Zoologica Scripta

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Phylogeny of the reptilian *Eimeria*: are *Choleoeimeria* and *Acroeimeria* valid generic names?

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Submitted: 27 January 2015
Accepted: 28 May 2015
doi:10.1111/zsc.12126

Megía-Palma, R., Martínez, J., Acevedo, I., Martín, J., García-Roa, R., Ortega, J., Peso-Fernández, M., Albaladejo, G., Cooper, R.D., Paranjpe, D.A., Sinervo, B.R., & Merino, S. (2015). Phylogeny of the reptilian *Eimeria*: are *Choleoeimeria* and *Acroeimeria* valid generic names?. — *Zoologica Scripta*, *00*, 000–000.

Reptiles are the animals with the most described coccidian species among all vertebrates. However, the co-evolutionary relationships in this host–parasite system have been scarcely studied. Paperna & Landsberg (*South African Journal of Zoology*, 24, 1989, 345) proposed the independent evolutionary origin of the *Eimeria*-like species isolated from reptiles based on morphological and developmental characteristics of their oocysts. Accordingly, they suggested the reclassification of these parasites in two new genera, *Choleoeimeria* and *Acroeimeria*. The validity of the genera proposed to classify reptilian *Eimeria* species remained unresolved due to the lack of species genetically characterized. In this study, we included *18S* rRNA gene sequences from seven *Eimeria*-like species isolated from five different lizard host families. The phylogenetic analyses confirmed the independent evolutionary origin of the *Eimeria*-like species infecting lizards. Within this group, most species were placed into two monophyletic clades. One of them included the species with ellipsoidal oocysts (i.e. *Choleoeimeria*-like oocysts), whereas the species with more spheroidal oocysts (i.e. *Acroeimeria*-like oocysts) were included in the second one. This result supports the taxonomic validity of the genera *Acroeimeria* and *Choleoeimeria*.

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Introduction

Schneider first described the genus *Eimeria* in 1875 in a rodent species. Since then, about 2000 species have been described parasitizing both vertebrate and invertebrate hosts (after Upton 2000; Zhao & Duszynski 2001a). However, 98% of *Eimeria* species were described from vertebrate hosts using the characteristic tetrasporocystic, dizoic exogenous oocyst (Asmundsson *et al.* 2006; Ghimire 2010). The implementation of molecular techniques rapidly advanced the knowledge of the phylogenetic relationships within the family Eimeriidae (e.g. Zhao & Duszynski 2001a,b; Jirků *et al.* 2002; Kvičerová *et al.* 2008). In this context, evolutionary trees showed not only the high specificity of these parasites to their vertebrate hosts (Honma *et al.* 2007; Power *et al.* 2009), but also the paraphyly of the genus *Eimeria* (Morrison 2009). In fact, genera such as *Cyclospora* Schneider 1881, *Caryospora* Léger 1904, *Isospora* Schneider 1881, *Lankesterella* Labbé 1899 and *Schellackia* Reichenow 1919 shared ancestor with *Eimeria* (see Megía-Palma *et al.* 2014). Based on previous molecular results (Jirků *et al.* 2002), *Eimeria*-like parasites found in reptiles were considered a sister taxon to Eimeriidae and phylogenetically distant from eimeriids isolated from birds and mammals (Jirků *et al.* 2002). However, the relationships among the *Eimeria*-like parasites infecting reptiles remained unresolved because only two closely related species were included in the phylogeny of the family (Jirků *et al.* 2002, 2009a,b).

Therefore, new species found in reptiles were classified based on characteristic of their life cycles, morphological features of the exogenous oocysts, and the ultrastructure of the different stages of their development (Paperna & Lainson 1999a,b, 2000; Paperna 2003, 2007; Al Nasr 2011). The species infecting reptiles undergo three different types of endogenous development (Lainson & Paperna 1999). On the one hand, parasites with endogenous development occurring in the gall bladder and biliary epithelium surface were proposed to form the genus *Choleoeimeria* (Paperna & Landsberg 1989). On the other hand, eimeriid species developed in the microvillous zone of the intestine might be classified within the genus *Acroeimeria* when the endogenous development is epicytoplasmic, or within the genus *Eimeria* when development is intracytoplasmic (Paperna & Landsberg 1989; Paperna 1994; Lainson & Paperna 1999; Paperna & Lainson 1999b; Modrý & Jirků 2006). Paperna & Landsberg (1989) proposed the genus *Choleoeimeria* including species with an oocyst shape index (OSI; Paperna & Landsberg 1989) threshold of >1.4 (usually 1.6–2.2). The validity of the OSI, in this case, was broadly discussed (Modrý *et al.* 2000; Jirků *et al.* 2002; Asmundsson *et al.* 2006). However, a relationship between OSI value (>1.4) and the location where oocysts undergo the endogenous development (i.e. the gall bladder) is supported for several species (Bovee & Telford 1965a; Asmundsson *et al.* 2006). Alternatively, OSI should be <1.25 for *Acroeimeria* parasites (Paperna & Landsberg 1989). Other authors preferred to adjust the OSI range for *Choleoeimeria* from 1.5 to 1.8, (but always above 1.4) and commented on the 'striking uniformity' of the oocyst morphology within the genus *Choleoeimeria* (Paperna & Landsberg 1989 in Jirků *et al.* 2002). Nevertheless, it was pointed out that typically some species of *Eimeria* showing an OSI average around 1.25 have measurement ranges that overlap with those of *Choleoeimeria*. These species could not be classified into any genus before more information became available (Paperna & Landsberg 1989). However, the name *Eimeria incertae sedis (i. s.)* was proposed for those species that did not fit either the amended definition of Eimeriidae (see Jirků *et al.* 2002), nor the definition of the genera *Choleoeimeria* or *Acroeimeria* based on the site of their endogenous development (Modrý & Jirků 2006).

Morphological features of the sporocysts were also used to identify eimeriids from poikilotherms. The absence of Stieda body and the presence of alternative opening structures (i.e. bivalve suture) in the sporocysts of these eimeriids (Paperna & Landsberg 1989) were highlighted as indicators of the ancestral origins of this group of parasites (Jirků *et al.* 2002, 2009a,b). Based on these features, some authors suggested the resurrection of the family Barrouxiidae *sensu* Levine (1983) including the genera *Goussia* Labbé 1896, *Choleoeimeria* Paperna & Landsberg 1989 and *Acroeimeria* Paperna & Landsberg 1989 (Berto *et al.* 2014). Thus, the presence of the typical suture in the genera *Goussia*, *Choleoeimeria* and *Acroeimeria* may represent a homoplasy rather than synapomorphy (Jirků *et al.* 2002).

There is an open debate about the use of certain characters including singularities of the life cycle and morphometric features of the oocyst to infer the evolutionary relationships among these eimeriids (see Paperna & Landsberg 1989; : Lainson & Paperna 1999; Paperna & Lainson 1999b, 2000; Asmundsson *et al.* 2006; Modrý & Jirků 2006; Abdel-Baki *et al.* 2008; Daszak *et al.* 2009). In this study, we explore the phylogenetic relationships of eimeriid species parasitizing lizards to help clarify the suitability of the genera *Choleoeimeria* and *Acroeimeria*. For this purpose, we use molecular techniques to characterize seven *Eimeria*-like species isolated from five different families of reptiles. We also include the *18*s rRNA gene sequence of other eimeriid species isolated from *Salamandra salamandra* Linnaeus 1758 (Amphibia: Caudata).

Material and methods

Faecal samples were collected from lizard species where various species of the genus *Eimeria* had previously been

described. Specifically, we tried to get species belonging to different host families. Fortunately, many of the lizard species known to be hosts for eimerian parasites were available in the pet trade. Apart from those exotic reptiles obtained from pet stores, we primarily sampled reptiles in the field. We obtained samples from the families Gekkonidae, Lacertidae, Phrynosomatidae, Scincidae and Trogonophidae (Table 1). Additionally, we included a coccidian found in the faeces of a Fire Salamander, *S. salamandra* (Caudata: Salamandridae) to contribute molecular data from a novel coccidian infection in the order Caudata. In all the cases, the faecal samples were obtained directly from the cloaca of the animals by briefly massaging their belly and collecting them in standard vials filled with 1 mL of 2% (w/v) potassium dichromate to facilitate sporulation (Duszynski & Wilber 1997). In the case of the Fire salamander faeces, we tried to aid sporulation of the oocysts by dividing the sample into two parts, one being preserved in tap water (Duszynski & Wilber 1997) and the remaining sample in potassium dichromate. After the process of sporulation, we homogenized the sample using a plastic pipette and used one part of the sample for the microscope identification of the sporulated oocysts. The remaining part of the sample was preserved at 4 °C for later molecular characterization.

Microscopic methods

For the microscopic screening of the samples, we followed the standard protocol of concentration of parasites by means of Sheather's sugar flotation technique (Levine 1973). Each sample was screened at 200× magnification with an optic microscope BX41TF (Olympus, Tokyo, Japan). In order to get representative photomicrographs and to measure the oocysts of the species that we found, we took photographs at 400, 600 and 1000× with an adjustable microscope camera (Olympus SC30). Unfortunately, due to the scarce sample from the Canarian geckonid and the salamander, we were unable to take pictures at 1000× magnification as is standard for research on eimeriids (Duszynski & Wilber 1997). Therefore, the microphotographs from the exogenous stages were scaled accordingly (Fig. 1) and line drawings of the newly described species were included as supplementary information online only (Fig. S1). The oocyst shape index was calculated as ratio of the length and the width of each parasite oocyst. Further, the species average OSI was calculated using these data. All measurements from the sporulated oocysts are expressed in micrometres and were taken using the MB-RULER 5.0 free software (http://www.markus-bader.de/MB-Ruler/).

Molecular methods

PowerFecal® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA 92010, USA) was used to extract DNA from the faecal samples. Thereafter, the DNA was purified using the NZYGelpure kit (NZYTECH, Lda – genes & enzymes). Partial amplification of the *18S* rRNA gene sequence (1626 bp) was performed using the primers BT-F1 (5′-GGT TGA TCC TGC CAG TAG T-3′)/hep1600R (5′-AAA GGG CAG GGA CGT AAT CGG-3′). These primers were previously used to amplify other coccidian species (Megía-Palma *et al.* 2014). Due to the insectivorous diet of some reptilian species, we amplified haemogregarines together with *Eimeria* in some faecal samples. To avoid this undesired amplification, the specific reverse primers EimIsoR1 (5′-AGG CAT TCC TCG TTG AAG ATT-3′) or EimIsoR3 (5′-GCA TAC TCA CAA GAT TAC CTA G-3′) were designed to substitute for the primer hep1600R. The size of the amplicons obtained with reverse primer EimIsoR1 and EimIsoR3 was 1580 and 1528 bp, respectively. PCR volume (20 μL) contained between 20 and 100 ng of DNA template. Supreme NZYTaq 2× Green Master Mix (NZYTECH, Lda – genes&enzymes) and 0.25 μM of each primer were routinely used. The reactions were cycled under the following conditions using the Verity thermal cycler (Applied Biosystems, Barcelona, Spain): 95 °C for 10 min (polymerase activation), 40 cycles at 95 °C for 30 s, annealing temperature for 58 °C for 30 s, 72 °C for 120 s and a final extension at 72 °C for 10 min.

The eight DNA sequences (*18*S rRNA) obtained in this study were aligned together with 79 other sequences

Table 1 Reptile host species included in this study classified by family, the origin of the samples and the coccidian parasite found

Species	Common name	Family	Locality	Coccidia found
Gekko gecko	Tokay gecko	Gekkonidae	Pet trade	*Eimeria tokayae*
Tarentola delalandii	Tenerife wall gecko	Gekkonidae	Tenerife, Canary Islands	*Acroeimeria* cf. *tarentolae*
Gallotia galloti	Tenerife lizard	Lacertidae	Tenerife, Canary Islands	*Choleoeimeria gallotiae* n. comb.
Sceloporus occidentalis	Western fence lizard	Phrynosomatidae	Santa Cruz, CA. USA	*Acroeimeria sceloporis*
Salamandra salamandra	Fire salamander	Salamandridae	Monchique, Portugal	*Eimeria steinhausi* n. sp.
Eutropis macularia	Bronze grass skink	Scincidae	Pet trade	*Eimeria* (i. s.) *eutropidis* n. sp.
Mabuya (s. l.) sp.*	Skink	Scincidae	Pet trade	*Choleoeimeria scincorum* n. sp.
Trogonophis wiegmanni	Checkerboard worm lizard	Trogonophidae	Chafarinas Islands	*Choleoeimeria wiegmanniana* n. sp.

*This individual was sampled in a pet store and we lack of accurate information about the host geographic origin and its specific identification.

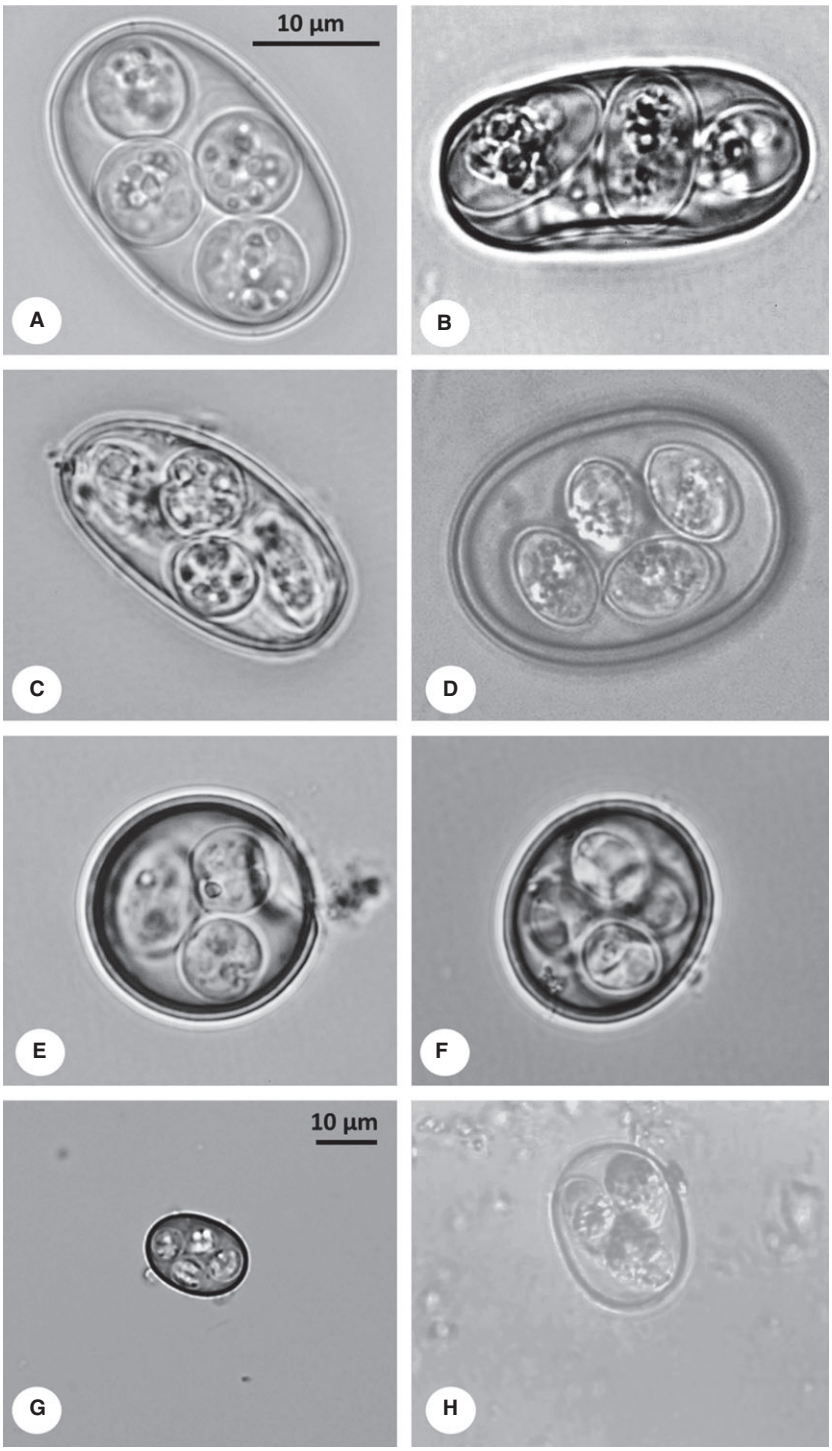

Fig. 1 A–H, exogenous oocysts of the *Eimeria*-like species found in the reptile hosts included in the phylogeny of this study. —A–F and G–H are shown at the same scale.—A. *Choleoeimeria wiegmanniana* n. sp. from *Trogonophis wiegmanni* (Trogonophidae). —B. *Choleoeimeria gallotiae* n. comb. from *Gallotia galloti* (Lacertidae). —C. *Choleoeimeria scincorum* n. sp. from *Mabuya* (s. l.) sp. —D. *Acroeimeria sceloporis* from *Sceloporus occidentalis* (Phrynosomatidae). —E. *Eimeria tokayae* from *Gekko gecko* (Gekkonidae). —F. *Eimeria* (i. s.) *eutropidis* n. sp. from *Eutropis macularia* (Scincidae). —G. *Acroeimeria* cf. *tarentolae* n. comb. from *Tarentola delalandii* (Gekkonidae). —H. *Eimeria steinhausi* n. sp. from *Salamandra salamandra* (Caudata: Salamandridae).

included in a previous study (Megía-Palma *et al.* 2014). The alignment was performed using PROBCONS (http://toolkit.tuebingen.mpg.de/probcons). Poorly aligned positions and divergent regions of the alignment were suppressed using GBlocks program (Talavera & Castresana 2007) selecting the following options: 'Minimum Number of Sequences for a Conserved Position' to 44, 'Minimum Number of Sequences for a Flank Position' to 44, 'Maximum Number of Contiguous Nonconserved Positions' to 8, 'Minimum Length of a Block' to 5 and 'Allowed Gap

Positions' to 'With Half'. The final alignment contained 1527 positions and 86 sequences. The substitution model GTR+I+G was selected using jModeltest 2.1.4 (Darriba *et al.* 2012) to perform the Bayesian analysis. This analysis consisted of two runs of four chains each, with 5 000 000 generations per run and a burn-in of 1 250 000 generations (37 500 trees for consensus tree). The final standard deviation of the split frequencies was 0.01 in both analyses. Convergence was checked using the TRACER v1.5 software (Rambaut & Drummond 2007). All of the model parameters exceeded 100.

In addition, the alignment was analysed using the maximum-likelihood inference (PhyML program; Guindon *et al.* 2010). The substitution models were those indicated above, the subtree pruning and regrafting (SPR) and the nearest-neighbour interchange (NNI) tree-rearrangements were selected, and a Bayesian-like transformation of aLRT (aBayes) was used to obtain the clade support (Anisimova *et al.* 2011).

Results

Microscopy results

We found oocysts of eimerian parasites in seven lizard species and one Fire salamander. Three of these host species were sampled in captivity and five of them in the field (Table 1). Three of the coccidian species were already described (*Eimeria gallotiae* Matuschka & Bannert 1987; *E. tokayae* Ball & Daszak 1995 and *Acroeimeria sceloporis* (Bovee & Telford 1965b) Paperna & Landsberg 1989). However, the endogenous development was only known for *A. sceloporis* Paperna & Landsberg 1989 (see Bovee & Telford 1965b). In the supplementary information [Appendix S1 (online)], we describe four new species of *Eimeria*-like parasites found in lizard hosts, we redescribed *E. gallotiae* Matuschka & Bannert 1987; *E. tropidura* Aquino-Shuster *et al.* 1990 and *Eimeria* cf. *tarentolae* Matuschka & Bannert 1986, and we describe a new species of *Eimeria*-like parasite found in Caudata hosts.

Phylogenetic results

All eimeriid species isolated from reptilian hosts, except *E. arnyi* Upton & Oppert 1991, form a well-supported monophyletic group (Fig. 2). This clade presented a basal position with respect to the rest of *Eimeria* species except *E. steinhausi* n. sp. Within this group of *Eimeria*-like parasites of reptiles, we found a strongly supported group with oocyst morphology consistent with *Acroeimeria*. *Acroeimeria sceloporis* was the sister taxa to *A. tropidura* n. comb. Both

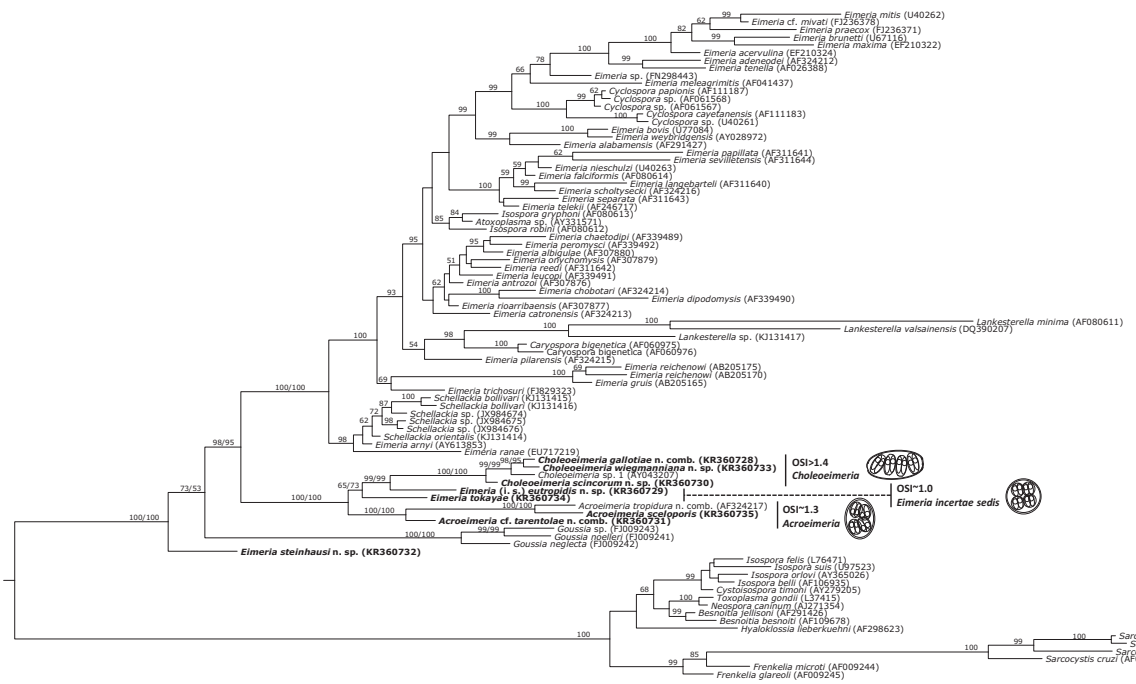

Fig. 2 Phylogenetic tree showing the evolutionary relationships among the Eimeriorina. The Bayesian inference used the GTR+G+I substitution model. This analysis consisted of two runs of four chains each, with 5 000 000 generations per run and a burn-in of 1 250 000 generations (37 500 trees for consensus tree). All branches were maintained, but support values <50% were suppressed. Where two numbers are shown in the branch, the first one indicates the supporting value achieved by Bayesian inference and the second one by maximum-likelihood inferences (ML). The ML inference was performed using PhyML program selecting the GTR+I+G substitution model. Bayesian-like transformation of aLRT (aBayes) was used to obtain the clade support. The length of the alignment was 1527 pb.

taxa were found in American lizards (Bovee & Telford 1965b; Aquino-Shuster *et al.* 1990). These two *18*s rRNA gene sequences were closely related to that from *A.* cf. *tarentolae* n. comb. found in *Tarentola delalandii* Duméril & Bribon 1836 from the Canary Islands.

On the other hand, we found four sequences of *Eimeria*-like species with oocyst morphology consistent with *Choleoeimeria sensu* Paperna & Landsberg 1989 (i.e. *Choleoeimeria* sp. 1, *C. gallotiae* n. comb., *C. wiegmanniana* n. sp. and *C. scincorum* n. sp.). These sequences formed a strongly supported clade and were also closely related to a species with a rounded oocyst, that is, *Eimeria* (i. s.) *eutropidis* n. sp. In relation with *E. steinhausi* n. sp. found in *S. salamandra*, the topology of the tree showed its ancestral origin in comparison with the rest of Eimeriidae species, including *Goussia* spp. from anuran and fish hosts. However, this relationship was moderately supported in the phylogeny (see Fig. 2).

Discussion

Based on characteristics of internal and external stages or the phylogenetic relationships studied thus far, the evolutionary origin of the *Eimeria*-like species that infect reptiles was considered independent from that of other eimeriids found in mammals and birds (Jirků *et al.* 2002, 2009a,b; Paperna 2007). In fact, all the species included in the present study grouped in a reptile-specific clade that supports the hypothesis of separate originations of these parasites. Within this clade, the species with OSI~1.3 and OSI >1.4 grouped with morphological consistency. *Acroeimeria tropidura* n. comb., *A. sceloporis* and *A.* cf. *tarentolae* n. comb. with OSI~1.3 grouped together. The high morphological and phylogenetic consistency (see Fig. 2) of this clade supports the monophyly and therefore the validity of the genus *Acroeimeria sensu* Paperna & Landsberg (1989). Nevertheless, *A.* cf. *tarentolae* n. comb. separated first from *A. sceloporis* and *A. tropidura* n. comb. (see Fig. 2), and therefore, the endogenous development of this species should be studied to confirm its consistency with *Acroeimeria* (Paperna & Landsberg 1989).

The four *Eimeria*-like species whose oocysts exhibited an OSI >1.4 formed a well-supported clade (see Fig. 2). If the morphology of the oocyst is related to site of endogenous development in the host, the three species with OSI >1.4 included in the phylogenetic analyses may develop in the host's gall bladder and the biliary epithelium (Bovee & Telford 1965a; Paperna & Landsberg 1989; Daszak & Ball 1991; Jirků *et al.* 2002; Asmundsson *et al.* 2006). The morphological consistency of the oocyst and the phylogenetic relationship of these species lend validity to the genus *Choleoeimeria.* In addition, the evolutionary tree indicated a recent origin of these *Choleoeimeria* species compared with its sister taxon, *Eimeria* (i. s.) *eutropidis*, which show an OSI of ~1.0. This morphometric feature could suggest that the ancestor of *Choleoeimeria* may resemble an *Eimeria*-like parasite with rounded oocysts and intestinal development. Thus, the ellipsoidal oocysts could be an adaptation to the physiognomy of the host's gall bladder. Alternatively, the spherical oocysts of *Eimeria (i. s.) eutropidis* could develop in the gall bladder indicating that this developmental characteristic would not be a synapomorphic character for *Choleoeimeria*. It is clearly necessary to investigate the endogenous development of the species with conflicting phylogenetic positions to confirm if the morphology of the oocyst is related to the location of the endogenous development in the host (Paperna & Landsberg 1989). In this sense, the uncertain phylogenetic position of *E. tokayae* along with its oocyst morphology with an OSI~1.0 prompted us to include it within the *Eimeria* incertae sedis *sensu* Paperna & Landsberg (1989).

The designation of separate genera with different monophyletic clades within Eimeriidae was encouraged by previous studies (Morrison 2009; Ghimire 2010). Therefore, we consider that the use of the genera *Acroeimeria* and *Choleoeimeria sensu* Paperna & Landsberg 1989 is justified even though we do not know their endogenous development. In fact, in previous studies of *Eimeria*-like parasites of reptiles, the morphology of the oocyst was related with the location of the endogenous development in the host's tissues (Bovee & Telford 1965a,b; Paperna & Landsberg 1989; Ball & Daszak 1995; Lainson & Paperna 1999; Paperna & Lainson 2000; Asmundsson *et al.* 2006; Paperna 2007; Al-Quraishy 2011; Abdel-Baki *et al.* 2013). Moreover, the use of sequencing data to determine other coccidian species without obtaining the characteristics of endogenous oocysts was implemented before in the genera *Schellackia* and *Lankesterella* (Merino *et al.* 2006; Biedrzycka *et al.* 2013; Megía-Palma *et al.* 2013, 2014). This method is particularly useful to avoid killing the reptile hosts, because it would concern conservation and ethical issues.

In regard to the family Eimeriidae, the tree indicates an evolutionarily ancestral position of the group formed by the *Eimeria*-like species from reptiles, in relation to other taxa in the family (excluding those of *Salamandra*). However, the phylogenetic position of this coccidian species isolated from Caudata hosts was poorly supported. The phylogenetic analyses showed that *Goussia* evolved independently from the *Eimeria*-like species found in reptiles (Jirků *et al.* 2009b) rejecting a recent hypothesis that placed both taxa under the family Barrouxidae (Berto *et al.* 2014). However, the oocysts of both groups shared morphological characteristics such as the occurrence of bivalved sporocysts (Jirků *et al.* 2002). The occurrence of this feature might be a plesiomorphy shared by *Eimeria*-like species from poiki-

lotherm hosts (amphibia, reptile, fish and invertebrate hosts). This characteristic also suggested an early evolution of the parasitic relationships between eimeriid coccidia and cold-blooded vertebrate hosts. However, *Eimeria arnyi* and *E. ranae* are exceptions to this rule. They are *Eimeria*-like species, parasitizing reptilian *(Diadophis punctatus arnyi)* and anuran hosts *(Rana temporaria)*, closely related to *Schellackia* species. However, it is necessary to resample data from these parasites to discard a hypothetical misidentification (Megía-Palma *et al.* 2014). Later on, evolutionary radiation of the family Eimeriidae could occur due to the emergence of the Stieda body. This structure located in sporocysts is an apomorphic trait of *Eimeria sensu* lato, and it might confer a preadaptation to parasitizing other groups of vertebrates (Jirků *et al.* 2009b).

Three of the samples that were included in the present study were obtained from reptiles kept in captivity. The risk of pseudoparasitization due to the inespecificity within Eimeriidae was reported before (see Ghimire 2010). Therefore, the parasites found in the pet trade might result in misidentification of their proper host species. However, in the present study, we selected reptile stores where the lizard species were housed separately to minimize the chances of cross-infection. Furthermore, we were able to find other coccidian species in neighbour terraria containing different host species (R. Megía-Palma, J. Martínez, I. Nasri, J. J. Cuervo, J. Martín, I. Acevedo, J. Belliure, J. Ortega, R. García-Roa, S. Selmi & S. Merino, in preparation), but we never found cross-infections either by microscopy or by molecular tools in different host species. This is not the first time that parasites have been described from reptile hosts in captivity evidencing the suitability of using imported species to detect indigenous parasites (e.g. McAllister *et al.* 1995, 2014; Megía-Palma *et al.* 2014). Moreover, the phylogenetic position, the morphology of the oocyst and the high number of oocysts (R. Megía-Palma, pers. obs.) of the *Eimeria*-like species found in the two species of skink and the Tokay gecko support this argument.

In conclusion, the reptilian *Eimeria* species form a well-supported monophyletic clade and the use of the genera *Choleoeimeria* and *Acroeimeria* proposed by Paperna & Landsberg 1989 seems to be justified from both a morphological and phylogenetic point of view.

Acknowledgements

We want to thank A. Acevedo, Dr. A. Martín, E. Serrano and C. Romero for their persistence during the field work to get *Gallotia* and *Tarentola* samples in Tenerife, all the people in the pet stores in Madrid for allowing us to collect samples from captive reptiles and the staff and facilities of the field station of the 'Refugio Nacional de Caza de las Islas Chafarinas' and 'El Ventorrillo' (MNCN-CSIC) for logistical support. I. Díez-Cortaberria for the line drawings presented here and the referees who contributed with comments that made possible the publication of this manuscript. Permissions for capturing reptiles in the wild and for collecting samples were obtained from the Área de Medio Ambiente, Sotenibilidad Territorial y Aguas, Cabildo Insular de Tenerife and the California Department of Fish and Wildlife. Financial support for field campaigns and laboratory analyses was provided by a contract from the Organismo Autónomo de Parques Nacionales (Spain), by the Spanish Ministerio de Economía y Competitividad (projects CGL2012-40026-C02-01 to S. M. and CGL2012-40026-C02-02 to J. Martínez and project CGL2011-24150 to J. Martín; and grant number BES-2010-038427 to R. M.-P.).

References

Abdel-Baki, A. S., El-Fayomi, H. M., Sakran, Th. & Abdel-Haleem, H. M. (2008). *Choleoeimeria saqanqouri* n. sp. (Apicomplexa: Eimeriidae) infecting the gallbladder of Scincus scincus scincus (Reptilia: Squamata) from Egypt. *Acta Protozoologica*, *47*, 143–147.

Abdel-Baki, A. S., Al-Quraishy, S. & Abdel-Haleem, H. M. (2013). A new species of *Choleoeimeria* (Apicomplexa: Eimeriidae) from the lizard, *Scincus hemprichii* (Sauria: Scincidae). *Folia Parasitologica*, *60*, 232–236.

Al Nasr, I. S. (2011). Reclassification of *Eimeria schmidti* Al-Yousif *et al.* (1997) (Apicomplexa: Eimeriidae) with description of its endogenous Stages. *Pakistan Journal of Zoology*, *43*, 1127–1133.

Al-Quraishy, S. (2011). A new *Choleoeimeria* species (Apicomplexa: Eimeriidae) infecting the gall bladder of *Scincus mitranus* (Reptilia: Scincidae) in Saudi Arabia. *Journal of Parasitology*, *97*, 1125–1128.

Anisimova, M., Gil, M., Dufayard, J. F., Dessimoz, C. & Gascuel, O. (2011). Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology*, *60*, 685–699.

Aquino-Shuster, A. L., Duszynski, D. W. & Snell, H. L. (1990). Three new coccidia (Apicomplexa) from the Hood Island lizard, *Tropidurus delanonis*, from the Galápagos Archipelago. *Journal of Parasitology*, *76*, 313–318.

Asmundsson, I. M., Duszynski, D. W. & Campbell, J. A. (2006). Seven new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from colubrid snakes of Guatemala and a discussion of what to call ellipsoid tetrasporocystic, dizoic coccidia of reptiles. *Systematic Parasitology*, *64*, 91–103.

Ball, S. J. & Daszak, P. (1995). Description of the oocysts of three new species of *Eimeria* (Apicomplexa: Eimeriidae) from geckoes (Sauria: Gekkonidae). *Systematic Parasitology*, *32*, 101–106.

Berto, B. P., McIntosh, D. & Gomes Lopes, C. W. (2014). Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Brazilian Journal of Veterinary Parasitology*, *23*, 115.

Biedrzycka, A., Kloch, A., Migalska, M. & Bielański, W. (2013). Molecular characterization of putative *Hepatozoon* sp. from the sedge warbler (*Acrocephalus schoenobaenus*). *Parasitology*, *140*, 695–698.

Bovee, E. C. & Telford, S. R., Jr (1965a). *Eimeria noctisauris* sp. n. a coccidian from the lizard, *Klauberina riversiana*. *Journal of Parasitology*, *51*, 325–330.

Bovee, E. C. & Telford, S. R., Jr (1965b). *Eimeria sceloporis* and *Eimeria molochis* spp. n. from lizards. *Journal of Parasitology*, *51*, 85–94.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*, 772.

Daszak, P. & Ball, S. J. (1991). Five new species of *Eimeria* (Apicomplexa: Eimeriidae) from lizards. *Systematic Parasitology*, *20*, 141–147.

Daszak, P., Ball, S. J., Jones, C. G., Streicker, D. G. & Snow, K. R. (2009). Six new species of coccidia (Apicomplexa: Eimeriidae) from endangered *Phelsuma* spp. geckoes (Sauria: Gekkonidae) of the Black River Gorges National Park, Mauritius. *Folia Parasitologica*, *56*, 233–241.

Duméril, A. M. C. & Bibron, G. (1836). *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles. Tome troisième*. Paris: Librairie Encyclopédique de Roret, iv + 518 pp.

Duszynski, D. W. & Wilber, P. G. (1997). A guideline for the preparation of species descriptions in the Eimeriidae. *Journal of Parasitology*, *83*, 333–336.

Ghimire, T. R. (2010). Redescription of Genera of Family Eimeriidae Minchin, 1903. *International Journal of Life Sciences*, *4*, 26–47.

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

Honma, H., Yokoyama, T., Inoue, M., Uebayashi, A., Matsumoto, F., Watanabe, Y. & Nakai, Y. (2007). Genetical identification of coccidian in red-crowned crane, *Grus japonensis*. *Parasitology Research*, *100*, 637–640.

Jirků, M., Modrý, D., Slapeta, J. R., Koudela, B. & Lukeš, J. (2002). The phylogeny of *Goussia* and *Choleoeimeria* (Apicomplexa: Eimeriorina) and the evolution of excystation structures in coccidia. *Protist*, *153*, 379–390.

Jirků, M., Jirků, M., Oborník, M., Lukeš, J. & Modrý, D. (2009a). A model for taxonomic work on homoxenous coccidia: redescription, host specificity, and molecular phylogeny of *Eimeria ranae* Dobell, 1909, with a review of anuran-host *Eimeria* (Apicomplexa: Eimeriorina). *Journal of Eukaryotic Microbiology*, *56*, 39–51.

Jirků, M., Jirků, M., Oborník, M., Lukeš, J. & Modrý, D. (2009b). Goussia Labbé, 1896 (Apicomplexa, Eimeriorina) in Amphibia: diversity, biology, molecular phylogeny and comments on the status of the genus. *Protist*, *160*, 123–136.

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

Labbe, A. (1896). Recherches zoologiques, cytologiques et biologiques sur les coccidies. *Archives de Zoologie Expérimentale et Générale*, *4*, 517–654.

Labbé, A. (1899). Microsporidiida. In O. Bütschli (Ed.) *Das Tierreich Vol. 5. Sporozoa* (pp. 104–112). Berlin, Germany: Friedländer und Sohn.

Lainson, R. & Paperna, I. (1999). Some coccidia from the gallbladder and intestine of the Teiid lizard *Ameiva ameiva ameiva* and the gecko *Hemidactylus mabouia* in North Brazil. *Parasite*, *6*, 151–162.

Léger, L. (1904). Protozoaires parasites des vipères. *B Mens Ass France Avang*, *9*, 208.

Levine, N. D. (1973). *Protozoan Parasites of Domestic Animals and of Man*. Minneapolis, Minnesota: Burgess Publishing Company, 406 pp.

Levine, N. D. (1983). The genera *Barrouxia*, *Defretinella*, *and Goussia* of the coccidian family Barrouxiidae (Protozoa, Apicomplexa). *Journal of Protozoology*, *30*, 542–547.

Matuschka, F. R. & Bannert, B. (1986). New eimeriid coccidia from the gecko *Tarentola delalandii* Dumeril and Bibron, 1836. *Protistologica*, *22*, 399–403.

Matuschka, F. R. & Bannert, B. (1987). New eimeriid coccidia from the Canarian Lizard, *Gallotia galloti* Oudart, 1839. *Journal of Protozoology*, *34*, 231–235.

McAllister, C. T., Upton, S. J., Jacobson, E. R. & Kopit, W. (1995). A description of *Isospora amphiboluri* (Apicomplexa: Eimeriidae) from the inland bearded dragon, *Pogona vitticeps* (Sauria: Agamidae). *Journal of Parasitology*, *81*, 281–284.

McAllister, C. T., Seville, R. S. & Connior, M. B. (2014). A new caryosporan and eimerian (Apicomplexa: Eimeriidae) from green anoles, *Anolis carolinensis* (Sauria: Dactyloidae), from Arkansas and Louisiana, with a summary of the coccidia of Dactyloidae. *Journal of Parasitology*, *100*, 480–484.

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

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

Merino, S., Martínez, J., Martínez-de la Puente, J., Criado-Fornelio, A., Tomás, G., Morales, J., Lobato, E. & García-Fraile, S. (2006). Molecular characterization of the *18*S rRNA gene of an avian *Hepatozoon* reveals that it is closely related to *Lankesterella*. *Journal of Parasitology*, *92*, 1330–1335.

Modrý, D. & Jirků, M. (2006). Three new species of coccidia (Apicomplexa: Eimeriorina) from the marble-throated skink, *Marmorosphax tricolor* Bavay, 1869 (Reptilia: Scincidae), endemic to New Caledonia with a taxonomic revision of *Eimeria* spp. from scincid hosts. *Parasitology Research*, *99*, 419–428.

Modrý, D., Slapeta, J. R. & Koudela, B. (2000). Six new species of coccidia (Apicomplexa: Eimeriidae) from east African chameleons (Sauria: Chamaeleonidae). *Journal of Parasitology*, *86*, 373–379.

Morrison, D. A. (2009). The evolution of the Apicomplexa: where are we now? *Trends in Parasitology*, *25*, 375–382.

Paperna, I. (1994). *Eimeria gastrosauris* n. sp. (Apicomplexa: Eimeriidae) from the stomach epithelium of Australian geckoes. *Systematic Parasitology*, *28*, 15–21.

Paperna, I. (2003). The endogenous development, described by light and electron microscopy, of *Eimeria jamescooki* sp. n. (Apicomplexa: Eimeriidae) from the skink *Cryptoblepharus virgatus*. *Folia Parasitologica*, *50*, 89–96.

Paperna, I. (2007). Ultrastructural review of *Choleoeimeria* spp., a coccidium infecting the gall-bladder epithelium of reptiles. *Parassitologia*, *49*, 247–256.

Paperna, I. & Lainson, R. (1999a). Fine structure of the epicytoplasmic eimerid coccidium Acroeimeria pintoi Lainson & Paperna, 1999, a gut parasite of the lizard *Ameiva ameiva* in north Brazil. *Parasite*, *6*, 359–364.

Paperna, I. & Lainson, R. (1999b). The ultrastructure of some endogenous stages of the coccidian *Eimeria boveroi* Carini & Pinto, 1926 in the gut epithelial cells of the gecko *Hemidactylus mabouia* from Brazil. *Parasite*, *6*, 237–242.

Paperna, I. & Lainson, R. (2000). Ultrastructural study of meronts and gamonts of *Choleoeimeria rochalimai* (Apicomplexa: Eimeriidae) developing in the gall bladder of the gecko *Hemidactylus mabouia* from Brazil. *Folia Parasitologica*, *47*, 91–96.

Paperna, I. & Landsberg, J. H. (1989). Description and taxonomic discussion of eimerian coccidia from African and Levantine geckoes. *South African Journal of Zoology*, *24*, 345–355.

Power, M. L., Richter, C., Emery, S., Hufschmid, J. & Gillings, M. R. (2009). *Eimeria trichosuri*: phylogenetic position of a marsupial coccidium, based on *18*s rDNA sequences. *Experimental Parasitology*, *122*, 165–168.

Rambaut, A. & Drummond, A. J. (2007). Tracer: MCMC trace analysis tool. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Reichenow, E. (1920). Der Entwicklungsgang der Hämococcidien Karyolysus und Schellackia nov. gen. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin*, *10*, 440–447.

Schneider, A. (1881). Sur les psorospermies oviformes ou coccidies. Espèces nouvelles ou peu connues. *Archives de Zoologie Expérimentale et Générale*, *9*, 387–404.

Talavera, G. & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*, 564–577.

Upton, S. J. (2000). Suborder Eimeriorina Léger, 1911. In J. J. Lee, G. F. Leedale & P. Bradbury (Eds) *The Illustrated Guide to the Protozoa*, 2nd edn, *Vol. 1* (pp. 318–369). Lawrence, Kansas: Allen Press, Inc.

Upton, S. J. & Oppert, C. J. (1991). Description of the oöcysts of *Eimeria arnyi* n. sp. (Apicomplexa: Eimeriidae) from the eastern ringneck snake Diadophis punctatus arnyi (Serpentes: Colubridae). *Systematic Parasitology*, *20*, 195–197.

Zhao, X. & Duszynski, D. W. (2001a). Molecular phylogenies suggest the oocyst residuum can be used to distinguish two independent lineages of *Eimeria* spp. in rodents. *Parasitology Research*, *87*, 638–643.

Zhao, X. & Duszynski, D. W. (2001b). Phylogenetic relationships among rodent *Eimeria* species determined by plastid ORF470 and nuclear *18*S rDNA sequences. *International Journal for Parasitology*, *31*, 715–719.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Line drawings of the newly described species of *Choleoeimeria* and *Eimeria* (i. s.) in this study.

Table S1. Species of tetrasporozoic, dizoic coccidia described in amphisbaenian lizards

Table S2. Species of tetrasporocystic, dizoic coccidia of African and Asian Scincidae with available morphological information

Table S3. Species of tetrasporozoic, dizoic coccidia described in European and Asiatic caudata of the family Salamandridae

Table S4. Species of tetrasporozoic, dizoic coccidia described in lizards of the family Lacertidae

Table S5. Species of tetrasporozoic, dizoic coccidia described in African geckoes

Appendix S1. Taxonomic section