

Molecular identification of *Eimeria* species in Spanish bats

Seila Couso-Pérez^a, Xosé Pardavila^b, Elvira Ares-Mazás^a, Hipólito Gómez-Couso^{a,c,*}

^a Laboratory of Parasitology, Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, A Coruña, Spain

^b Sorex Ecoloxía e Medio Ambiente S.L.U., Rúa das Barreiras 80, 15702 Santiago de Compostela, A Coruña, Spain

^c Research Institute on Chemical and Biological Analysis, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, A Coruña, Spain

ARTICLE INFO

Keywords:

Eimeria
Chiroptera
Molecular characterization
Spain

ABSTRACT

This is the first study reporting the detection and molecular characterization of *Eimeria* in bats in Spain, specifically in 12 of 32 chiropteran species described in the Iberian Peninsula. A total of 76 faecal samples were collected from different bat roosting sites across Spanish territory. The DNA was extracted from the samples and sequenced by targeting the *Eimeria* SSU-rRNA gene. Two *Eimeria* species were detected in 29 of the 76 faecal samples (38%), and the bat-specific *Eimeria rioarribaensis* and rodent-specific *Eimeria jerfinica* were detected in 4 (5%) and 25 (33%) of the samples, respectively. This is the first report of *E. rioarribaensis* in the bats *Rhinolophus euryale*, *Myotis myotis* and *Nyctalus lasiopterus*, extending the host and geographical ranges for this bat coccidian parasite. The identification of the rodent-specific parasite species *E. jerfinica* in bats indicates the occurrence of this species in Spain, although its presence has not previously been reported in wild rodents in this country. Considering that most of the *Eimeria* spp. reported in bats were described only on the basis of morphometric data, molecular studies are required to determine which *Eimeria* species occur in bats, to complete the identification of these species and to clarify the phylogeny.

1. Introduction

The order Chiroptera, which comprises about 1400 known bat species distributed throughout the world, is the second most numerous mammalian order [1]. Bats live in large colonies, inhabiting foliage, caves, rock crevices and tree hollows. They become active at night, feeding on diverse food items such as insects (mainly in the orders Coleoptera, Diptera, Lepidoptera and Hymenoptera), nectar, fruits, seeds, frogs, fish and small mammals like rodents [2]. Bats are naturally infected by different types of microorganisms and can act as potential reservoirs of many pathogens and play an important role in the transmission of emerging infectious diseases worldwide [3]. In the Iberian Peninsula, the order Chiroptera is the best represented mammalian order, with 32 different species belonging to 11 genera and 4 families [4,5]. All bat species are protected by state legislation in Spain as some serious environmental problems have led to bats being endangered [6].

Members of the genus *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) are obligate protozoan parasites that typically infect the digestive tracts of various vertebrates (mammals, birds, reptiles, amphibians and fish). These coccidian parasites have direct life cycles,

completing their development in a single host species. They are ubiquitous in vertebrates and represent some of the most prevalent parasites known. However, *Eimeria* species are less common in chiropteran hosts than in other mammalian orders (i.e. >400 *Eimeria* species are known to infect rodents) [7].

The first description of *Eimeria* in bats was made by Wheat [8] in the tri-colored bat (*Perimyotis subflavus* Cuvier, 1832; formerly *Pipistrellus subflavus*) in Alabama, USA. Since then, 40 *Eimeria* species have been described in bats worldwide, although molecular data are only available for six species: *Eimeria antrozoi*, *Eimeria catronensis*, *Eimeria hessei*, *Eimeria macyi*, *Eimeria pilarensis* and *Eimeria rioarribaensis* [9–11].

The aim of this work was to investigate the presence of *Eimeria* spp. in bat faecal samples obtained in several locations in Spain using molecular methods (amplification and sequencing of the small subunit rRNA gene) and analyse their phylogenetic relationship with other species.

* Corresponding author at: Laboratory of Parasitology, Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, A Coruña, Spain.

E-mail address: hipolito.gomez@usc.es (H. Gómez-Couso).

<https://doi.org/10.1016/j.parint.2022.102621>

Received 24 January 2022; Received in revised form 29 June 2022; Accepted 30 June 2022

Available online 7 July 2022

1383-5769/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

2. Materials and methods

2.1. Sample origin and processing

Between December 2019 and June 2021, a total of 76 faecal samples were mainly collected from captured bats by the authors and some of them were also provided by the Bat Evolutionary Ecology and Conservation Group (Doñana Biological Station, Spanish National Research Council) and DROSERA Association for the Study and Conservation of the Natural Environment. The samples came from different bat roosting sites across Spain: Galicia ($n = 9$), Castile-Leon ($n = 2$), Murcia ($n = 53$) and Andalusia ($n = 12$) (Fig. 1). The roosting sites belong to 15 different insectivorous bat species: the Isabelline serotine bat (*Eptesicus isabellinus* Temminck, 1839, $n = 1$); the serotine bat (*Eptesicus serotinus* Schreber, 1774, $n = 1$); Savi's pipistrelle bat (*Hypsugo savii* Bonaparte, 1837, $n = 1$); Schreiber's bat (*Miniopterus schreibersii* Kuhl, 1817, $n = 17$); the lesser mouse-eared bat (*Myotis blythii* Tomes, 1857, $n = 6$); the long-fingered bat (*Myotis capaccinii* Bonaparte, 1837, $n = 7$); Daubenton's bat (*Myotis daubentonii* Kuhl, 1817, $n = 1$); Geoffroy's bat (*Myotis emarginata* Geoffroy, 1806, $n = 1$); the greater mouse-eared bat (*Myotis myotis* Borkhausen, 1797, $n = 18$); the greater noctule bat (*Nyctalus lasiopterus* Schreber, 1780, $n = 14$); Leisler's bat (*Nyctalus leisleri* Kuhl, 1817, $n = 1$); the common pipistrelle bat (*Pipistrellus pipistrellus* Schreber, 1774, $n = 2$); the Mediterranean horseshoe bat (*Rhinolophus euryale* Blasius, 1853, $n = 1$); the greater horseshoe bat (*Rhinolophus ferrumequinum* Schreber, 1774, $n = 4$); and the lesser horseshoe bat (*Rhinolophus hipposideros* Bechstein, 1800, $n = 1$) (Fig. 1; Table 1). The captured bats were identified by Xosé Pardavila and other bat specialists belonging to the group and the association cited previously. All samples were stored at 4 °C until analysis.

The faecal samples were processed using a previously described diphasic concentration method [12]. Briefly, 0.1–0.5 g of each sample was individually ground in a mortar with 30 mL of 0.04 M phosphate buffered saline (PBS) pH 7.2, filtered through a set of two sieves (150 and 45 µm of mesh size), shaken vigorously with diethyl ether (2:1) and finally concentrated by centrifugation at 1250 ×g, 4 °C, for 15 min. The supernatant was carefully removed and discarded. The pellets were

resuspended in 500–1000 µL of PBS 0.04 M pH 7.2 and stored at –20 °C.

2.2. Molecular characterization

Total genomic DNA was extracted from 200 µL aliquots of the sediments by using the Stool DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. The extracted DNA was stored at –20 °C until use. A heminested PCR technique was used to amplify a ~ 420 bp fragment of the SSU-rRNA gene of *Eimeria* [12]. In all PCR runs, genomic DNA of *Eimeria tenella* from naturally infected chickens was used as a positive control, and molecular biology grade water was used as a negative control. The PCR products were electrophoresed on 2% agarose gels and stained with Real Safe (Real Laboratory S.L., Paterna, Valencia, Spain). Those amplicons corresponding to the expected size were purified using the Nucleospin® gel and PCR clean-up kit (Macherey-Nagel GmbH & Co KG, Düren, Germany) and sequenced in both directions.

The sequences obtained were aligned using SeqMan™ 7.0 software (DNASTAR®, Madison, WI, USA) and the computed sequences were edited using BioEdit 7.2.3 software (©1997–2013 Tom Hall, Ibis Therapeutics, Carlsbad, CA, USA). The derived consensus sequences were compared with other *Eimeria* sequences deposited in the GenBank® database [13], by using the public web interface of the BLAST® 2.12.0 program [14]. Phylogeny analyses were conducted in MEGA X [15] and were constructed by the neighbour-joining method on the basis of the genetic distances calculated by the two-parameter model proposed by Kimura [16], with discrete Gamma distribution.

3. Results

By analysis of the SSU-rRNA gene, *Eimeria* spp. were detected in 29 of 76 faecal samples (38%) from 12 of 15 bat species considered in the study, being not detected in samples from *R. ferrumequinum*, *R. hipposideros* and *M. emarginata* (Table 1). The bat-specific *Eimeria* species, *E. rioarribaensis*, was identified in 4 of 76 (5%) faecal samples from the following species: the Mediterranean horseshoe bat (*R. euryale*) (1/1; 100%) in Galicia; the greater mouse-eared bat (*M. myotis*) (2/18;

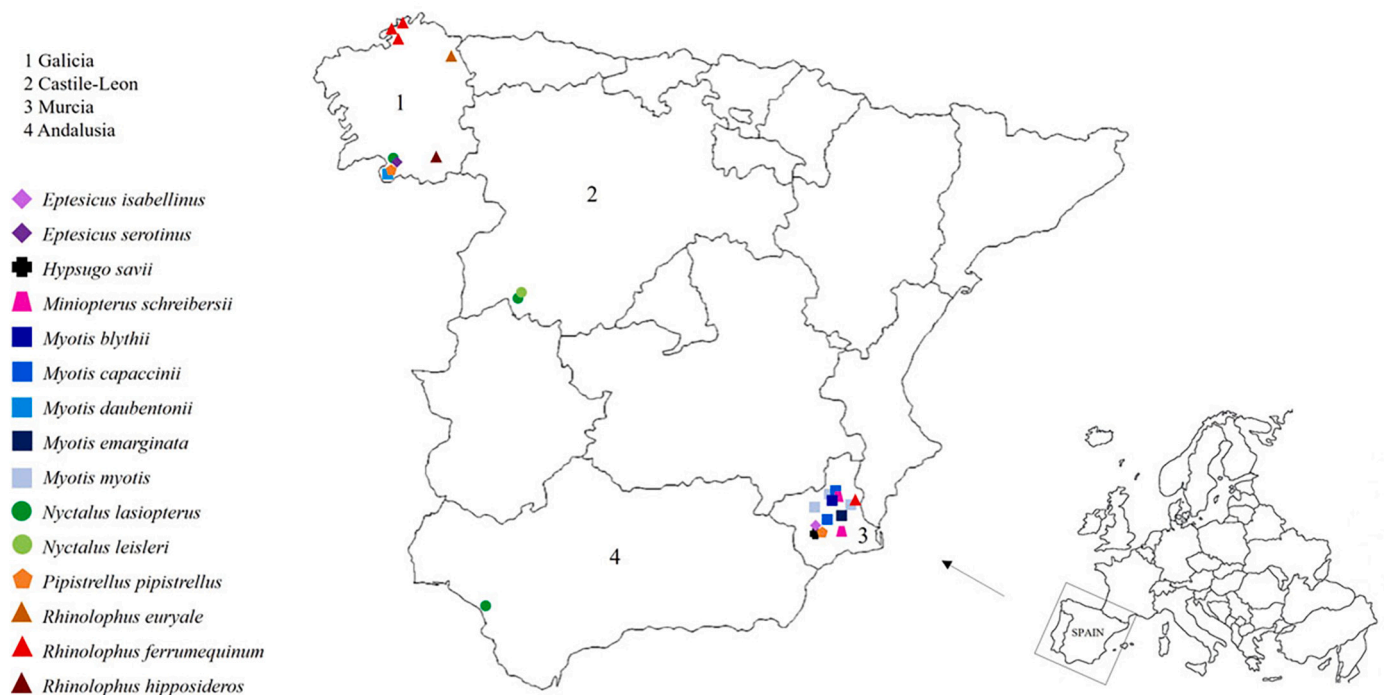


Fig. 1. Geographical locations of roosting sites where the bat faecal samples were collected.

Table 1
Molecular characterization of *Eimeria* spp. in bat faecal samples from several locations in Spain.

Location	Host scientific name	Host common name	n analysed	n positive	Molecular characterization	GenBank® AN
Galicia	<i>Eptesicus serotinus</i>	Serotine bat	1	1	<i>Eimeria jerfinica</i>	MW182398
	<i>Myotis daubentonii</i>	Daubenton's bat	1	1	<i>Eimeria jerfinica</i>	MW192396
	<i>Nyctalus lasiopterus</i>	Greater noctule bat	1	1	<i>Eimeria jerfinica</i>	MT813028
	<i>Pipistrellus pipistrellus</i>	Common pipistrelle bat	1	1	<i>Eimeria jerfinica</i>	MW182397
	<i>Rhinolophus euryale</i>	Mediterranean horseshoe bat	1	1	<i>Eimeria rioarribaensis</i>	MT598820
	<i>Rhinolophus ferrumequinum</i>	Greater horseshoe bat	3	0	–	–
	<i>Rhinolophus hipposideros</i>	Lesser horseshoe bat	1	0	–	–
Castile-Leon	<i>Nyctalus lasiopterus</i>	Greater noctule bat	1	1	<i>Eimeria jerfinica</i>	MT813028
	<i>Nyctalus leisleri</i>	Leisler's bat	1	1	<i>Eimeria jerfinica</i>	MW182395
	<i>Eptesicus isabellinus</i>	Isabelline serotine bat	1	1	<i>Eimeria jerfinica</i>	MW182403
	<i>Hypsugo savii</i>	Savi's pipistrelle bat	1	1	<i>Eimeria jerfinica</i>	MW182402
	<i>Miniopterus schreibersii</i>	Schreiber's bat	17	2	<i>Eimeria jerfinica</i> (n = 2)	MW182405
	<i>Myotis blythii</i>	Lesser mouse-eared bat	6	2	<i>Eimeria jerfinica</i> (n = 2)	MW182406
	<i>Myotis capaccinii</i>	Long-fingered bat	7	1	<i>Eimeria jerfinica</i>	MW182399
	<i>Myotis emarginata</i>	Geoffroy's bat	1	0	–	–
	<i>Myotis myotis</i>	Greater mouse-eared bat	18	4	<i>Eimeria rioarribaensis</i> (n = 2)	MW182400
Murcia	<i>Pipistrellus pipistrellus</i>	Common pipistrelle bat	1	1	<i>Eimeria jerfinica</i> (n = 2)	OL588525
	<i>Rhinolophus ferrumequinum</i>	Greater horseshoe bat	1	0	<i>Eimeria jerfinica</i>	MW182401
					–	–
					<i>Eimeria rioarribaensis</i> (n = 1)	MW182393
					<i>Eimeria jerfinica</i> (n = 9)	MT813028

n = number of samples; AN = accession number.

11%) in Murcia, and the greater noctule bat (*N. lasiopterus*) (1/14; 7%) in Andalusia (Table 1; Fig. 1). Three consensus sequences were 100% identical, and the remaining sequence showed 1 nucleotide difference, exhibiting 99.5% similarity relative to the GenBank® sequence AF307877 (the newly obtained sequences have been deposited in GenBank® database under accession numbers MT598820, MW182393, MW182400 and MW182404).

A rodent-specific *Eimeria* species, *Eimeria jerfinica*, was identified in 25 of 29 *Eimeria*-positive samples (86%) from all locations sampled. Thus, this coccidian was identified in faecal samples from the Isabelline serotine bat (*E. isabellinus*) (1/1; 100%), in the serotine bat (*E. serotinus*) (1/1; 100%), in Savi's pipistrelle bat (*H. savii*) (1/1; 100%), in Schreiber's bat (*M. schreibersii*) (2/17; 12%), in the lesser mouse-eared bat (*M. blythii*) (2/6; 33%), in the long-fingered bat (*M. capaccinii*) (1/7; 14%), in Daubenton's bat (*M. daubentonii*) (1/1; 100%), in the greater mouse-eared bat (*M. myotis*) (2/18; 11%), in the greater noctule bat (*N. lasiopterus*) (11/14; 79%), in Leisler's bat (*N. leisleri*) (1/1; 100%) and in the common pipistrelle bat (*P. pipistrellus*) (2/2; 100%) (Table 1; Fig. 1). A total of 12 consensus sequences were obtained, nine of which were 100% identical and the three remaining sequences showed 1–2 nucleotide differences. The consensus sequences exhibited similarities of 99.3–99.8% relative to the GenBank® sequence KU192975 (the consensus sequences obtained in the present study were deposited in GenBank® database under accession numbers MT813028, MW182395-MW182399, MW182401-MW182403, MW182405-MW182406 and OL588525).

Phylogenetic analysis revealed that three isolates were 100% homologous to *E. rioarribaensis*, whereas the other isolate clustered with this bat-specific *Eimeria* species exhibited 0.5% genetic distance. Moreover, 25 isolates were 99% homologous to *E. jerfinica*, exhibiting genetic distances ranging between 0.2% and 0.7% (Fig. 2).

4. Discussion

To our knowledge, this is the first time that the coccidian *Eimeria* was investigated in bat faecal samples in Spain, which belongs to 15 of 32 chiropteran species (7 genera and 2 families) described in the Iberian Peninsula [4,5].

Molecular analysis of the SSU-rRNA gene identified *Eimeria* spp. in 29 of 76 (38%) faecal samples from insectivorous bats. This prevalence rate is within the range described for *Eimeria* in different bat species

(4–100%) by various authors [17–22]. Remarkably, this coccidian parasite was more predominant in insectivorous bats than in fruit bats as was previously reported by Adhikari et al. [22].

A bat-specific *Eimeria*, *E. rioarribaensis*, was detected in only 4 of the 76 samples analysed (5%). This species has been described in the western small-footed bat (*Myotis ciliolabrum* Merriam, 1886) in North America with prevalence rates ranging from 5% to 18% [18]. Furthermore, it was reported in 1 of 3 (33%) northern bats (*Eptesicus nilssonii* Keyserling and Blasius, 1839) in Japan after histopathological and molecular studies [23]. Therefore, to our knowledge, this is the first report of the presence of *E. rioarribaensis* in *R. euryale* (100%), *M. myotis* (11%) and *N. lasiopterus* (7%), thus indicating new hosts and geographical distribution records for this bat-infecting coccidian species.

The identification of *E. jerfinica* in 25 of the 76 (33%) faecal samples belonging to 11 of 15 chiropteran species from all locations sampled is remarkable. This *Eimeria* species was described by Musaev and Veisov [24] in the wood mouse (*Apodemus sylvaticus*) in Azerbaijan and it was recently detected by molecular characterization in rodents, specifically *A. sylvaticus*, *Apodemus flavicollis* and *Apodemus agrarius*, from several countries in Europe [25]. Data from the present study suggests that *E. jerfinica* may be a parasite species on both rodents and bats, being needed further histological studies to confirm a true infection in chiropteran hosts. However, we believe that this finding may be explained by a spurious infection acquired through the trophic chain, as the bat species studied are insectivores and mainly feed on Lepidoptera, Diptera and Coleoptera, the last of which includes coprophagous beetles of the Scarabaeidae and Geotrupidae families, which could act as mechanical carriers of *Eimeria* oocysts [26–28]. In addition, possible direct contamination via faeces from infected hosts cannot be ruled out, as insectivorous bats share roosting sites with other animals, including rodents [4]. Although the presence of *E. jerfinica* has not been confirmed officially in *Apodemus* species in Spain, the identification of this rodent-specific *Eimeria* species in bats likely demonstrates its existence in rodents in the Iberian Peninsula.

Eimeria species have traditionally been identified on the basis of the morphology of sporulated oocysts, host specificity, geographical distribution and pathology. However, these characteristics are often insufficient for identification purposes, as they may overlap among *Eimeria* species [29]. Amplification and sequencing of the SSU-rRNA gene is currently one of the most extensively used techniques for characterizing *Eimeria* species and examining their phylogenetic relationships [9]. In

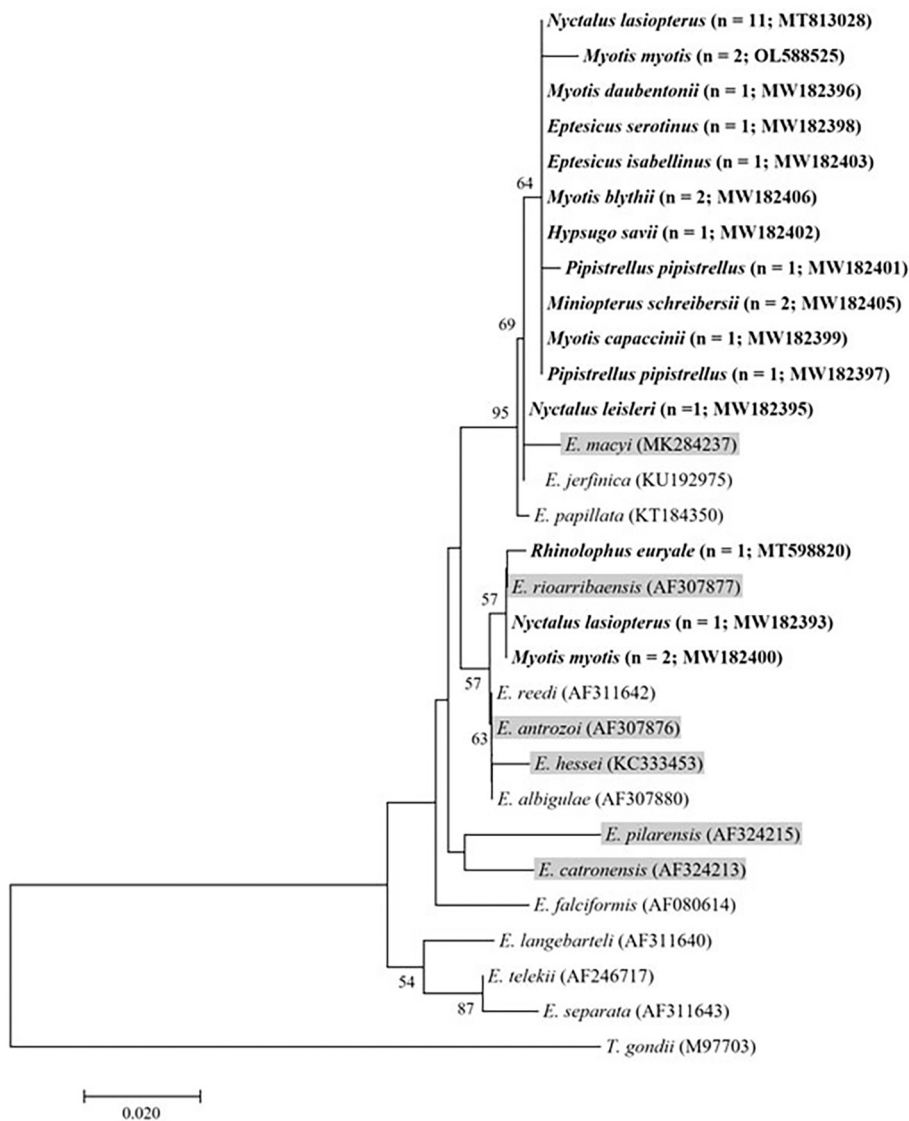


Fig. 2. Phylogenetic relationships between *Eimeria* isolates from bats and other *Eimeria* spp. inferred by neighbour-joining analysis of the small subunit ribosomal RNA (SSU-rRNA) locus on the basis of genetic distances calculated by Kimura's two parameter model with discrete Gamma distribution. The tree was generated using a total of 322 positions in the final data set and the percentages of replicate trees in which associated taxa clustered together in the bootstrap test (10,000 replicates) are shown at the internal nodes for distance (>50%). The *Eimeria* species found to infect bats are indicated by shading. The isolates and their corresponding accession numbers obtained in this study are highlighted in bold type.

the present study, the phylogenetic analysis revealed that one *Eimeria* isolate from the Mediterranean horseshoe bat (*R. euryale*), two isolates from the greater mouse-eared bat (*M. myotis*) and one isolate from the greater noctule bat (*N. lasiopterus*) were grouped with the bat-infecting species *E. rioarribaensis*, whereas the remaining 25 *Eimeria* isolates obtained from 12 different bat species formed part of a clade along with the rodent-derived species *E. jerfunica* and with the bat coccidian *E. macyi* (Fig. 2). This finding is consistent with the results of phylogenetic analyses reported by several authors, who observed that *Eimeria* species detected in bats did not form a different clade from the *Eimeria* species isolated from rodents [9,23,29,30].

In conclusion, this is the first report of the occurrence of *E. rioarribaensis* in *R. euryale*, *M. myotis* and *N. lasiopterus*, thereby increasing the hosts and geographical distribution ranges of this bat-infecting coccidian parasite. The identification of *E. jerfunica*, a rodent-specific species, in faecal samples from the roosting sites of 12 different bat species in all locations sampled indicates the likely presence of this species in wild rodents in Spain. Moreover, it would be necessary to increase the number of samples and species examined to provide epidemiological data more reliable. Considering the high number of *Eimeria* species described in bats only on the basis of morphometric analysis, molecular characterization should be conducted in order to complete their identification and to clarify the phylogeny.

Declaration of Competing Interest

None.

Acknowledgements

The authors are grateful to the Bat Evolutionary Ecology and Conservation Group (Doñana Biological Station-Spanish National Research Council, Seville, Spain) and DROSER Association for the Study and Conservation of the Natural Environment (As Pontes, A Coruña, Spain) for providing faecal samples of bats. SC-P is granted by the Programme for the requalification, international mobility and attraction of talent in the Spanish university system, modality Margarita Salas. The study was funded by the Xunta de Galicia (grant ED431C 2021/26) and the samples from Murcia were collected during the project "Programa de Seguimiento Biológico de la Fauna Vertebrada Amenazada de la Región de Murcia. Seguimiento de Refugios Prioritarios EXP2/16 Región de Murcia, Consejería de Agua, Agricultura, Ganadería, Pesca y Medio Ambiente, Dirección General del Medio Natural, Subdirección General del Patrimonio Natural y Cambio Climático".

References

- [1] D.E. Wilson, R.A. Mittermeier, *Handbook of the Mammals of the World*, Lynx Edicions, Barcelona, 2019.
- [2] M. Kasso, M. Balakrishnan, Ecological and economic importance of bats (Order Chiroptera), *ISRN Biodivers.* 187415 (2013) 1–9, <https://doi.org/10.1155/2013/187415>.
- [3] D. Crowley, D. Becker, A. Washburne, R. Plowright, Identifying suspect bat reservoirs of emerging infections, *Vaccines* 8 (2020) 228, <https://doi.org/10.3390/vaccines8020228>.
- [4] L.J. Palomo, J. Gisbert, J.C. Blanco, Atlas y libro rojo de los mamíferos terrestres de España, in: Ministerio de Medio Ambiente, Dirección General para la Biodiversidad-Sociedad Española para la Conservación y Estudio de los Mamíferos (SECEM), Sociedad Española para la Conservación y Estudio de los Murciélagos (SECEMU), Madrid, 2nd ed, 2007.
- [5] Asociación Española para la Conservación y el Estudio de los Murciélagos (SECEMU), Especies de murciélagos de España, Portugal y Gibraltar. <http://secemu.org/especies-murcielagos-espana-portugal-gibraltar/>, 2022 (Accessed 7 July 2022).
- [6] Royal Decree 139/2011, of 4 February, for the development of the List of Wild Species under Special Protection and the Spanish Catalogue of Threatened Species, *Off. State Gaz* 46, 2011, pp. 1–30.
- [7] D.W. Duszynski, S.J. Upton, The common coccidia of wild mammals. *Cyclospora*, *Eimeria* (Eimeriidae) and *Cryptosporidium* (Cryptosporidiidae) spp, in: W. Samuel, M. Pybus, A. Kocan (Eds.), *Parasitic Diseases of Wild Mammals*, Iowa State University Press, Ames, 2001, pp. 416–433.
- [8] B.E. Wheat, *Eimeria macyi* sp. n. (Protozoa: Eimeriidae) from the eastern pipistrelle, *Pipistrellus subflavus*, from Alabama, *J. Parasitol.* 61 (1975) 920–922, <https://doi.org/10.2307/3279236>.
- [9] T.P. Miles, S.A. Rush, T.G. Rosser, Morphological, molecular and phylogenetic characterisation of *Eimeria macyi* Wheat, 1975 (Apicomplexa: Eimeriidae) in the eastern red bat *Lasiurus borealis* (Müller) from Mississippi, USA, *Syst. Parasitol.* 96 (2019) 245–255, <https://doi.org/10.1007/s11230-019-09844-0>.
- [10] D.W. Duszynski, Coccidia (Apicomplexa: Eimeriidae) of the mammalian order Chiroptera, *Spec. Publ. Museum Southwest. Biol.* 5 (2002) 1–45.
- [11] M.T. de Santana Miglionico, L.M. Costa, E.M. Mota, H.G. Bergallo, D. Dias, A new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from *Myotis riparius* Handley, 1960 (Chiroptera: Vespertilionidae) in the Atlantic Forest of Brazil, with a checklist of *Eimeria* spp. reported from bats, *Acta Parasitol.* 65 (2020) 496–503, <https://doi.org/10.2478/s11686-020-00182-6>.
- [12] S. Couso-Pérez, E. Ares-Mazás, H. Gómez-Couso, First molecular data on *Eimeria truttae* from brown trout (*Salmo trutta*), *Parasitol. Res.* 118 (2019) 2121–2127, <https://doi.org/10.1007/s00436-019-06320-y>.
- [13] E.W. Sayers, J. Beck, J.R. Brister, E.E. Bolton, K. Canese, D.C. Comeau, K. Funk, A. Ketter, S. Kim, A. Kimchi, P.A. Kitts, A. Kuznetsov, S. Lathrop, Z. Lu, K. McGarvey, T.L. Madden, T.D. Murphy, N. O'leary, L. Phan, V.A. Schneider, F. Oise Thibaud-Nissen, B.W. Trawick, K.D. Pruitt, J. Ostell, Database resources of the national center for biotechnology information, *Nucleic Acids Res.* 48 (2020) D9–D16, <https://doi.org/10.1093/nar/gkz899>.
- [14] M. Johnson, I. Zaretskaya, Y. Raytselis, Y. Merezhuik, S. McGinnis, T.L. Madden, NCBI BLAST: a better web interface, *Nucleic Acids Res.* 36 (2008) W5–W9, <https://doi.org/10.1093/nar/gkn201>.
- [15] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, *Mol. Biol. Evol.* 35 (2018) 1547–1549, <https://doi.org/10.1093/molbev/msy096>.
- [16] M. Kimura, A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences, *J. Mol. Evol.* 16 (1980) 111–120, <https://doi.org/10.1007/bf01731581>.
- [17] C.T. McAllister, S.J. Upton, Two new species of *Eimeria* (Apicomplexa: Eimeriidae) from eastern red bats, *Lasiurus borealis* (Chiroptera: Vespertilionidae), in Arkansas and North Carolina, *J. Parasitol.* 95 (2009) 991–993, <https://doi.org/10.1645/GE-1967.1>.
- [18] D.W. Duszynski, D.T. Scott, J. Aragon, A. Leach, T. Perry, Six new six new *Eimeria* species from vespertilionid bats of North America, *J. Parasitol.* 85 (1999) 496–503, <https://doi.org/10.2307/3285785>.
- [19] V.F. Santana Lima, P.A. Rocha, M.A. Dias Silva, R. Beltrão-Mendes, R.A.N. Ramos, A. Giannelli, L. Rinaldi, G. Cringoli, P.C. Estrela, L.C. Alves, Survey on helminths and protozoa of free-living Neotropical bats from northeastern Brazil, *Acta Trop.* 185 (2018) 267–272, <https://doi.org/10.1016/j.actatropica.2018.06.002>.
- [20] E. Afonso, P.E. Baurand, P. Tournant, N. Capelli, First amplification of *Eimeria hessei* DNA from the lesser horseshoe bat (*Rhinolophus hipposideros*) and its phylogenetic relationships with *Eimeria* species from other bats and rodents, *Exp. Parasitol.* 139 (2014) 58–62, <https://doi.org/10.1016/j.exppara.2014.02.013>.
- [21] C.T. McAllister, R.S. Seville, Z.P. Roehrs, A new species of *Eimeria* (Apicomplexa: Eimeriidae) from the northern myotis, *Myotis septentrionalis* (Chiroptera: Vespertilionidae), in Oklahoma, *J. Parasitol.* 98 (2012) 1003–1005, <https://doi.org/10.1645/GE-3119.1>.
- [22] R.B. Adhikari, M. Maharjan, T.R. Ghimire, Prevalence of gastrointestinal parasites in the frugivorous and the insectivorous bats in Southcentral Nepal, *J. Parasitol. Res.* (2020), 8880033, <https://doi.org/10.1155/2020/8880033>.
- [23] F. Murakoshi, K. Koyama, T. Akasaka, N. Horiuchi, K. Kato, Molecular and histopathological characterization of *Cryptosporidium* and *Eimeria* species in bats in Japan, *J. Vet. Med. Sci.* 80 (2018) 1395–1399, <https://doi.org/10.1292/jvms.18-0130>.
- [24] M.A. Musaev, A.M. Viesov, Coccidia occurring in *Apodemus sylvaticus* in Azerbaidjan, *Izv. Akad. Nauk Azerbaidzhanskoi SSR, Seriya Biol. Med.* 5 (1963) 3–14.
- [25] A. Mácová, A. Hoblíková, V. Hypša, M. Stanko, J. Martinň, J. Kvičerová, Mysteries of host switching: diversification and host specificity in rodent-coccidia associations, *Mol. Phylogenet. Evol.* 127 (2018) 179–189, <https://doi.org/10.1016/j.ympev.2018.05.009>.
- [26] A. Alberdi, O. Razgour, O. Aizpuru, R. Novella-Fernández, J. Aihartzar, I. Budinski, I. Garin, C. Ibáñez, E. Izagirre, H. Rebelo, D. Russo, A. Vlaschenko, V. Zhelyazkova, V. Zrnčić, M.T.P. Gilbert, DNA metabarcoding and spatial modelling link diet diversification with distribution homogeneity in European bats, *Nat. Commun.* 11 (2020) 1–8, <https://doi.org/10.1038/s41467-020-14961-2>.
- [27] J. Tiede, M. Diepenbruck, J. Gadau, B. Wemheuer, R. Daniel, C. Scherber, Seasonal variation in the diet of the serotine bat (*Eptesicus serotinus*): a high-resolution analysis using DNA metabarcoding, *Basic Appl. Ecol.* 49 (2020) 1–12, <https://doi.org/10.1016/j.baec.2020.09.004>.
- [28] O. Aizpuru, I. Budinski, P. Georgiakakis, S. Gopalakrishnan, C. Ibáñez, V. Mata, H. Rebelo, D. Russo, F. Szodoray-Parádi, V. Zhelyazkova, V. Zrnčić, M.T.P. Gilbert, A. Alberdi, Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: evidence from DNA metabarcoding, *Mol. Ecol.* 27 (2018) 815–825, <https://doi.org/10.1111/mec.14474>.
- [29] X. Zhao, D.W. Duszynski, E.S. Loker, Phylogenetic position of *Eimeria antrozoi*, a bat coccidium (Apicomplexa: Eimeriidae) and its relationship to morphologically similar *Eimeria* spp. from bats and rodents based on nuclear 18S and plastid 23S rDNA sequences, *J. Parasitol.* 87 (2001) 1120–1123, [https://doi.org/10.1645/0022-3395\(2001\)087\[1120:PPOEAA\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2001)087[1120:PPOEAA]2.0.CO;2).
- [30] J. Kvičerová, V. Hypša, Host-parasite incongruences in rodent *Eimeria* suggest significant role of adaptation rather than cophylogeny in maintenance of host specificity, *PLoS One* 8 (2013), e63601, <https://doi.org/10.1371/journal.pone.0063601>.