about the geographic distribution and the number of known host species of some reptile apicomplexan parasites, highlighting the great unexplored diversity of them in this region.

Section Editor: Charlotte Oskam

✉ Sergio Magallanes
sergio.magallanes@ebd.csic.es

[1] Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ronda de Toledo, 12, 13005 Ciudad Real, Spain

[2] Departamento de Anatomía Biología Celular y Zoología, Universidad de Extremadura, Avda. Elvas s/n, 06006 Badajoz, Spain

[3] Grupo de Investigaciones en Fauna Silvestre, Universidad Nacional de San Martín, Jr. Maynas 1777, 22021 Tarapoto, Perú

[4] Department of Wetland Ecology (EBD-CSIC), Estación Biológica de Doñana, Avda. Américo Vespucio 26, E-41092 Seville, Spain

Introduction

Parasites are one of the most ubiquitous and abundant organisms in the world having a wide range of negative effects on their host life-histories (Schmid-Hempel 2011). Moreover, parasites play key roles not only on individual hosts, but also on many ecology interactions, host population dynamics, and community structure in many ecosystems (Price et al. 1986; Wood et al. 2007). In this scenario, the knowledge of the diversity and geographic distributions of parasite species at various spatial scales and host groups is the first step towards understanding processes of global epidemiology and species conservation (du Toit et al. 2013; Wells et al. 2015). The phylum Apicomplexa is a highly diverse protist group of obligatory parasitic organisms. The medical and veterinary importance of some of its genera (e.g., *Plasmodium*, *Toxoplasma*, and *Babesia*, among others) has contributed to the increase in the number of the studies on this group of parasites in the last two decades (Yabsley and Shock 2012; Santiago-Alarcón and Marzal 2020). Even so, the number

of formally described species is far from the existing reality (Votýpka et al. 2016). In addition, many of these species were described on mammal and avian hosts, while studies of amphibians' and reptiles' apicomplexan diversity are even scarcer (Telford 2009).

Amphibians and reptiles are hosts to a wide variety of hemoparasitic apicomplexan groups, including hemococidians, haemogregarines, haemosporidians, piroplasms, and Sarcocystidae (Davies and Johnston 2000; Telford 2009; Muriel et al. 2021). Haemogregarines of the genus *Hepatozoon* are the most common apicomplexans infecting these animals, although other haemogregarine genera such as *Haemogregarina*, *Hemolivia*, and *Karyolysus* can be also found (Smith 1996; Davies and Johnston 2000; Telford 2009). Haemogregarines have a heteroxenous life cycle with two different hosts: a vertebrate host in which asexual reproduction occurs, and an invertebrate vector in which sexual reproduction happens (Telford 2009). Recorded invertebrate vectors of apicomplexan parasites of reptiles and amphibians include ticks, mites, biting flies, mosquitoes, and leeches (Smith 1996). Likewise, haemosporidians of the genera *Plasmodium*, *Haemoproteus*, and *Haemocystidium* are also heteroxenous parasites with similar life cycles transmitted by hematophagous dipterans (Garnham 1966; Valkiūnas 2005). Among them, *Plasmodium* and *Haemocystidium* have been reported infecting reptiles, whereas *Haemoproteus* has been found in both amphibians and reptiles (Davies and Johnston 2000; Valkiūnas 2005; Telford 2009; Muriel et al. 2021). However, the genus *Haemocystidium* has not been fully accepted by the academic community due to its limited knowledge, and it was previously synonymized with the genus *Plasmodium* first and later with the genus *Haemoproteus* (Javanbakht et al. 2015; Austen et al. 2020). Nevertheless, molecular studies have shown that *Haemocystidium* species reported from reptiles are a distinct group from avian *Haemoproteus* and thus all reptilian *Haemoproteus* should be reclassified within the genus *Haemocystidium* (Maia et al. 2016a; Pineda-Catalan et al. 2013).

The establishment of molecular techniques for the detection of blood parasites (Vilcins et al. 2009) has revealed unexpectedly high levels of apicomplexan parasite diversity in amphibians and reptiles worldwide (O'Dwyer et al. 2013; Tomé et al. 2014; Harris et al. 2015). For example, many new lineages and species of *Hepatozoon* of certain regions and host species have been identified using these techniques (e.g., Maia et al. 2012; Rajabi et al. 2017; Gutiérrez-Liberato et al. 2021). In contrast, studies about amphibian and reptile haemosporidians are scarcer in the literature compared to those of haemogregarines (e.g., Fantham et al. 1942; Martinele et al. 2016; Matta et al. 2018). The pattern of distribution and genetic diversity of parasite species is shaped by the range of hosts in which they occur (Poulin et al. 2011). However, it is still unknown a large share of the diversity of

amphibian and reptile haemogregarine and haemosporidian fauna from many regions and host species. Therefore, the screening of different species and in different countries is needed in order to reveal new lineages and new parasite-host interactions.

In the Iberian Peninsula, haemogregarine parasites have been reported from 14 species of reptiles such as *Algyroides marchi*, *Psammodromus algirus*, or *Timon lepidus* (Amo et al. 2005a, 2007; Maia et al. 2012), and to a greater extent in species of the genus *Podarcis* (Amo et al. 2005b; Roca and Galdón 2010; Maia et al. 2012), while none from amphibians (Seabra-Babo et al. 2015). In contrast, to the best of our knowledge, no studies have been conducted on amphibian and reptile haemosporidians in this area. This study aimed to characterize the diversity of haemosporidian and haemogregarine parasites in some species of amphibians and reptiles in the southwestern Iberian Peninsula by using molecular approaches. Furthermore, the phylogenetic relationships of the haemosporidian and haemogregarine species detected with those found to date are analyzed.

Materials and methods

Sample collection

The study was carried out across eight localities in the Extremadura region (southwestern Iberian Peninsula) (Fig. 1) in 2021. These areas were mainly composed of holm oak woodlands (*Quercus ilex* L.), grasslands, and farmlands of typically Mediterranean climate, characterized by temperate, rainy winters, and dry, hot summers (average annual temperature and rainfall 16 °C, 615 mm) (García and Mateos 2010). Samplings were conducted sporadically and non-systematically throughout the whole year (from January to December). Amphibians and reptiles were collected opportunistically by hand or with nets searching under stones used for shelter, or among aquatic vegetation. They were handled by experienced staff and held in plastic containers for on-site blood collection immediately in the field in aseptic conditions. A blood sample of each individual was taken using heparinized needles from different body areas depending on the species. Amphibian blood samples were taken from the ventral tail vein in newts and the ventral abdominal vein in frogs and toads (Allender and Fry 2008). Blood from all reptiles was collected from caudal tail vein (Sykes and Klaphake 2008), except for turtles, in which blood was drawn from the caudal sinus at the base of the tail (Polo-Cavia et al. 2010). Samples were immediately stored in 500 µl of SET buffer (0.015 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) (Sambrook et al. 1989) for subsequent molecular analysis. All the individuals were safely released and at the same point of capture after ensuring that

Fig. 1 Map displaying the sampling locations in Extremadura region, Iberian Peninsula. Size of circles and the number written in them show sample size collected in each location. Locations with infected individuals found are shown in red, whereas locations without any infected individuals are shown in gray
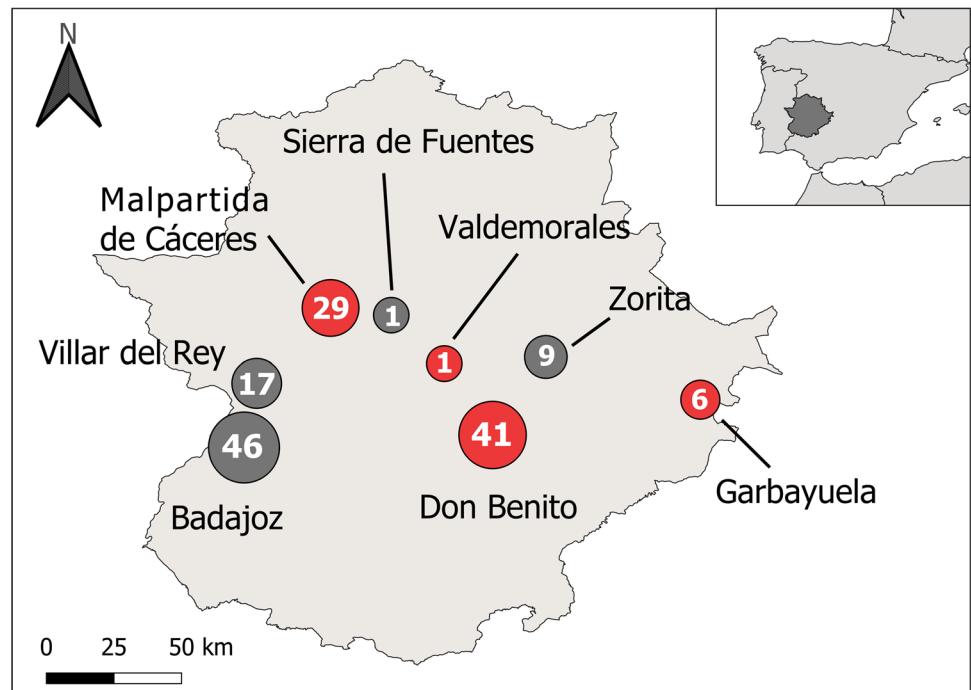
they were in perfect condition. We obtained blood samples of 86 amphibians belonging to five species and 59 reptiles belonging to 13 species (Table 1).

Molecular detection of blood parasite infections

Total genomic DNA from blood samples was extracted with the Thermo KingFisher purification system™, using the MagMAX™ Cell-Free DNA Isolation kit (Thermo Fisher, Ref. A32700) and PK buffer for MagMAX™-96 DNA Multi sample kit (Thermo Fisher, Ref. 4,489,111). DNA samples were screened to identify haemosporidian parasites following the protocol by Hellgren et al. (2004), as it has been previously used to identify *Haemocystidium* in reptiles (Oliveira et al. 2018; Austen et al. 2020). We used a nested polymerase chain reaction (PCR) that targets a 478-bp fragment (excluding primers) of the mitochondrial cytochrome b gene of these parasites following Hellgren et al. (2004). This procedure is based on a first PCR using primers Haem-NFI (5′-CAT ATA TTA AGA GAA ITA TGG AG-3′) and HaemNR3 (5′-ATA GAA AGA TAA GAA ATA CCA TTC-3′), followed by a second PCR using primers HaemF (5′-ATG GTG CTT TCG ATA TAT GCA TG-3′) and HaemR2 (5′-GCA TTA TCT GGA TGT GAT AAT GGT-3′). To detect the presence of haemogregarine parasites, we amplified the 18S RNA gene of *Hepatozoon* spp., as it has been previously used in other studies in reptiles (Harris et al. 2011; Marzal et al. 2017). This method uses a nested PCR amplification targeting a 600 bp of the 18S rRNA gene of apicomplexan parasites initially using the primers HEMO1

(5′-TAT TGG TTT TAA GAA CTA ATT TTA TGA TTG-3′) and HEMO2 (5′-CTT CTC CTT CCT TTA AGT GAT AAG GTT CAC-3′) (Perkins and Keller 2001) and then primers HepF300 (5′-GTT TCT GAC CTA TCA GCT TTC GAC G-3′) and HepR900 (5′-CAA ATC TAA GAA TTT CAC CTC TGA C-3′) (Ujvari et al. 2004). Negative and positive controls were run with each reaction. Parasites detected by a positive amplification were purified using the Genejet PCR Purification Kit (Thermo-Fisher Scientific) and then sequenced in both directions on an ABI 3130 genetic analyzer (provided by the Service of Bioscience Applied Techniques of the University of Extremadura, SAIUEx). A consensus sequence was created by combining the two partially overlapping regions of the forward and reverse sequences of each sample with Geneious Prime 2019.2.3 (Kearse et al. 2012). Obtained consensus sequences were then compared against published ones to find the best match on GenBank using BLAST. Three different sequences matched 100% with haemogregarine parasites previously found in reptiles. The new sequences found were deposited in GenBank under the accession numbers ON458135, ON332722, ON332723, and ON332724.

The genetic relationships of the *Haemocystidum* and haemogregarine parasites were investigated separately by analyzing the haemosporidian cytochrome b and the 18S rDNA sequence divergence respectively using the program MEGA11 v. 11.0.11 (Tamura et al. 2021). All available *Haemocystidium* sequences from GenBank were included to build the phylogenetic tree. For the haemogregarines, only those found in Mediterranean hosts were selected.

Table 1 Samples analyzed for apicomplexan parasites (haemosporidians and haemogregarines) in amphibians and reptiles from southwestern Iberian Peninsula. "Haemos" and "Haemog" represent the number of *Haemocystidium* and haemogregarine positive sequences, respectively. Shown are also accession numbers of positive sequences in GenBank

Host species	N	Haemos	Haemog	Accession number
Amphibians				
Fam. Bufonidae				
Epidalea calamita	41			
Fam. Hylidae				
Hyla meridionalis	1			
Fam. Ranidae				
Pelophylax perezi	27			
Fam. Salamandridae				
Pleurodeles waltl	9			
Triturus pygmaeus	8			
Reptiles				
Fam. Blanidae				
Blanus cinereus	3			
Fam. Colubridae				
Natrix maura	13			
Fam. Gekkonidae				
Hemidactylus turcicus	1			
Fam. Geoemydidae				
Mauremys leprosa	3		**1**	KJ740753
Fam. Lacertidae				
Acanthodactylus erythrurus	2			
Podarcis virescens	3		**2**	ON332722, JX531954
Psammodromus algirus	6			
Psammodromus occidentalis	3			
Timon lepidus	5			
Fam. Lamprophiidae				
Malpolon monspessulanus	6		**3**	ON332723, ON332724, KC696567
Fam. Phyllodactylidae				
Tarentola mauritanica	10	**2**		ON458135
Fam. Scincidae				
Chalcides bedriagai	1			
Chalcides striatus	3			

The *Haemocystidium* and haemogregarine phylogenetic trees were rooted with a sequence of *Plasmodium falciparum* (GeneBank M76611) and *Adelina bambarooniae* (GeneBank AF494059), respectively. We generated an alignment of 33 sequences, with a total length of 470 bp for *Haemocystidium* and an alignment of 68 sequences, with a total length of 508 bp for haemogregarines. The AIC criterion was used to choose the best model of sequence evolution and the parameters employed for our set of sequences. Maximum likelihood analyses were performed using a general time reversible model (GTR) with a Gamma distribution and invariant sites for the *Haemocystidium* sequences and a Tamura 3-parameter model (T92) with a Gamma distribution for the haemogregarine sequences. Support for nodes was estimated using the bootstrap technique (Felsenstein 1985) with 1000 replicates. Estimates of evolutionary divergences were also calculated (Supplementary material).

Results

Of the 86 amphibians analyzed, we did not find any infected individual neither by haemosporidian nor haemogregarine parasites. On the other hand, of 59 reptiles screened, two individuals were infected by *Haemocystidium*, one individual was infected by *Haemogregarina* and five by *Hepatozoon* (Table 1).

After comparing our sequences with those published in GenBank, we found seven parasite lineages infecting sampled individuals: one *Haemocystidium*, one *Haemogregarina*, and five *Hepatozoon* lineages. From

them, four parasite sequences had not been previously detected in prior studies, and therefore were considered as new haplotypes: one *Haemocystidium* sequence was detected in *Tarentola mauritanica* (ON458135), two *Hepatozoon* lineages were found infecting *M. monspessulanus* individuals (ON332723 and ON332724), and one *Hepatozoon* lineage was detected in a *P. virescens* (ON332722). The other three haplotypes (KC696567, JX531954, and KJ740753) had been found in prior studies infecting reptiles.

The *Haemocystidium* phylogenetic analyses revealed the affiliation of the new haplotype from *T. mauritanica* with *Haemocystidium* sequences from geckos and snakes, which were separated from lineages found in chelonians (Fig. 2). The haemogregarine phylogenetic analyses revealed that the new *Hepatozoon* lineages from *M. monspessulanus* were closely related to other *Hepatozoon* sequences found in Iberian and North African snakes forming an individual clade. The new lineage obtained from *P. virescens* formed a clustered with other *Hepatozoon* lineages found in *Podarcis* spp. (Fig. 3).

Fig. 2 Phylogenetic relationships derived from a maximum likelihood analysis (GTR + G + I model) of *Haemocystidium* sp. mitochondrial cytochrome b sequences available in Gen-Bank used in the phylogenetic analyses of this study. The tree was rooted with *Plasmodium falciparum* (M76611). Numbers at the branches show consensus support (%) below 40%. Gen-Bank accession numbers and associated host are shown. New sequences found in this study are in bold. The silhouette of the host corresponds to turtles, geckos, and snakes

Discussion

The knowledge of apicomplexan parasite diversity in amphibians and reptiles has increased notably in the last decade due to the use of molecular methods (O'Dwyer et al. 2013; Tomé et al. 2014; Harris et al. 2015). Here, we analyzed 145 individuals of 18 amphibian and reptile species from the Iberian Peninsula to characterize the genetic diversity of haemosporidian and haemogregarine parasites. None of the 86 individuals from five amphibian species were infected by these blood parasites. In contrast, of the 59 reptiles analyzed, one *Haemogregarina*, five *Hepatozoon*, and one *Haemocystidium* lineages were found infecting four reptile species. Notably, more than a half of these haplotypes had not been previously recorded in former studies. Moreover, our outcomes also revealed six new reptile—parasite records. These findings highlight the great unexplored diversity of apicomplexan parasites in reptiles from this region.

Amphibians are hosts of a wide variety of hemoparasites including *Haemogregarina*, *Hepatozoon*, *Lankesterella*, or *Schellackia* among others (Muriel et al. 2021). The presence of *Haemoproteus* in these animals is very unusual, having been identified only three species to date (Fantham et al. 1942), while haemogregarine parasites, and *Hepatozoon* species particularly, are more frequently recorded (e.g., Leal et al. 2009; Netherlands et al. 2015; Maia et al. 2016b). However, although some haemogregarine infections have been found in reptiles from nearby localities (e.g., Marzal et al. 2017; this study), none of the amphibians analyzed in this study was infected. The absence of infected amphibians could be explained by the methodological approach used. Seabra-Babo et al. (2015) failed to amplify DNA of any amphibian haemogregarines using the Hep primers (Ujvari et al. 2004), while they were clearly identified on visual screening of blood smears. On the other hand, Harris et al. (2013) and Maia et al. (2016b) detected some positive amphibians infected by *Hepatozoon* using the same primers. Therefore, based on these contradictory results, Haem and Hep primers may not be effective in detecting haemogregarine and haemosporidian parasites in amphibians. Alternatively, these differences could be also explained by other factors. While reptiles are mostly terrestrial, amphibians spend much of their time into the water. The absence of infected individuals in our samples could be due to the absence of appropriate aquatic vectors infecting amphibians in the study area or due to the short exposure time to terrestrial vectors (Ball 1967; Barta and Desser 1984; Bennett et al. 1992). In addition, the available vectors or parasite haplotypes in the area may have a high host-specificity infecting only reptile species instead of amphibians (Apperson et al. 2004; Maia et al. 2016b; Fecchio et al. 2019). Finally, amphibians can recognize both physical and chemical cues from some potential parasites, avoiding sites where vector or parasite densities are high (Kiesecker and Skelly 2000; Ferguson and Smith 2012). Nevertheless, our results should be taken with caution due to low sample sizes collected *per* species and which make it difficult to draw accurate conclusions. This highlights the need for studies with a larger sample size or with other specific primers in order to corroborate the absence or presence of parasites in Iberian amphibians.

The genus *Plasmodium* was not found in this study, in contrast with other studies in reptiles (e.g., Matta et al. 2018; Harris et al. 2019). On the contrary, two lineages of *Haemocystidium* sp. and six of *Hepatozoon* sp. were found among reptiles studied. The reports of *Haemocystidium* sp. worldwide are still limited in literature, being found almost exclusively infecting some gecko and turtle species (Telford 2005; Javanbakht et al. 2015; Maia et al. 2016a). Thus, to our knowledge, it represented the first report of *Haemocystidium* in western Europe and the first in the species *Tarentola mauritanica*. The phylogenetic analysis was similar to those previously described by González et al. (2019) and Austen et al. (2020), with a clade formed by parasites found in chelonians and other clade formed by parasites found in lacertids and snakes. The new lineage ON458135 found from *T. mauritanica* could be placed together with this second clade with haplotypes from other gecko and a snake species.

Six haplotypes of haemogregarines from six infected reptiles were found in this study. Haemogregarines are frequently reported infecting reptiles worldwide, with usually high prevalence (e.g., Ujvari et al. 2004; Maia et al. 2012, 2016b). In spite of the low number of individuals collected per species, our results seem to show that the occurrence of haemogregarines varied among species, as we only detected parasites in three of the thirteen species studied. Differences in susceptibility to infection among species could be associated with host immune defenses that can lead to prevent or better tolerate the infection (Klein 2004; Lindström et al. 2004). Alternatively, both the parasites and the vectors that transmit them may have a high degree of specificity for certain species of reptiles (Maia et al. 2016b; Fecchio et al. 2019). This may explain why some host species were more likely to be infected than others. Moreover, the host-related variables, for example, age or size (Brown et al. 2006; Salkeld and Schwarzkopf 2005), methodological aspects (Harris et al. 2011), or ecological factors (Sehgal et al. 2011; Gupta et al. 2013), must be also considered when analyzing infection levels from different species and from different geographical areas. However, as in amphibians, the limitations of these results have to be taken into account since some host species were represented by only one individual and because of the low sample size collected *per* species the probabilities of detecting infection were generally low and probably a bit random.

Fig. 3 Phylogenetic relationships derived from a maximum likelihood analysis (T92 + G model) of a representative set of haemogregarine 18S rDNA sequences available in GenBank used in the phylogenetic analyses of this study. The tree was rooted with *Adelina bambarooniae* (AF494059). Numbers at the branches show consensus support (%) below 40%. GenBank accession numbers and associated host are shown. New sequences found in this study are in bold and those previously reported in other host species are underlined. The silhouette of the host corresponds to anurans, (geckos and lizards), mammals, snakes, and turtles
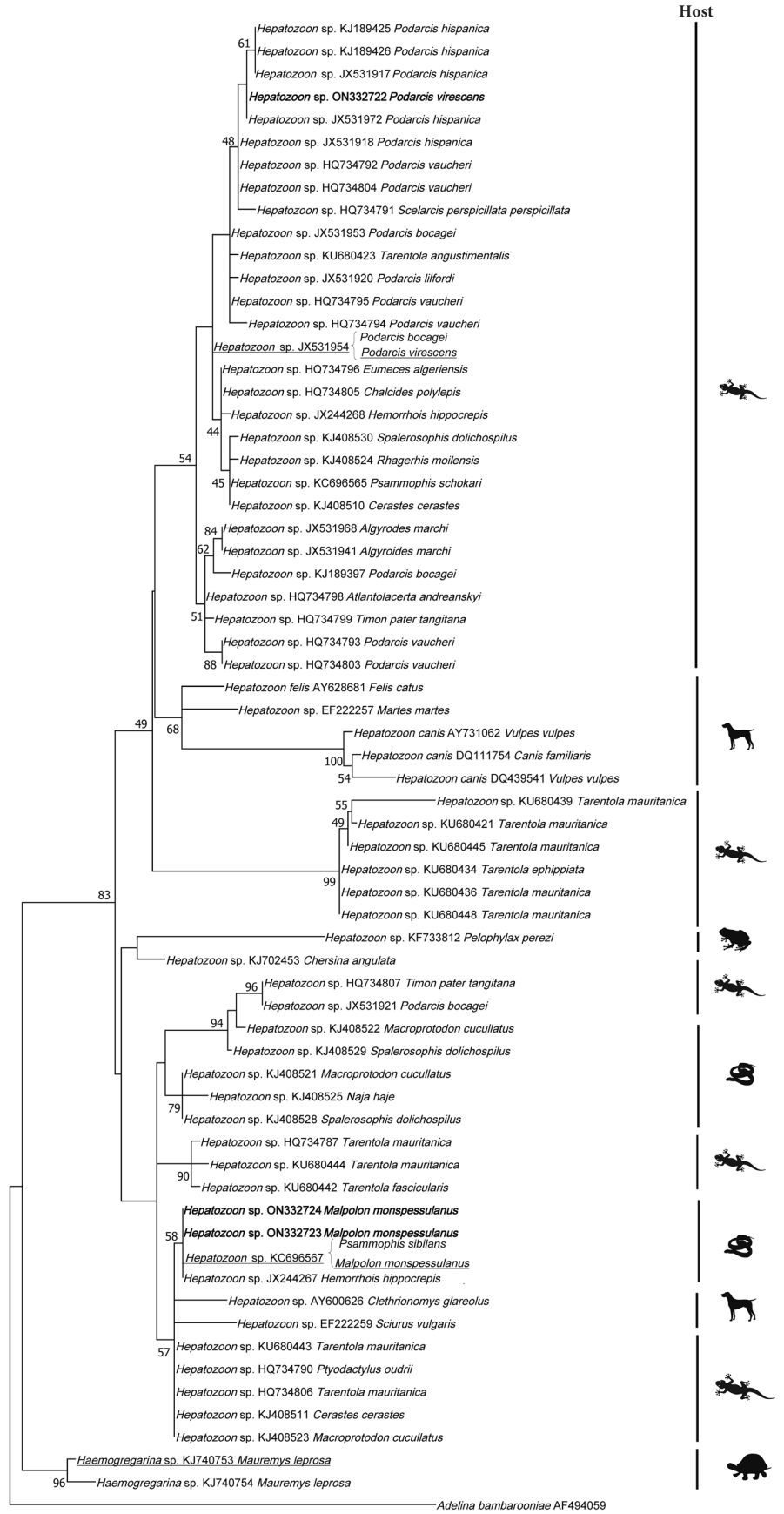
Among the infected species, we found two *Hepatozoon* haplotypes infecting the lacertid *Podarcis virescens* in this study. One of them (JX531954) was previously reported infecting *P. bocagei* from Tanes (Spain) (Maia et al. 2012). Phylogenetic analyses revealed that the haplotype was closely related to other North African lacertid haplotypes. The other new isolate (ON332722) fell in a group with other *Podarcis* species from North Africa and the Iberian Peninsula (*P. hispanica* and *P. vaucheri*). Our findings represented the first report of *Hepatozoon* parasites infecting the species *P. virescens*.

Moreover, the haplotypes found infecting *Malpolon monspessulanus* in this study also represented the first report of *Hepatozoon* sp. in this species. According to the phylogenetic analyses, the two new isolates appeared to be closely related to each other forming part of a group with other haplotypes found in snakes from North Africa and the Iberian Peninsula. The third haplotype identified was previously reported by Tomé et al. (2013) from *Psammophis sibilans* in North Africa and was also included in the snake group previously mentioned. This result followed the idea that some *Hepatozoon* parasites are not host-specific (Maia et al. 2016b; Tomé et al. 2021). The apparent switch of host genus may be facilitated by the hosts similarity (both are snakes), and therefore it is assumed that they will have more similar immune defenses to deal with (Medeiros et al. 2013; Maia et al. 2016b; Clark and Clegg 2017). Furthermore, this may be more likely to occur when these hosts share a habitat (Maia et al. 2016b). However, both records take place in different regions separated by a geographical barrier. In this sense, other studies with avian haemosporidians have reported the same patterns across the Strait of Gibraltar, finding the *Plasmodium* lineage LK6 (H158) in birds of Spain and Africa (Mata et al. 2015).

Finally, the *Haemogregarina* haplotype from *Mauremys leprosa* found here belonged to a previously reported lineage from the same species in a nearby locality (Marzal et al. 2017) and was very closely related to another *M. leprosa* haplotype of the same locality.

In conclusion, despite the low number of individuals infected by haemogregarine parasites, a high number of new lineages were detected, pointing out the lack of knowledge about the diversity of these parasites in the Iberian Peninsula. It is for this reason that much information is still required. More studies with higher sample sizes are needed to understand the true diversity of parasites in this area and the differences in prevalence between hosts. The knowledge of this information can be critical to understand their impact on biodiversity and can also have implications for conservation, especially in host groups as threatened and vulnerable as amphibians and reptiles.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-023-07814-6.

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Capture and manipulation of amphibians and reptiles were authorized by the "Consejería para la Transición Ecológica y Sostenibilidad, Junta de Extremadura" (CN0043/20/ACA). Daniel Parejo-Pulido (functions a, b, c, Junta de Andalucía) and Sergio Magallanes (category C, Junta de Extremadura) had the permits for animal experimentation that guarantee animal handling and taking biological samples.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Allender MC, Fry MM (2008) Amphibian haematology. Vet Clin North Am Exot Anim Pract 11:463–480. https://doi.org/10.1016/j.cvex.2008.03.006

Amo L, Fargallo JA, Martíñez-Padilla J, Millán J, López P, Martín J (2005a) Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*. Parasitol Res 96:413–417. https://doi.org/10.1007/s00436-005-1355-1

Amo L, López P, Martín J (2005b) Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. Parasitol Res 96:378–381. https://doi.org/10.1007/s00436-005-1354-2

Amo L, López P, Martín J (2007) Habitat deterioration affects anti-predatory behavior, body condition, and parasite load of female *Psammodromus algirus* lizards. Can J Zool 85:743–751. https://doi.org/10.1139/Z07-052

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR (2004) Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. Vector Borne Zoonotic Dis 4:71–82. https://doi.org/10.1089/153036604773083013

Austen JM, Hall J, Zahedi A, Goften A, Ryan U (2020) Further characterisation of *Haemocystidium chelodinae*-like Haemoproteidae isolated from the Bellinger River snapping turtle (*Myuchelys georgesi*). Parasitol Res 119:601–609. https://doi.org/10.1007/s00436-019-06547-9

Ball GH (1967) Blood sporozoans from East African amphibia. J Protozool 14:521–527. https://doi.org/10.1111/j.1550-7408.1967.tb02037.x

Barta JR, Desser SS (1984) Blood parasites of amphibians from Algonquin Park, Ontario. J Wildl Dis 20:180–189. https://doi.org/10.7589/0090-3558-20.3.180

Bennett GF, Montgomerie R, Seutin G (1992) Scarcity of haematozoa in birds breeding on the arctic tundra of North America. Condor 94:289–292. https://doi.org/10.2307/1368821

Brown GP, Shilton CM, Shine R (2006) Do parasites matter? Assessing the fitness consequences of haemogregarine infection in snakes. Can J Zool 84:668–676. https://doi.org/10.1139/z06-044

Clark NJ, Clegg SM (2017) Integrating phylogenetic and ecological distances reveals new insights into parasite host specificity. Mol Ecol 26(11):3074–3086. https://doi.org/10.1111/mec.14101

Davies AJ, Johnston MRL (2000) The biology of some intraerythrocytic parasites of fishes, amphibia and reptiles. Adv Parasitol 45:1–107. https://doi.org/10.1016/s0065-308x(00)45003-7

du Toit N, van Vuuren BJ, Matthee S, Matthee CA (2013) Biogeography and host-related factors trump parasite life history: limited congruence among the genetic structures of specific ectoparasitic lice and their rodent hosts. Mol Ecol 22:5185–5204. https://doi.org/10.1111/mec.12459

Fantham HB, Porter A, Richardson LH (1942) Some haematozoa observed in vertebrates in eastern Canada. Parasitology 34:199–226. https://doi.org/10.1017/S0031182000016176

Fecchio A, Wells K, Bell JA, Tkach VV, Lutz HL, Weckstein JD, Clegg SM, Clark N (2019) Climate variation influences host specificity in avian malaria parasites. Ecol Lett 22:547–557. https://doi.org/10.1111/ele.13215

Felsenstein J (1985) Confidence and phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.2307/2408678

Ferguson LV, Smith TG (2012) Reciprocal trophic interactions and transmission of blood parasites between mosquitoes and frogs. Insects 3:410–423. https://doi.org/10.3390/insects3020410

García R, Mateos AB (2010) El Clima de Extremadura. In: Schnabel S, Lavado JF, Gómez A, García R (ed) Aportaciones a la Geografía Física de Extremadura: con especial referencia a las dehesas. Asociación Profesional para la Ordenación del Territorio, el Ambiente y el Desarrollo Sostenible (Fundicotex), Cáceres, Spain, pp 25–52

Garnham PCC (1966) Malaria parasites and other haemosporidian. Blackwell Scientific, Oxford

González LP, Pacheco MA, Escalante AA, Maldonado ADJ, Cepeda AS, Rodríguez-Fandiño OA, Vargas-Ramírez M, Matta NE (2019) *Haemocystidium* spp., a species complex infecting ancient aquatic turtles of the family Podocnemididae: first report of these parasites in *Podocnemis vogli* from the Orinoquia. Int J Parasitol 10:299–309. https://doi.org/10.1016/j.ijppaw.2019.10.003

Gupta N, Bhaskar M, Gupta DK (2013) Ecological attributes of *Hepatozoon lacertilis* susceptibility in Indian lizards, *Hemidactylus flaviviridis* (Gekkonidae) and *Calotes versicolor* (Agamidae). Trop Biomed 30:97–104

Gutiérrez-Liberato GA, Lotta-Arévalo IA, Rodríguez-Almonacid CC, Vargas- Ramírez M, Matta NE (2021) Molecular and morphological description of the first *Hepatozoon* (Apicomplexa: Hepatozoidae) species infecting a neotropical turtle, with an approach to its phylogenetic relationships. Parasitology 148:747–759. https://doi.org/10.1017/S0031182021000184

Harris DJ, Maia JP, Perera A (2011) Molecular characterization of *Hepatozoon* species in reptiles from the Seychelles. J Parasitol 97:106–110. https://doi.org/10.1645/GE-2470.1

Harris DJ, Spigonardi MP, Maia JP, Cunha RT (2013) Molecular survey of parasites in introduced *Pelophylax perezi* (Ranidae) water frogs in the Azores. Acta Parasitol 58(4):607–611. https://doi.org/10.2478/s11686-013-0176-0

Harris DJ, Borges-Nojosa DM, Maia JP (2015) Prevalence and diversity of *Hepatozoon* in Native and Exotic Geckos from Brazil. J Parasitol 101:80–85. https://doi.org/10.1645/14-522.1

Harris DJ, Santos JL, Borges-Nojosa DM, Castro DP (2019) Molecular screening of *Plasmodium* (Haemosporidia: Plasmodiidae) parasites from reptiles in Brazil. J Parasitol 105:913–917. https://doi.org/10.1645/18-149

Hellgren O, Waldenström J, Bensch S (2004) A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J Parasitol 90:797–802. https://doi.org/10.1645/GE-184R1

Javanbakht H, Kvičerová J, Dvořáková N, Mikulíček P, Sharifi M, Kautman M, Maršíková A, Široký P (2015) Phylogeny, diversity, distribution, and host specificity of *Haemoproteus* spp. (Apicomplexa: Haemosporida: Haemoproteidae) of palaearctic tortoises. J Eukaryot Microbiol 62:670–678. https://doi.org/10.1111/jeu.12227

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, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kiesecker JM, Skelly DK (2000) Choice of oviposition site by gray treefrogs: the role of potential parasitic infection. Ecology 81:2939–2943. https://doi.org/10.2307/177354

Klein SL (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 26:247–264. https://doi.org/10.1111/j.0141-9838.2004.00710.x

Lindström KM, Foufopoulos J, Pärn H, Wikelski M (2004) Immunological investments reflect parasite abundance in island populations of Darwin's finches. Proc R Soc B Biol Sci 271:1513–1519. https://doi.org/10.1098/rspb.2004.2752

Leal DDM, O'Dwyer LH, Ribeiro VC, Silva RJ, Ferreira VL, Rodrigues RB (2009) Hemoparasites of the genus *Trypanosoma* (Kinetoplastida: Trypanosomatidae) and haemogregarines in anurans of the São Paulo and Mato Grosso do Sul States-Brazil. An Acad

Bras Cienc 81:199–206. https://doi.org/10.1590/s0001-37652 009000200006

Maia JP, Perera A, Harris DJ (2012) Molecular survey and microscopic examination of *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina) in lacertid lizards from the western Mediterranean. Folia Parasitol 59:241–248. https://doi.org/10.14411/fp.2012.033

Maia JP, Harris DJ, Carranza S (2016a) Reconstruction of the evolutionary history of Haemosporida (Apicomplexa) based on the cytbgene with characterization of *Haemocystidium* in geckos (Squamata: Gekkota) from Oman. Parasit Int 65:5–11. https://doi.org/10.1016/j.parint.2015.09.003

Maia JP, Harris DJ, Carranza S, Goméz-Díaz E (2016b) Assessing the diversity, host-specificity and infection patterns of apicomplexan parasites in reptiles from Oman, Arabia. Parasitology 143:1730–1747. https://doi.org/10.1017/S0031182016001372

Martinele I, Tostes R, Castro R, D'Agosto M (2016) Prevalence of *Haemoproteus* spp. (Apicomplexa: Haemoproteidae) in tortoises in Brazil and its molecular phylogeny. Parasitol Res 115:249–254. https://doi.org/10.1007/s00436-015-4741-3

Marzal A, Ibáñez A, González-Blázquez M, López P, Martín J (2017) Prevalence and genetic diversity of blood parasite mixed infections in Spanish terrapins, *Mauremys leprosa*. Parasitology 144:1449–1457. https://doi.org/10.1017/S0031182017000889

Mata VA, da Silva LP, Lopes RJ, Drovetski SV (2015) The Strait of Gibraltar poses an effective barrier to host-specialised but not to host-generalised lineages of avian Haemosporidia. Int J Parasitol 45:711–719. https://doi.org/10.1016/j.ijpara.2015.04.006

Matta NE, González LP, Pacheco MA, Escalante AA, Moreno AM, González AD, Calderón-Espinosa ML (2018) *Plasmodium* parasites in reptiles from the Colombia Orinoco-Amazon basin: a re-description of *Plasmodium kentropyxi* Lainson R, Landau I, Paperna I, 2001 and *Plasmodium carmelinoi* Lainson R, Franco CM, da Matta R, 2010. Parasitol Res 117:1357–1370. https://doi.org/10.1007/s00436-018-5815-9

Medeiros MCI, Hamer GL, Ricklefs RE (2013) Host compatibility rather than vector-host-encounter rate determines the host range of avian *Plasmodium* parasites. Proc R Soc B Biol Sci 280(1760):20122947. https://doi.org/10.1098/rspb.2012.2947

Muriel J, González-Blázquez M, Matta NE, Vargas-León CM, Marzal A (2021) Parasitas Sanguíneos de Anfíbio*s*. Editora da Universidade Federal do Piauí, Teresina

Netherlands EC, Cook CA, Kruger DJ, du Preez LH, Smit NJ (2015) Biodiversity of frog haemoparasites from sub-tropical Northern Kwazulu-Natal, South Africa. Int J Parasitol Parasites Wildl 4:135–141. https://doi.org/10.1016/j.ijppaw.2015.01.003

O'Dwyer LH, Moço TC, Paduan KDS, Spenassatto C, da Silva RJ, Ribolla PEM (2013) Description of three new species of *Hepatozoon* (Apicomplexa, Hepatozoidae) from Rattlesnakes (*Crotalus durissus terrificus*) based on molecular, morphometric and morphologic characters. Exp Parasitol 135:200–207. https://doi.org/10.1016/j.exppara.2013.06.019

Oliveira JP, André MR, Alves Júnior JRF, Lustosa APG, Werther K (2018) Molecular detection of hemogregarines and haemosporidians in Brazilian free-living testudines. Int J Parasitol Parasites Wildl 7(1):75–84. https://doi.org/10.1016/j.ijppaw.2018.01.008.PMID:30050752;PMCID:PMC6058349

Perkins SL, Keller AK (2001) Phylogeny of nuclear small subunit rRNA genes of haemogregarines amplified with specific oligonucleotídeos. J Parasitol 87:870–876. https://doi.org/10.1645/0022-3395(2001)087[0870:PONSSR]2.0.CO;2

Pineda-Catalan O, Perkins SL, Peirce MA, Engstrand R, Garcia-Davila C, Pinedo-Vasquez M, Aguirre AA (2013) Revision of hemoproteid genera and description and redescription of two species of chelonian hemoproteid parasites. J Parasitol 99:1089–1098. https://doi.org/10.1645/13-296.1

Polo-Cavia N, Engstrom T, López P, Martín J (2010) Body condition does not predict immunocompetence of western pond turtles in altered versus natural habitats. Anim Conserv 13:256–264. https://doi.org/10.1111/j.1469-1795.2009.00329.x

Poulin R, Krasnov BR, Mouillot D (2011) Host specificity in phylogenetic and geographic space. Trends Parasitol 27:355–361. https://doi.org/10.1016/j.pt.2011.05.003

Price PW, Westoby M, Rice B, Atsatt PR, Fritz RS, Thompson JN, Mobley K (1986) Parasite mediation in ecological interactions. Annu Rev Ecol Syst 17:487–505

Rajabi F, Javanbakht H, Sajjadi SS (2017) A preliminary study of haemoparasites in marsh frogs, *Pelophylax ridibundus* (Ranidae) from Iran. J Entomol Zool Stud 5:1314–1317

Roca V, Galdón MA (2010) Haemogregarine blood parasites in the lizards *Podarcis bocagei* (Seoane) and *P. carbonelli* (Pérez-Mellado) (Sauria: Lacertidae) from NW Portugal. Syst Parasitol 75:75–79. https://doi.org/10.1007/s11230-009-9206-6

Salkeld DJ, Schwarzkopf L (2005) Epizootiology of blood parasites in an Australian lizard: a mark-recapture study of a natural population. Int J Parasitol 35:11–18. https://doi.org/10.1016/j.ijpara.2004.09.005

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Plainview

Santiago-Alarcón D, Marzal A (2020) Avian malaria and related parasites in the tropics: ecology, evolution and systematics. Springer, Cham

Schmid-Hempel P (2011) Evolutionary parasitology: the integrated study of infections, immunology, ecology and genetics. Oxford University Press, Oxford

Seabra-Babo J, Maia JP, Harris DJ (2015) Scanning for apicomplexan parasites (Suborder Adeleorina) in five Holarctic anuran species. Herpetozoa 27(3/4):168–172

Sehgal RNM, Buermann W, Harrigan RJ, Bonneaud C, Loiseau C, Chasar A, Sepil I, Valkiūnas G, Iezhova T, Saatchi S, Smith TB (2011) Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. P Proc R Soc B Biol Sci 278:1025–1033. https://doi.org/10.1098/rspb.2010.1720

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

Sykes JM IV, Klaphake E (2008) Reptile hematology. Vet Clin North Am Exot Anim Pract 11:481–500. https://doi.org/10.1016/j.cvex.2014.09.011

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msab120

Telford SR (2005) A *Haemocystidium* species from the east african gecko *Lygodactylus capensis grotei*. J Parasitol 91:135–138. https://doi.org/10.1645/GE-3395

Telford SR (2009) Hemoparasites of the Reptilia: color atlas and text. CRC Press, Boca Raton

Tomé 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? J Parasitol 99:883–887. https://doi.org/10.1645/12-95.1

Tomé B, Maia JP, Salvi D, Brito JC, Carretero MA, Perera A, Meimberg H, Harris DJ (2014) Patterns of genetic diversity in *Hepatozoon* spp. infecting snakes from North Africa and the Mediterranean Basin. Syst Parasitol 87:249–258. https://doi.org/10.1007/s11230-014-9477-4

Tomé B, Maia JP, Perera A, Carranza S, Vasconcelos R (2021) Parasites in a hotspot: diversity and specificity patterns of apicomplexans infecting reptiles from the Socotra Archipelago. Parasitology 148:42–52. https://doi.org/10.1017/S0031182020002000

Ujvari B, Madsen T, Olsson M (2004) High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in water pythons

(*Liasis fuscus*) from tropical Australia. J Parasitol 90:670–672. https://doi.org/10.1645/GE-204R

Valkiūnas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton

Vilcins IE, 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. J Parasitol 95:434–442. https://doi.org/10.1645/GE-1725.1

Votýpka J, Modrý D, Oborník M, Šlapeta J, Lukeš J (2016) Apicomplexa. In: Archibald JM, Simpson AGB and Slamovits CH et al. (ed) Handbook of the Protists. Springer, Cham, pp 1–58. https://doi.org/10.1007/978-3-319-32669-6_20-1

Wells K, O'Hara RB, Morand S, Lessard J-P, Ribas A (2015) The importance of parasite geography and spillover effects for global patterns of host–parasite associations in two invasive species. Divers Distrib 21:477–486. https://doi.org/10.1111/ddi.12297

Wood CL, Byers JE, Cottingham KL, Altman I, Donahue MJ, Blakeslee AMH (2007) Parasites alter community structure. Proc Natl Acad Sci USA 104:9335–9339. https://doi.org/10.1073/pnas.0700062104

Yabsley MJ, Shock BC (2012) Natural history of Zoonotic Babesia: role of wildlife reservoirs. Int J Parasitol Parasites Wildl 22:18–31. https://doi.org/10.1016/j.ijppaw.2012.11.003