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First report of *Eimeria myoxi* in the garden dormouse (*Eliomys quercinus* Linnaeus, 1766) from Doñana Natural Area (Andalusia, SW Spain)

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ABSTRACT

This work reports for the first time the presence and molecular characterization of *Eimeria myoxi* in the garden dormouse (*Eliomys quercinus*) from the Doñana Natural Area (Andalusia, SW Spain). Fresh faecal samples were collected from a total of 28 garden dormice, which were caught following current guidelines for the ethical use of animals in research, and processing by a standard flotation technique with saturated saline solution. Then, wet drops were examined microscopically, and the number of oocysts was semi-quantified. *Eimeria* oocysts were observed in 16 of the 28 (57.1%) faecal samples, showing most of them a very low number of oocysts (≤1 oocyst per microscopic field × 400). The unsporulated oocysts visualized in 16 faecal samples were subspherical and of length 19.2 ± 1.2 μm and width 17.4 ± 1.1 μm, being morphologically compatible with *E. myoxi*. This finding was supported by molecular analysis of the small subunit ribosomal RNA (SSU-rRNA) gene, identifying the same species in 22 of the 28 (78.6%) dormice, including 15 samples in which oocyst size was compatible with *E. myoxi*. Moreover, the subsequent analyses of the apicoplast open reading frame 470 (ORF470) and the mitochondrial cytochrome *c* oxidase subunit I (COI) genes confirmed the molecular identification of the isolates as *E. myoxi*. The phylogeny analyses were consistent with previous phylogenetic studies and support the existence of three lineages of rodent-infecting *Eimeria* species.

1. Introduction

The genus *Eimeria* (Apicomplexa, Eimeriidae) comprises obligate intracellular protozoan parasites that mainly infect the intestinal cells of a wide range of animals (fish, amphibians, reptiles, birds and mammals), causing enteric disease [1]. This genus includes several pathogenic species that can led to high levels of morbidity and/or mortality and thus important economic losses in animal production industries [2,3]. *Eimeria* parasites have complex life cycles and complete their development in a single host species. The sporulated oocysts are the infective forms of the parasite, which are very resistant, spread rapidly on soil, vegetation and water, and under favourable conditions, can remain viable in the environment for several months [4].

More than 400 *Eimeria* species have been described in rodents [5]. However, data on this protozoan parasite in members of the dormouse family, Gliridae (28 species belonging to 9 genera) [6], are very scarce, and only nine *Eimeria* species have been reported in four glirid hosts: *Dryomys nitedula* Pallas, 1778; *Eliomys melanurus* Wagner, 1840; *Eliomys quercinus* Linnaeus, 1766; and *Glis glis* Linnaeus, 1766 [7–10]. The first *Eimeria* species reported to infect the garden dormouse (*E. quercinus*), was *Eimeria myoxi*, described by Galli-Valerio in 1940 in Switzerland [11]. The species was later detected in the same host in the Czech Republic and re-described by Kvičerová et al. [10]. Moreover, mixed infections of *Eimeria melanuri* and *E. myoxi* have also been reported in the Asian garden dormouse (*E. melanurus*) in Syria [8] and in the garden dormouse (*E. quercinus*) in Italy [9].

The garden dormouse is a rodent inhabiting throughout Europe (except in the British Isles), Asia Minor, North Arabia, Egypt and in an

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Fig. 1. Geographical location of the Doñana Natural Area (Andalusia, SW Spain) where faecal samples from the garden dormouse (*Eliomys quercinus*) were collected.

area ranging from North Africa to the Sahara Dessert [12]. This species is currently listed as "near threatened" globally by the International Union for Conservation of Nature's Red List [13] and it has been declared extinct in Finland, Lithuania and Slovakia [12]. *Eliomys quercinus* is a nocturnal animal and hibernates during the winter months. It is capable of living in very different environments, such as pine, holm oak, beech and other deciduous forests, Mediterranean bushes, rocky places, sandy dunes and semi-arid zones, at elevations ranging from sea level to 1500 m and above [14]. The garden dormouse is essentially an insectivorous species, but it can also consume seeds, nuts, fruit, birds and their eggs, and even small rodents such as members of the genera *Apodemus* and *Mus* [12].

The aim of the present study was to investigate the presence of *Eimeria* in the garden dormouse (*E. quercinus*) from the Doñana Natural Area (Andalusia, SW Spain) by microscopic and molecular analysis.

2. Materials and methods

2.1. Collection and processing of faecal samples

In October 2020 and April 2021, faecal samples from the garden dormouse *E. quercinus* were collected in the Doñana Natural Area (Andalusia, SW Spain), which is considered one of the most important protected areas in Spain and the largest ecological reserve in Europe (Fig. 1) [15]. A total of 28 garden dormice were caught during their daily resting activity in wooden nest boxes or live trapped with Sherman traps placed on the ground following current guidelines for the ethical use of animals in research. Fresh faecal samples were collected directly from each individual or from the live trap (in which only one garden dormouse was caught) and stored at 4 °C until analysis. All sampled animals were then released at the trapping site after being earmarked.

The faecal samples were processed using a standard flotation technique with saturated saline solution (NaCl; Sigma-Aldrich, St. Louis, MO, United States), and wet drops were examined under bright-field microscopy (× 400 magnification) to detect *Eimeria* oocysts using a microscope AX70 (Olympus Optical Co., Ltd., Tokyo, Japan). The number of oocysts was scored semi-quantitatively according to the mean number of oocysts in 20 randomly selected fields: 0 (no oocysts), 1 (≤1 oocyst), 2 (2–10 oocysts) and 3 (>10 oocysts). Positive samples (0.5 g) were individually placed in 2.5% (w/v) potassium dichromate solution ($K_2Cr_2O_7$; Sigma-Aldrich) and left for maximum of 2 weeks at 23 °C to sporulate in order to enable morphological identification. The oocysts were measured in a light microscope (AX70, Olympus Optical Co., Ltd.) by using an ocular micrometer and DP Controller software 2.1.1.183 (©2001–2004 Olympus Optical Co., Ltd.).

2.2. Molecular analysis

Genomic DNA was extracted from approximately 0.5 g of each faecal sample with the Stool DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. Samples were screened using PCR techniques which amplified fragments of ~420 bp, ~ 810 bp and ~ 780 bp of the small subunit ribosomal RNA (SSU-rRNA), the apicoplast open reading frame 470 (ORF470) and the mitochondrial cytochrome *c* oxidase subunit I (COI) genes of *Eimeria*, respectively [16–18]. The amplicons were electrophoresed on 2% agarose gels and stained with Real Safe (Real Laboratory S.L., Paterna, Valencia, Spain). The PCR products of 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.

Table 1
Detection of *Eimeria myoxi* in faecal samples from the garden dormouse (*Eliomys quercinus*) collected in Doñana Natural Area (Andalusia, SW Spain) by microscopic examination (including semi-quantification of oocysts) and molecular analysis at the small subunit ribosomal RNA (SSU-rRNA), the apicoplast open reading frame 470 (ORF470) and the mitochondrial cytochrome *c* oxidase subunit I (COI) genes.

Sample	Microscopic examination			Molecular analysis			
	Result	Score	Morphological compatibility	SSU-rRNA	ORF470	COI	Species
M1	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M2	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M3	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M4	+	2	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M5	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M6	+	3	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M7	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M8	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M9	+	2	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M10	+	1	*Eimeria myoxi*	−	−	−	
M11	−	0		+	−	−	*Eimeria myoxi*
M12	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M13	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M14	−	0		+	−	−	*Eimeria myoxi*
M15	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M16	−	0		−	−	−	
M17	+	3	*Eimeria myoxi*	+	+	−	*Eimeria myoxi*
M18	−	0		−	−	−	
M19	−	0		+	+	+	*Eimeria myoxi*
M20	−	0		+	−	−	*Eimeria myoxi*
M21	−	0		−	−	−	
M22	−	0		−	−	−	
M23	−	0		+	−	−	*Eimeria myoxi*
M24	−	0		+	−	−	*Eimeria myoxi*
M25	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M26	−	0		−	−	−	
M27	+	1	*Eimeria myoxi*	+	−	−	*Eimeria myoxi*
M28	−	0		+	+	−	*Eimeria myoxi*

Score: 0 = (no oocysts); 1 (≤1 oocyst); 2 (2–10 oocysts); 3 (>10 oocysts).

Table 2
Comparison of morphometric data (μm) of *Eimeria* oocysts isolated from faecal samples of the garden dormouse (*Eliomys quercinus*) from the Doñana Natural Area (Andalusia, SW Spain) with previously published data for *Eimeria myoxi*.

	Galli-Valerio [11]	Golemansky and Darwish [8]	Bertolino and Canestri-Trotti [9]	Kvičerová et al. [10]	Present study
Oocyst length					
Mean ± SD	18.0	18.1 ± 1.0	18.9 ± 1.2	18.1	19.2 ± 1.2
Range		16.2–20.0	–	16.0–20.0	17.5–20.0
Oocyst width					
Mean ± SD	15.0	16.4 ± 1.2	17.3 ± 1.0	16.9	17.4 ± 1.1
Range		13.0–19.0	–	15.0–18.0	15.0–20.0
Sporocyst length					
Mean ± SD	7.5	–	8.0 ± 1.1	10.2	–
Range			–	9.0–11.0	
Sporocyst width					
Mean ± SD	6.0	–	6.0 ± 0.7	6.5	–
Range			–	6.0–8.0	
Prevalence (%)	–	100	54.6–64.7	85.2	57.1
Country	Switzerland	Syria	Italy	Czech Republic	Spain

SD = standard deviation.

Sequence data were analysed using SeqMan™ 7.0 software (DNASTAR®, Madison, WI, USA) and 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 via the public web interface of the BLAST® 2.12.0 program [19,20]. Phylogeny analyses were conducted using MEGA X software [21].

3. Results

Microscopic examination revealed the presence of *Eimeria* oocysts in 16 of the 28 (57.1%) garden dormouse faecal samples, most of which showed very low number of oocysts (≤1 oocyst per microscopic field × 400) (Table 1). Unfortunately, the attempts to sporulate *Eimeria* oocysts were unsuccessful.

The oocysts visualized in 16 faecal samples were subspherical and of

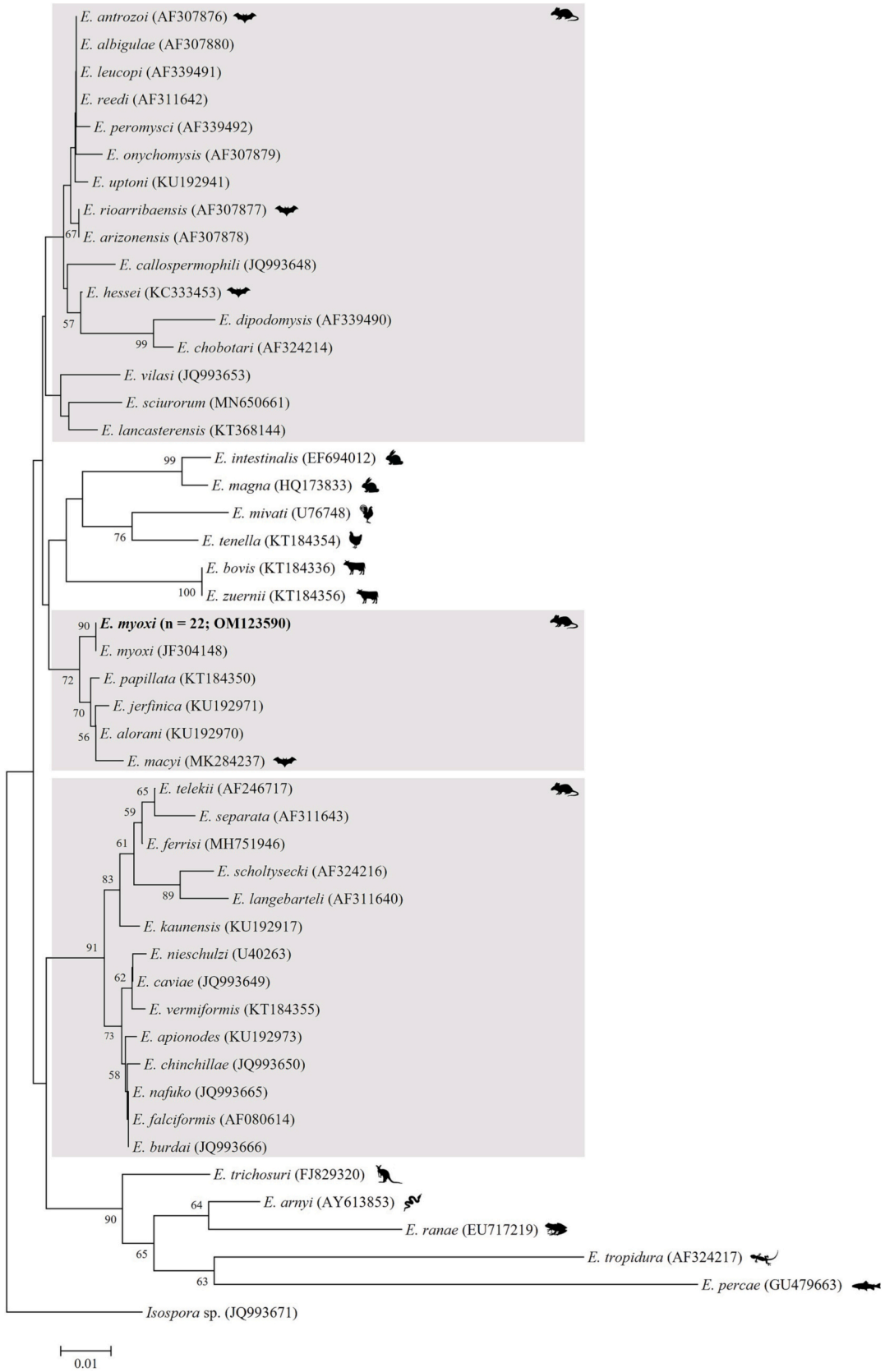

Fig. 2. Phylogenetic relationships between *Eimeria myoxi* isolated from the garden dormouse (*Eliomys quercinus*) and other *Eimeria* spp. inferred by neighbour-joining analysis of the small subunit ribosomal RNA (SSU-rRNA) gene on the basis of genetic distances calculated by Kimura's two parameter model [37] (gamma distributed with 5 rate categories). The tree was generated using a total of 374 positions in the final data set. 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 three lineages of rodent-infecting *Eimeria* are shown in shaded boxes. The isolate and its corresponding accession number obtained in this study is highlighted in bold.

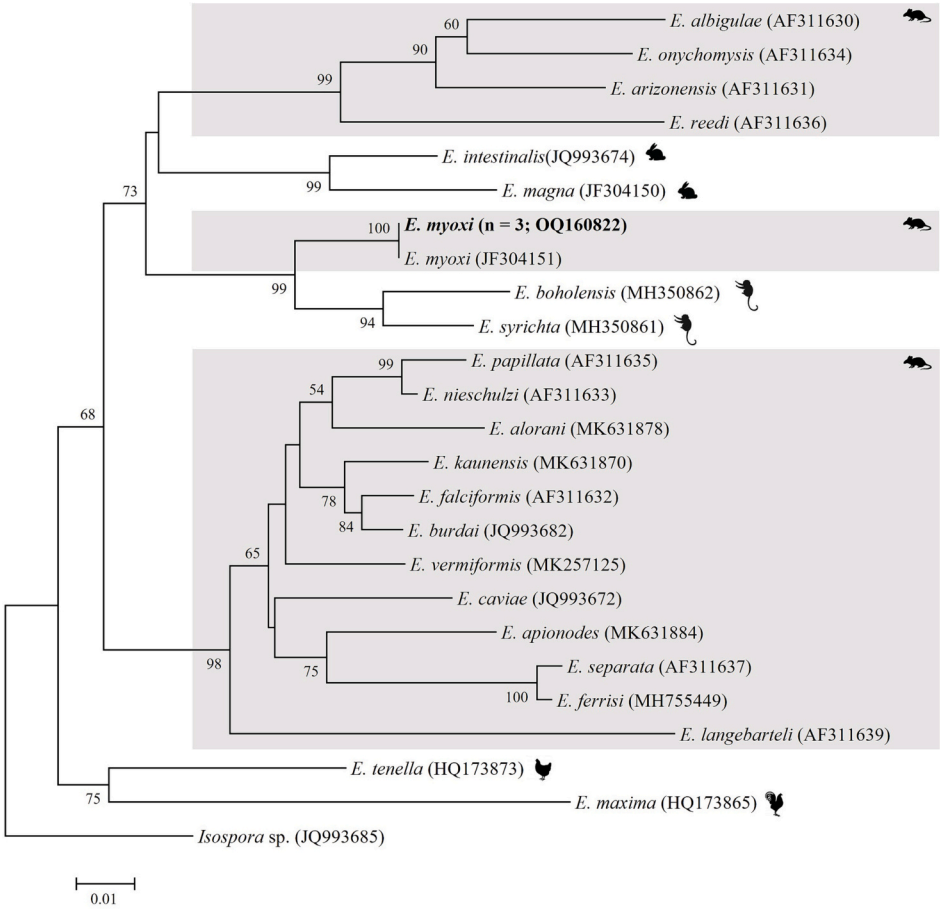

Fig. 3. Phylogenetic relationships between *Eimeria myoxi* isolated from the garden dormouse (*Eliomys quercinus*) and other *Eimeria* spp. inferred by neighbour-joining analysis of the apicoplast open reading frame 470 (ORF470) gene on the basis of genetic distances calculated by Tamura's three parameter model [38] (gamma distributed with 5 rate categories). The tree was generated using a total of 450 positions in the final data set. 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 three lineages of rodent-infecting *Eimeria* are shown in shaded boxes. The isolate and its corresponding accession number obtained in this study is highlighted in bold.

length 19.2 ± 1.2 μm and width 17.4 ± 1.1 μm (Table 2). The size and shape of the oocysts detected in the faecal samples from garden dormice were consistent with the original and subsequent descriptions of *E. myoxi* (Table 2). We therefore consider that the oocysts belong to the species *E. myoxi*.

Partial sequences of the *Eimeria* SSU-rRNA gene were obtained from 22 of the 28 (78.6%) faecal samples. *Eimeria myoxi* was identified in all garden dormice samples (including 15 samples in which oocyst size was compatible with *E. myoxi*) (Table 1). The same consensus sequence was obtained from all of these samples and displayed a similarity of 100% relative to the *E. myoxi* sequence deposited in the GenBank® database under accession number JF304148. The subsequent analyses of the ORF470 and COI genes allowed to obtain partial sequences from three (10.7%) and one (3.6%) of the 28 faecal samples, respectively, identifying again *E. myoxi* in all of them (Table 1). The same consensus sequence was obtained from the three ORF470 positive samples, which showed a similarity of 100% relative to the *E. myoxi* sequence deposited in the GenBank® database under accession number JF304151. The COI sequence obtained displayed an identity of 100% relative to the *E. myoxi* sequence of 404 bp deposited in the GenBank® database under accession number JQ993696. The phylogenetic analyses carried out in this work revealed that *Eimeria* isolates from the garden dormice were 100% homologous and clustered with *E. myoxi* (Figs. 2, 3 and 4). The sequences obtained in the present study were deposited in the GenBank® database under accession numbers OM123590, OQ160822 and OQ320778.

4. Discussion

This work reports for the first time the presence and molecular characterization of *E. myoxi* in the garden dormouse (*E. quercinus*) from the Doñana Natural Area (Andalusia, SW Spain), obtaining prevalence values of 57.1% and 78.6% after microscopic and molecular analysis, respectively. In previous studies, prevalence values ranged between 54.6% and 100%, although the maximum rate corresponds to a study in which only one specimen was examined (see Table 2) [8–10]. Several authors suggested that the high prevalence of parasites, including *Eimeria*, in dormice may be due to group hibernation; however, other factors dependent on group density or the status of parasite infection should not be ruled out [9,22].

All attempts to sporulate *Eimeria* oocysts were unsuccessful, probably as a consequence of the faecal samples being stored at 4 °C for several months from collection to processing. The temperature has a strong effect on the sporulation of the oocysts of different *Eimeria* species as reported by Christensen [23] and Pyziel and Demiaszkiewiez [24]. Thus, the percentage of sporulated oocysts decreases significantly when faecal samples are stored at 3–5 °C [24]. In any case, the measurements of the unsporulated oocysts observed in the present study (19.2 ± 1.2 μm × 17.4 ± 1.1 μm) were similar to those previously reported for *E. myoxi* by several authors in Switzerland, Syria, Italy and Czech Republic (Table 2) [8–11]. The size of *Eimeria* oocysts can vary, as morphometric polymorphism has been reported in the same host from several geographical locations [25,26]. Although Pellérdy [7] reported the presence of *E. myoxi* in *E. quercinus* from Hungary, the oocysts described were considerably larger (20–27 μm × 19–25 μm), exceeding the variability observed for this *Eimeria* species in other studies. Subsequently, Golemansky and Darwish [8] suggested that the large dimensions of the oocysts indicated that they belonged to *E. melanuri* (21.5 ± 1.1 μm × 19.4 ± 0.8 μm).

Molecular data are available for a relatively small number of the

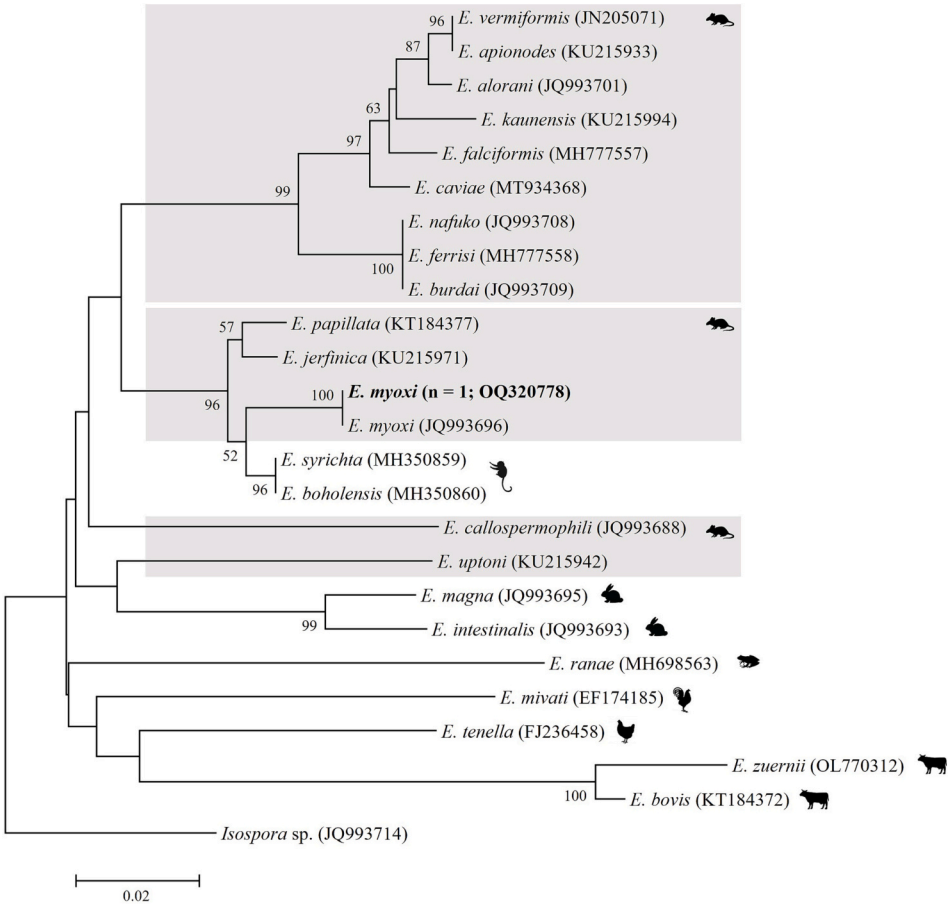

Fig. 4. Phylogenetic relationships between *Eimeria myoxi* isolated from the garden dormouse (*Eliomys quercinus*) and other *Eimeria* spp. inferred by neighbour-joining analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene on the basis of genetic distances calculated by Tamura-Nei model [39] (gamma distributed with 5 rate categories). The tree was generated using a total of 399 positions in the final data set. 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 three lineages of rodent-infecting *Eimeria* are shown in shaded boxes. The isolate and its corresponding accession number obtained in this study is highlighted in bold.

Eimeria species described in rodents. Amplification and sequencing of the SSU-rRNA, ORF470 and COI genes are currently being used to detect and characterise *Eimeria* in multiple host species [16,27–30]. In the present study, molecular analysis of a fragment of the SSU-rRNA gene confirmed that the oocysts visualized in 15 faecal samples from *E. quercinus* belong to *E. myoxi* and allowed to identify this coccidian species in other seven garden dormouse samples, increasing the prevalence value obtained by microscopical examination. The subsequent analyses of fragments of the ORF470 and COI genes confirmed the identification of *E. myoxi*.

One of the main problems in the reconstruction of the *Eimeria* evolution is the lack of reliable genetic resolution of the deeper nodes within the phylogenetic trees, being needed to establish a proper molecular marker to approach this question. Some authors reported instability among *Eimeria* lineages when phylogenetic markers such as SSU-rRNA and COI are used because they would not be variable enough to differentiate isolates of the parasite that would be considered separate species based on the host [10,30]. Recently, Jarquín-Díaz et al. [30] suggested that the rarely used marker ORF470 from the apicoplast genome appears to provide slightly better resolution.

Previous phylogenetic studies showed that *Eimeria* species that infect rodents tend to cluster in two monophyletic but unrelated groups and that their relationships within the clusters are not dependent on the taxonomic position of the host [10,17,31–33]. The first molecular analysis of *E. myoxi* was carried out by Kvičerová et al. [10], who reported that the *E. myoxi* sequence is located outside the two 'rodent' branches and that it would constitute the third lineage of rodent-infecting eimerians. As only a few *Eimeria* species have been characterized at the molecular level, some authors believe that host-dependent clustering may be consequence of inadequate taxonomic sampling and, therefore, additional sequences obtained from other rodent genera may alter the pattern of the two rodent-specific groups [10,34]. In the present study, the results of the phylogenetic analyses performed with *Eimeria* sequences obtained from different animal classes (including rodent-specific species such as *Eimeria jerfinica* and *Eimeria alorani*) show that the SSU-rRNA, ORF470 and COI sequences of *E. myoxi* obtained from the garden dormouse are placed outside the two recognized rodent clades. These findings are consistent with previous phylogenetic analyses conducted by several authors [17,30–33,35,36] and support the existence of three lineages of rodent-infecting *Eimeria*, as previously suggested by Kvičerová et al. [10].

5. Conclusion

The present study contributes to current knowledge about *Eimeria* in rodent species, identifying *E. myoxi* in the garden dormouse (*E. quercinus*) from Doñana Natural Area (Andalusia, SW Spain) and providing further evidence that the phylogeny of rodent-infecting *Eimeria* is constituted by three lineages.

Ethics statement

The sampling was performed according to European (EC Directive 86/609/EEC) and Spanish laws (RD 223/1988; RD 1021/2005), current guidelines for the ethical use of animals in research (ASAB, 2006), the Animal Experiment Committee of CSIC and the Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible, Junta de Andalucía (19/03/2017/133).

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CRediT authorship contribution statement

Seila Couso-Pérez: Investigation, Writing – original draft, Writing – review & editing. **Xosé Pardavila:** Resources. **Francisco Carro:** Resources. **Elvira Ares-Mazás:** Investigation, Writing - original draft, Writing - review & editing. **Hipólito Gómez-Couso:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Data availability

The original contributions presented in the study are included in the article. Data will be made available on request to authors.

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References

[1] H.D. Chapman, J.R. Barta, D. Blake, A. Gruber, M. Jenkins, N.C. Smith, X. Suo, F. M. Tomley, A selective review of advances in coccidiosis research, Adv. Parasitol. 83 (2013) 93–171, https://doi.org/10.1016/B978-0-12-407705-8.00002-1.

[2] S. Aarthi, G. Dhinakar Raj, M. Raman, S. Gomathinayagam, K. Kumanan, Molecular prevalence and preponderance of *Eimeria* spp. among chickens in Tamil Nadu, India, Parasitol. Res. 107 (2010) 1013–1017, https://doi.org/10.1007/s00436-010-1971-2.

[3] D. Sharma, N.K. Singh, H. Singh, A. Joachim, S.S. Rath, D.P. Blake, Discrimination, molecular characterisation and phylogenetic comparison of porcine *Eimeria* spp. in India, Vet. Parasitol. 255 (2018) 43–48, https://doi.org/10.1016/J.VETPAR.2018.03.020.

[4] J.D. Lima, Coccidiose dos rumiantes domésticos, Rev. Bras. Parasitol. Vet. 13 (2004), 9–3.

[5] D.W. Duszynski, S.J. Upton, *Cyclospora*, *Eimeria*, *Isospora*, and *Cryptosporidium* spp., in: W.M. Samuel, M.J. Pybus, A.A. Kocan (Eds.), Parasitic Diseases of Wild Mammals, Iowa State University Press, Ames, 2001, pp. 416–459.

[6] D.E. Wilson, D.M. Reeder, Mammal Species of the World: A Taxonomic and Geographic Reference, second ed., Smithsonian Institution Press, Washington D. C, 1993.

[7] L.P. Pellérdy, Coccidia and coccidiosis, Akademiai Kiadó, Budapest, 1974.

[8] V. Golemansky, A. Darwish, *Eimeria melanuri* sp. n. (Coccidia, Eimeriidae), an intestinal parasite of *Eliomys melanurus* Wagner, 1840 (Rodentia, Gliridae) from Syria, Acta Protozool. 32 (1993) 269–270.

[9] S. Bertolino, G. Canestri-Trotti, *Eimeria* species (Apicomplexa: Eimeriidae) infecting *Eliomys quercinus* in an alpine habitat, J. Wildl. Dis. 41 (2005) 442–445, https://doi.org/10.7589/0090-3558-41.2.442.

[10] J. Kvičerová, V. Mikeš, V. Hypša, Third lineage of rodent eimerians: morphology, phylogeny and re-description of *Eimeria myoxi* (Apicomplexa: Eimeriidae) from *Eliomys quercinus* (Rodentia: Gliridae), Parasitology 138 (2011) 1217–1223, https://doi.org/10.1017/S0031182011001107.

[11] B. Galli-Valerio, Notes de parasitologie et de technique parasitologique, Schweiz. Arch. Tierheilkd. 82 (1940) 352–358.

[12] S. Moreno, Lirón careto - *Eliomys quercinus*. http://www.vertebradosibericos.org/mamiferos/elique.html, 2017 (accessed 30 January 2023).

[13] S. Bertolino, G. Amori, H. Henttonen, I. Zagorodnyuk, J. Zima, R. Juškaitis, H. Meinig, B. Kryštufek, *Eliomys quercinus*. The IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/7618/12835766, 2008 (accessed 30 January 2023).

[14] S. Moreno, Lirón careto - *Eliomys quercinus* (Linnaeus, 1766), in: A. Salvador, J. Cassinello (Eds.), Enciclopedia virtual de los vertebrados españoles, Museo Nacional de Ciencias Naturales, Madrid, 2012, pp. 1–16.

[15] Junta de Andalucía, Espacio Natural de Doñana. https://www.juntadeandalucia.es/medioambiente/portal/landing-page-índice/-/asset_publisher/zX2ouZa4r1Rf/content/espacio-natural-de-do-c3-b1ana-1/20151, 2022 (accessed 30 January 2023).

[16] 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.

[17] X. Zhao, D.W. Duszynski, Phylogenetic relationships among rodent *Eimeria* species determined by plastid ORF470 and nuclear 18S rDNA sequences, Int. J. Parasitol. 31 (2001) 715–719, https://doi.org/10.1016/S0020-7519(01)00136-9.

[18] J.D. Ogedengbe, R.H. Hanner, J.R. Barta, DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata), Int. J. Parasitol. 41 (2011) 843–850, https://doi.org/10.1016/J.IJPARA.2011.03.007.

[19] E.W. Sayers, M. Cavanaugh, K. Clark, J. Ostell, K.D. Pruitt, I. Karsch-Mizrachi, GenBank, Nucleic Acids Res. 47 (2019) D94–D99, https://doi.org/10.1093/nar/gky989.

[20] 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.

[21] 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.

[22] J. Bussy, Observations écologiques et hibernation des lérots (*Eliomys quercinus*) au cours des hivers 1966-67 et 1967-68, Bull. Mens. Soc. Linn. Lyon 37 (1968) 255–258, https://doi.org/10.3406/LINLY.1968.6026.

[23] J.F. Christensen, Sporulation and viability of oocysts of *Eimeria arloingi* from the domestic sheep, J. Agric. Res. 59 (1939) 527–534.

[24] A.M. Pyziel, A.W. Demiaszkiewicz, Observations on sporulation of *Eimeria bovis* (Apicomplexa: Eimeriidae) from the European bison *Bison bonasus*: effect of temperature and potassium dichromate solution, Folia Parasitol. 62 (2015) 020, https://doi.org/10.14411/FP.2015.020.

[25] D.W. Duszynski, Increase in size of *Eimeria separata* oocysts during patency, J. Parasitol. 57 (1971) 948–952.

[26] S.L. Gardner, D.W. Duszynski, Polymorphism of eimerian oocysts can be a problem in naturally infected hosts: an example from subterranean rodents in Bolivia, J. Parasitol. 76 (1990) 805–811.

[27] A. Mácová, A. Hoblíková, V. Hypša, M. Stanko, J. Martinů, 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.

[28] 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.

[29] O.B. Mohammed, M.M. Aljedaie, M.S. Alyousif, N. Amor, *Eimeria tamimi* sp. n. (Apicomplexa: Eimeriidae) from the rock hyrax (*Procavia capensis jayakari*) in central Saudi Arabia, Folia Parasitol. (Praha) 67 (2020), https://doi.org/10.14411/fp.2020.001, 2020.001.

[30] V.H. Jarquín-Díaz, A. Balard, A. Mácová, J. Jost, T.R. von Szepesbéla, K. Berktold, S. Tank, J. Kvičerová, E. Heitlinger, Generalist *Eimeria* species in rodents: multilocus analyses indicate inadequate resolution of established markers, Ecol. Evol. 10 (2020) 1378–1389, https://doi.org/10.1002/ece3.5992.

[31] X. Zhao, D.W. Duszynski, Molecular phylogenies suggest the oocyst residuum can be used to distinguish two independent lineages of *Eimeria* spp. in rodents, Parasitol. Res. 87 (2001) 638–643, https://doi.org/10.1007/s004360100393.

[32] 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.

[33] 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.

[34] 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.

[35] J.A. Hnida, D.W. Duszynski, Taxonomy and systematics of some *Eimeria* species of murid rodents as determined by the ITS1 region of the ribosomal gene complex, Parasitology 119 (1999) 349–357, https://doi.org/10.1017/S0031182099004849.

[36] L. Hofmannová, M. Jirků, M. Řeháková, J. Kvičerová, Two new species of *Eimeria* (Apicomplexa: Eimeriidae) in Philippine tarsier (*Tarsius syrichta*), Eur. J. Protistol. 66 (2018) 77–85, https://doi.org/10.1016/J.EJOP.2018.08.003.

[37] 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.

[38] K. Tamura, Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases, Mol. Biol. Evol. 9 (1992) 678–687, https://doi.org/10.1093/oxfordjournals.molbev.a040752.

[39] K. Tamura, M. Nei, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol. Biol. Evol. 10 (1993) 512–526, https://doi.org/10.1093/oxfordjournals.molbev.a040023.