Research Article

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Eimeria tamimi sp. n. (Apicomplexa: Eimeriidae) from the rock hyrax (*Procavia capensis jayakari*) in central Saudi Arabia

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Abstract: Faecal samples from the rock hyrax (*Procavia capensis jayakari* Thomas) were collected from the Ibex Reserve in central Saudi Arabia. Eimerian oocysts, which are believed to represent a new species described here as *Eimeria tamimi* sp. n., were detected in 40 out of 93 samples. Oocysts were fully sporulated in 24–48 hours at 25 ± 2 °C. Sporulated oocysts of *E. tamimi* sp. n. were ovoid, measuring 35–42 × 19–25 µm (39 × 23 µm), a length/width ratio 1.5–2 (1.7). Oocyst wall was bilayered and measured 1.5 µm in thickness. Micropyle, oocyst residuum and polar granules were not present. Sporocysts are elongate, measuring 12–18 × 9–12 µm (15 × 10 µm), with a length/width ratio 1.1–1.8 (1.5) prominent Stieda bodies and sporocyst residuum. Experimental infection of two clinically healthy rock hyraxes with sporulated oocysts of *E. tamimi* sp. n. resulted in shedding unsporulated oocysts 5–10 days post infection. Partial sequences of 18S ribosomal RNA (18S rDNA) and cytochrome C oxidase subunit 1 (COI) regions were amplified using the polymerase chain reaction (PCR) and sequenced. Phylogenetic analysis based on 18S rDNA using maximum likelihood (ML) and Bayesian inference (BI) methods revealed that *E. tamimi* sp. n. grouped with *Eimeria quokka* Barker, O'Callaghan et Beveridge, 1988, *E. mundayi* Barker, O'Callaghan et Beveridge, 1988, *E. potoroi* Barker, O'Callaghan et Beveridge, 1988 and *E. gaimardi* Barker, O'Callaghan et Beveridge, 1988 marsupials. Eimerian species have been regarded as a paraphyletic group and the present investigation confirmed the conflict between phenotypic traits, used widely in the classification of this group of parasites.

Keywords: coccidia, Howtat Bani Tamim, Hyracoidea, morphology, molecular characterisation.

The rock hyrax *Procavia capensis* (Pallas) (Mammalia: Hyracoidea), also known as dassie or rock rabbit, is a small-hoofed ungulate. It is neither lagomorph nor entirely rock dweller. Hyraxes are rodent-like in appearance, with a short neck, ears, and tail. Three genera of hyrax are known, the bush hyrax (*Heterohyrax* Gray), the rock hyrax (*Procavia* Storr), and the tree hyrax (*Dendrohyrax* Gray). The first two genera are terrestrial animals that live in groups among rocks and are active by day, whereas the third one is arboreal, solitary, and nocturnal (Shoshani 2005). All hyraxes feed on grasses and leaves; however, they may also feed on insects and lizards (Jones 1984, Feldhamer et al. 1999).

The phylogenetic relationships of the order Hyracoidea with other mammalian groups are not fully understood. Morphologically they are related to ungulates, but fossils of 30 million years ago indicated most of these hyraxes were large in size. The closest extant relatives of the Hyracoidea are Proboscidae (elephants) and Sirenia (manatees and dugongs) (Rohland et al. 2007, Kuntner et al. 2011, Meredith

et al. 2011). The rock hyrax is distributed in the sub-Saharan and northern Africa as well as in the Middle East including Saudi Arabia (Skinner and Chimimba 2005, Butynski et al. 2015). Only two subspecies of *P. capensis* occur outside Africa, *Procavia capensis jayakari* Thomas, which is believed to occur in Saudi Arabia, and *Procavia capensis syriaca* Schreber, which occurs in the Levant including Syria, Jordan, and Palestine (Kingdon 1971, Harrison and Bates 1991, Kamal 1996). In Africa, the common subspecies *Procavia capensis capensis* (Pallas) is distributed throughout its range although other subspecies are recognised based on morphological characteristics and pelage colour differences (Springer 2003, Seiffert 2007).

Several helminths and protozoan parasites have been reported from the rock hyrax. Hall (1916) listed the following nematodes as parasitising *P. capensis*, *Crossophorus eouaris* Hemprich et Ehrenberg, 1828 (Ascaridida), *Crossophorus tentaculatus* Hemprich et Ehrenberg, 1828, *Crossophorus collaris* Hemprich et Ehrenberg, 1828, *Oxy-*

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uris flagellum Ehrengerg, 1828 (Oxyurida), *Oxyuris pugio* Ehrengerg, 1828 and *Physaloptera spirula* Hemprich et Ehrengerg, 1828 (Spirurida).

The tapeworms *Inermicapsifer beveridgei* Schmidt et Wertheim, 1988 and *Inermicapsifer hyracis* (Rudolphi, 1810) (Cestoidea: Anoplocephalidae) were detected from *P. capensis* (Hyracoidea) in Japan and Middle East (Schmidt and Wertheim 1988, Saitoh et al. 2004). The nematode, *Grassenema procaviae* Petter, 1959, has also been described from the rock hyrax in Japan (Saitoh et al. 2004).

Few protozoan parasites have been reported from Hyracoidea: only two species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriorina: Eimeriidae) have been reported from Procaviidae: *Eimeria dendrohyracis* van de Berghe et Chardome, 1953 and *Eimeria dorsalisi* van de Berghe et Chardome, 1962. The first species was described from the Eastern tree hyrax (*Dendrohyrax arboreus* [Smith]) whereas the latter was described from the Western tree hyrax (*Dendrohyrax dorsalis* [Fraser]). Apart from those reports, Saitoh et al. (2004) reported *E. dendrohyracis* from a captive rock hyrax (*P. capensis*) in Guma Safari Park, Japan. *Toxoplasma gondii* (Nicolle et Manceaux, 1908) was reported from *P. capensis* in Japan (El-Dakhly et al. 2013). *Cryptosporidium muris* Tyzzer, 1907 has been reported from experimentally infected *P. capensis* (Xiao et al. 1999).

From the order Hyracoidea, only two species of *Eimeria* have been reported and none has been described from the rock hyrax (*P. capensis*) apart from single report of *E. dendrohyracis* from Japan based on morphological appearance (Saitoh et al. 2004). Here a new species of *Eimeria* is described from *P. capensis jayakari* based on morphological as well as molecular characteristics using both partial 18S rDNA and COI genes.

MATERIALS AND METHODS

Faecal samples from 93 rock hyrax (*Procavia capensis jayakari*) were collected from the Ibex Reserve in Howtat Bani Tamim, south of Riyadh, central Saudi Arabia. The samples were moist when they were collected indicating that they were fresh. Oocysts were left in shallow layer of 2.5% $K_2Cr_2O_7$ to allow to sporulate and examined daily to check the progress of sporulation. In the laboratory, a small amount of the faecal sample was ground and strained through a sieve and underwent flotation technique using saturated sugar solution (Anonymous 1986). Oocysts detected were examined microscopically and measured using eyepiece fitted calibrated micrometer. Measurements of the sporulated oocysts, the sporocysts and the wall thickness are given in micrometres (μm). Oocysts were left in a shallow layer of 2.5% $K_2Cr_2O_7$ allowing sporulation to take place and the suspension was examined on daily basis to check the progress of the sporulation process (Mohammed and Hussein 1992). Digital images of unsporulated and sporulated oocysts were taken using a digital camera attached to the microscope (Nikon, DP 72, Tokyo, Japan).

Experimental infection

Three two-month-old rock hyraxes were obtained from nearby the Ibex Reserve. The animals were brought to the Department of Zoology animal facility. Animals were checked for the presence

of gastrointestinal parasites, particularly for eimerian oocysts for a period of 15 days prior to the experiment and proved to be negative. Clean food and water were provided *ad libitum*. Thoroughly washed sporulated oocysts of the new species of *Eimeria* were administered using a stomach tube to two animals and the third one was left as an uninfected control. The inoculum contained 5,000 sporulated oocysts and each experimental animal received this amount. Daily faecal samples were collected from the animals fed sporulated oocysts and examined microscopically. When oocysts started to show in faeces a modified McMaster technique was used to quantify the number of oocysts per gram (opg) of faeces (Anonymous 1986).

DNA extraction and Polymerase Chain Reaction

Sporulated oocysts were purified by washing in distilled water for 3–5 times till the supernatant was clear and free of potassium dichromate. Purified oocysts were subjected to DNA extraction using the technique outlined in Zhao et al. (2001) using the method involving CTAB buffer treatment with slight modification. Purified oocysts were treated with sodium hypochlorite and Cetyl-Trimethyl Ammonium Bromide (CTAB) buffer (2% w/v CTAB, 1.4 M NaCl, 0.2% b-mercaptoethanol, 20 mM EDTA, 100 mM TRIS) was added. DNA extraction from the lysate was completed using QiaAmp DNA Micro extraction kit (Qiagen, Hilden, Germany).

Extracted genomic DNA was subjected to PCR amplification using primers which amplify part of the 18S rDNA gene (636 bp) with the forward primer F1E and reverse primer R2B (Orlandi et al. 2003). Partial COI gene (650 bp) was also amplified using the primers cocci-COI1F and cocci-COI1R as described by Ogedengbe et al. (2011).

The Polymerase Chain Reaction (PCR) was set up in 25 μl using Bioline Buffer (Bioline, London, UK) containing 200 μM concentrations each of dNTPs. The reaction mixture was adjusted to a final concentration of 2 mM $MgCl_2$ and 0.2 μM concentrations of each primer. DNA Taq polymerase (Bioline) was added to the PCR mixture. The PCR amplifications were performed in a Multigene™ thermocycler (Labnet International, Inc., Edison, NJ, USA). The amplification program began with one cycle of 2 minutes at 94°C as an initial denaturation step. The cycling conditions consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at (50°C for 18S rDNA; 52°C for COI) for 30 s, and primer extension at 72°C for 30 s. A final extension at 72°C for 7 min was allowed, and followed by keeping at 4°C.

PCR products were visualised using a gel documentation system and digital images of the PCR product were obtained. PCR products were cut from the gel and purified using Isolate II PCR and Gel purification kit (Bioline) and sequenced using Macrogen sequencing facility (Macrogen Inc, Seoul, South Korea).

Data analysis

Phylogenetic relationships between the new species and related *Eimeria* spp. were inferred using 18S rDNA, and COI loci available in GenBank database. Seaview 3.2 (Galtier et al. 1996) was used to perform multiple alignments for each genetic marker. COI sequences were translated into amino acids to check for possible amplification of pseudogenes. Representative sequences of the Eimeriidae available in GenBank were included in both datasets (18S rDNA and COI) (Table 1).

Fig. 1. Sporulated oocysts of *Eimeria tamimi* sp. n. with double layered wall and no micropyle. No sporocyst residuum and no polar body are present. **A** – sporocyst residuum in the form scattered fine granules covering the sporozoites; **B** – a composite line drawing. *Abbreviations:* DSR – diffuse sporocyst residuum; IL – oocysts inner layer; N – sporozoite nucleus; OL – oocysts outer layer; RB – retractile body of the sporozoite; SB – stieda body; SZ – sporozoite.

The best-fitting models of molecular evolution applied to the datasets in the phylogenetic reconstructions were inferred with jModeltest 2.1.8 (Posada 2008), based on the Akaike information criterion (Akaike 1974).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out to infer the relationships among 18S rDNA and COI datasets using Phyml v. 2.4 (Guindon and Gascuel 2003) and MrBayes (Posada 2009), respectively. *Toxoplasma gondii* (M97703) was included as an outgroup. In ML tree constructions, bootstrap support was evaluated by 1,000 replicates. A consensus topology was generated using 50% of the resulting trees. For BI analysis, simulations were run for 5 million generations with trees sampled every 1,000 generations. The first 25% was discarded as burnin. Stationarity was considered to be reached when the average standard deviation of split frequencies was below 0.01. The trees were visualised using TreeGraph 2.14.0-771 (Stöver and Müller 2010).

RESULTS

Eimeria tamimi sp. n.

Fig. 1

Eimerian oocysts were detected in 40 (43%) faecal samples out of 93 rock hyrax. Oocysts detected were tetrasporocystic disporozoic with a bilayered wall. Oocysts were ovoid, oocyst wall composed of a thin light green outer layer (~0.5) and a thicker light brown inner layer (~1.0), with average size of $35.0\text{--}41.5 \times 18.5\text{--}24.5$ (38.7×23.0), a length/width ratio 1.7 (1.5–2.0), micropyle, oocyst residuum, and polar granules were all absent. Sporocyst are elongate, $12.0\text{--}18.0 \times 8.5\text{--}11.5$ (14.9×10.1), with a length/width ratio

Fig. 2. A consensus phylogenetic tree constructed with maximum-likelihood (ML) and Bayesian inference (BI) methods, showing phylogenetic relationships among *Eimeria* spp. and other eimerid coccidia, with *Toxoplasma gondii* (Nicolle et Manceaux, 1908) as an outgroup inferred from 18S rDNA gene sequence data generated from *Eimeria tamimi* sp. n. and other taxa from GenBank. Numbers indicated at branch nodes are bootstrap values and the posterior probability (ML /BI). Only bootstraps > 50% are shown. The new species is printed in bold.

1.1–1.8 (1.5), a Stieda body was present, a prominent sporocyst residuum and retractile granules (Fig. 1). Sporocyst residuum is composed of small granules dispersed between sporozoites. Sporozoites are sausage-shaped with larger and smaller refractile bodies. Species description was performed according to Duszynski and Wilber (1997).

Experimental animals started shedding unsporulated oocysts of *Eimeria tamimi* sp. n. five days post infection. Shedding of the oocysts continued for a period of another five days till they ceased shedding oocysts. Oocyst output was 3,950 opg on the first day and continued to rise till it peaked on the third day and showed 9,250 opg then declined to 3,050 opg on the fifth day. No oocysts were recovered from faeces after the fifth day of infection. The animals did not show any signs of diarrhoea or soft droppings which indicated that the parasite is of no remarkable pathogenicity.

Taxonomic summary

Type host: Rock hyrax (*Procapra capensis jayakari*).

Other hosts: Unknown.

Type locality: Howtat Bani Tamim (23°46'48"N; 46°59'55"E) Ibex Reserve, Saudi Arabia.

Other localities: Unknown.

The site of infection: Unknown, oocysts detected in faeces. Prevalence: Oocysts detected in 40 out of 93 rock hyraxes (43%).

Prepatent period: Five days.

Patent period: Five days.

Sporulation time: 24 to 48 hours.

Material deposited: Photoneosyntype (see Duszynski 1999 for terminology) deposited at the Museum of the Department of Zoology, College of Science, King Saud University (Acc. No. 931). Sporulated and unsporulated oocysts in 10% formalin were kept at the same collection together with the photoneosyntype. DNA sequences have been deposited in GenBank under accession numbers MK075988 and MK075989 for the 18S rDNA and COI loci, respectively.

Etymology: The specific name of *Eimeria tamimi* sp. n. was derived from the name of the tribe which inhabits Howtat Bani Tamim the area where the host occurs.

Remarks

The oocysts described from the rock hyrax (*P. capensis jayakari*) in the present study are different from those described from the eastern (*Dendrohyrax arboreus*) and western tree (*Dendrohyrax dorsalis*) hyrax. Oocysts of *Eimeria dendrohyracis* from *D. arboreus* are elliptical and measure 23.8–26.8 × 16.8–18.2 µm and the sporocyst measures 7.0–8.4 µm. Oocysts of *Eimeria dorsalis* from the western tree hyrax (*D. dorsalis*) are spherical and measure 19

Fig. 3. A consensus phylogenetic tree constructed with maximum-likelihood (ML) and Bayesian inference (BI) methods, showing phylogenetic relationships among *Eimeria* spp. and other eimerid coccidia, with *Toxoplasma gondii* (Nicolle et Manceaux, 1908) (outgroup) inferred from the COI sequences data generated from *Eimeria tamimi* sp. n. and other taxa from GenBank. Numbers indicated at branch nodes are bootstrap values and the posterior probability (ML/BI). Only bootstraps > 50% are shown. The new species is printed in bold.

in diameter (Table 2). The prepatent period, as well as the patent period of the parasite, were found to be 5 days. The parasite did not appear to be associated with pathology since the faecal material voided by infected animals did not show any sign of diarrhoea. The control animal remained negative for oocysts till the end of the experiment.

Molecular data analyses

A PCR product of 636 bp was successfully amplified from the 18S rDNA region of *E. tamimi* sp. n. and sequenced. A fragment of a length of 650 bp was also successfully amplified and sequenced from the COI of the mitochondrial genome. PCR products from two isolates produced identical sequences for the 18S rDNA and COI. Final alignment of 598 bp (18S rDNA) and 583 bp (COI) were used for the phylogenetic analyses. 18S rDNA and COI datasets included 92 and 79 sequences, respectively. Edited sequences input files were concatenated resulting in the inclusion of 67 sequences and 1,184 characters.

The optimum evolutionary models selected by jModel-Test were GTR + I, HKY + I and HKY for 18S rDNA, COI and concatenated datasets, respectively. Both phylogenetic analyses showed similar overall topologies; however, the position of several taxa varies when comparing the different trees. Node support values within the text consist of ML bootstrap values and Bayesian posterior probabilities.

In all trees, species of *Eimeria* appeared as paraphyletic with several clades characterised by well-defined spectrum of host taxa (rodents, rabbits, livestock and poultry). Other genera included in the analysis, such as *Isospora* Schneider, 1881, appeared polyphyletic. For all observed clades, the range of node support was higher in the Bayesian analysis.

Eimeria tamimi did not fall within one of these monophyletic clades. The 18S rDNA tree (Fig. 2) indicates that *E. tamimi* forms a clade with *E. quokka*, *E. mudayi*, *E. potoroi* and *E. gaimardi* of the marsupials. However, in the COI tree (Fig. 3), *E. tamimi* appeared to belong to a different clade including one of the paraphyletic *Isospora* (*Isospora* sp. ex *Talpa europaea* Linnaeus). The concatenated tree showed a clade including *E. tamimi*, *E. quokka*, *Isospora* sp. ex *T. europaea*, *Cyclospora cayetanensis* Ortega, Gilman et Sterling, 1994 and *Caryospora bigenetica* Wacha et Christiansen, 1982, with a high bootstrap separating *E. tamimi* from the marsupial eimerian parasites (*E. quokka*, *E. mudayi*, *E. potoroi* and *E. gaimardi*) (Fig. 4).

This analysis showed that several taxa require revision regarding their phylogenetic position. Although the major clades within *Eimeria* were well supported, especially in

Fig. 4. A consensus phylogenetic tree constructed with maximum-likelihood (ML) and Bayesian inference (BI) methods, showing phylogenetic relationships among *Eimeria* spp. and other eimerid coccidia, with *Toxoplasma gondii* (Nicolle et Manceaux, 1908) (outgroup) inferred from concatenated sequences from both 18S rDNA and COI genes generated from *Eimeria tamimi* sp. n. and other taxa from the GenBank. Numbers indicated at branch nodes are bootstrap values and the posterior probability (ML/BI). Only bootstraps > 50% are shown. *E. tamimi* sp. n. is printed in bold.

Table 1. Taxa and their GenBank accession numbers used in the present analyses.

Species	18S rRNA GenBank Accession number	COX1 GenBank Accession number			
<i>E. tamimi</i> sp. n.	MK075988	MK075989	<i>Eimeria meleagridis</i> 1	HG793040	HG793047
<i>Atoxoplasma</i> sp.	AY331569	-	<i>Eimeria meleagrimitis</i>	KC305191	-
<i>Atoxoplasma</i> sp. 1	AY331571	-	<i>Eimeria meleagrimitis</i> 1	KC305197	-
<i>Atoxoplasma</i> sp.2	AY331566	-	<i>Eimeria mitis</i>	FR775303	FR796699
<i>Caryospora</i>	KJ651445	-	<i>Eimeria mundayi</i>	MK182526	MK202808
<i>Caryospora bigenetica</i>	KT184331	KP658102	<i>Eimeria myoxi</i>	JF304148	JQ993696
<i>Cyclospora cayetanensis</i>	KX618190	MG831586	<i>Eimeria nafuko</i>	JQ993665	JQ993708
<i>Eimeria acervulina</i>	DQ538351	-	<i>Eimeria necatrix</i>	KT184349	KT184349
<i>Eimeria acervulina</i> 1	U67115	-	<i>Eimeria necatrix</i> 1	EU025108	JN022588
<i>Eimeria adenoeides</i>	FR745914	FR84620	<i>Eimeria nkaka</i>	JQ993651	JQ993697
<i>Eimeria adenoeides</i> 1	KC305172	FR846202	<i>Eimeria papillata</i>	KT184350	KT184377
<i>Eimeria alabamensis</i>	AF291427	KU351690	<i>Eimeria piriformis</i>	HQ173836	JQ993698
<i>Eimeria alabamensis</i> 1	AB769554	KT184335	<i>Eimeria potoroi</i>	MK182527	MK202807
<i>Eimeria alorani</i>	KU192970	JQ993701	<i>Eimeria praecox</i>	KT184352	KT184378
<i>Eimeria apionodes</i>	KU192973	KU255441	<i>Eimeria praecox</i> 1	-	JQ659301
<i>Eimeria arloingi</i>	KX845686	KX857470	<i>Eimeria praecox</i> 2	-	KX094943
<i>Eimeria boholensis</i>	-	MH350860	<i>Eimeria purpureicephali</i>	KU140597	KU140598
<i>Eimeria bovis</i>	KT184336	KT184372	<i>Eimeria quokka</i>	KF225636	KF225637
<i>Eimeria bovis</i> 1	KU641156	KU351696	<i>Eimeria rioarribaensis</i>	LC371915	-
<i>Eimeria bukidnonensis</i>	AB769600	KU351700	<i>Eimeria scabra</i>	AF279668	-
<i>Eimeria bukidnonensis</i> 1	AB769596	-	<i>Eimeria sciurorum</i>	KT360994	KT361027
<i>Eimeria bukidnonensis</i> 2	AB769595	-	<i>Eimeria sciurorum</i> 1	-	KT361025
<i>Eimeria cahiriniensis</i>	JQ993647	JQ993687	<i>Eimeria</i> sp. <i>Alectoris graeca</i>	HM070378	HM117020
<i>Eimeria callospermophili</i>	MG595728	JN021270	<i>Eimeria</i> sp. ex <i>Apodemus agrarius</i>	JQ993660	JQ993702
<i>Eimeria caviae</i>	JQ993649	JQ993689	<i>Eimeria</i> sp. ex <i>Apodemus agrarius</i> 1	KU192962	-
<i>Eimeria cf. ictidea</i>	MF860827	MF860823	<i>Eimeria</i> sp. ex <i>Apodemus flavicollis</i>	KU192939	-
<i>Eimeria cf. mivati</i>	FJ236378	-	<i>Eimeria</i> sp. ex <i>Apodemus sylvaticus</i>	KU192943	JQ993706
<i>Eimeria cf. mivati</i> 1	FJ236377	-	<i>Eimeria</i> sp. ex <i>Numida meleagris</i>	KJ547707	-
<i>Eimeria cf. mivati</i> 2	FJ236376	-	<i>Eimeria</i> sp. ex <i>Perdix perdix</i>	-	KJ547709
<i>Eimeria cf. vison</i>	MG011726	-	<i>Eimeria</i> sp. ex <i>Phasianus colchicus</i>	-	KJ547708
<i>Eimeria christenseni</i>	KX857468	KX845684	<i>Eimeria</i> sp. ex <i>Sorex araneus</i>	-	JQ993710
<i>Eimeria cylindrica</i>	KU641163	KU35170	<i>Eimeria</i> sp. <i>Meleagris gallopavo</i>	HM117014	-
<i>Eimeria dispersa</i>	KT184338	KT184338	<i>Eimeria</i> sp. strain Lezajsk	-	MG595960
<i>Eimeria dispersa</i> 1	HG793048	HG793028	<i>Eimeria subspherica</i>	LC271171	KU351704
<i>Eimeria exigua</i>	KT360996	JQ993691	<i>Eimeria tamiasciuri</i>	-	KT184375
<i>Eimeria ferrisi</i>	KT360995	KT361028	<i>Eimeria tenella</i>	EU025113	EU025109
<i>Eimeria flavescens</i>	JX406873	JQ993692	<i>Eimeria tenella</i> 1	DQ136180	FJ236396
<i>Eimeria flavescens</i> 1	HQ173830	KP025693	<i>Eimeria tenella</i> 2	DQ136184	FJ236385
<i>Eimeria furonis</i>	AB329724	MF795598	<i>Eimeria uptoni</i>	KU192953	KU216026
<i>Eimeria gaimardi</i>	MK182525	MK202809	<i>Eimeria vejdoskyi</i>	HQ173838	JQ993699
<i>Eimeria gallopavonis</i>	KT184343	KT184343	<i>Eimeria vermiformis</i>	KT184355	KT184355
<i>Eimeria hirci</i>	KX845685	KX857469	<i>Eimeria zuernii</i>	KT184356	KT184356
<i>Eimeria innocua</i>	HG793045	HG793049	<i>Isoospora butcheriae</i>	KY801685	KY801687
<i>Eimeria jerfinica</i>	KU192975	KU216033	<i>Isoospora greineri</i>	KR108298	KR108298
<i>Eimeria lancasterensis</i>	KT368144	KT361043	<i>Isoospora gryphoni</i>	KF854254	-
<i>Eimeria macyi</i>	MK284237	MK284238	<i>Isoospora</i> sp. ex <i>Microtus arvalis</i>	MH698573	-
<i>Eimeria magna</i>	JQ071391	JQ993695	<i>Isoospora</i> sp. ex <i>Plectrophenax nivalis</i>	-	MH698551
<i>Eimeria media</i>	HQ173834	HQ173884	<i>Isoospora</i> sp. MAH 2013	KF648870	-
<i>Eimeria meleagridis</i>	HG793039	HG793046	<i>Isoospora</i> sp. ex <i>Myodes glareolus</i>	MH698575	MH698560
			<i>Isoospora</i> sp. ex <i>Myodes glareolus</i> 1	MH698576	MH698561
			<i>Isoospora</i> sp. ex <i>Talpa europaea</i>	JQ993669	JQ993712
			<i>Isoospora streperae</i>	KJ634021	-
			<i>Toxoplasma gondii</i>	M97703	HM771690

the case of the Bayesian analysis, the relationships between different sister clades remain unresolved.

DISCUSSION

Rock hyrax is an omnivore and it primarily feeds on grass, insects, bird eggs and lizards (Jones 1984, Feldhamer et al. 1999). The distinctive morphological characters and the dimensions of the oocysts described from the rock hyrax (*Procavia capensis jayakari*) showed that this species is distinct and hitherto undescribed. Oocysts measured $35.0\text{--}41.5 \times 18.5\text{--}24.5 \mu\text{m}$ ($38.7 \times 23 \mu\text{m}$), with a length/width ratio of 1.5–2.0 (1.7). It lacks the micropyle and

the oocyst residual body. It was morphologically different from the *Eimeria dendrohyracis* described from the Eastern tree hyrax (*Dendrohyrax arboreus*) which measured $23.8\text{--}26.8 \times 16.8\text{--}18.2 \mu\text{m}$ in dimension. Furthermore, it was also different from the spherical oocysts of *Eimeria dorsalis* described from the Western tree hyrax (*Dendrohyrax dorsalis*) which measures 19.0 in diameter (van de Berghe and Chardome 1953, 1962). Sporocysts of *E. dorsalis* are spherical and measure 9.5. Hence based on these morphological characters and distinctiveness from *E. dorsalis* and *E. dendrohyracis* the species recovered from the rock hyrax was described as *Eimeria tamimi* sp. n.

Table 2. Morphological differences between *Eimeria tamimi* sp. n. and other eimerian species reported from the members of the family Procavidae.

Eimerian species	Type host	Oocysts shape, size features	Sporocyst size	Reference
<i>Eimeria tamimi</i> sp. n.	<i>Procavia capensis jayakari</i>	ovoid 38.7 × 23.0 (35.0–41.5 × 18.5–24.5) M, OR, PG: all – OW: 2 layers, 1.5 thick	14.9 × 10.1 (12.0–18.0 × 8.5–11.5) SB, +, SSB, PSB – SR: +, large granules	This study
<i>Eimeria dendrohyracis</i>	<i>Dendrohyrax arboreus</i>	ovoid 23.8–26.6 × 16.8–18.2 M, OR, PG: all –	11.2 × 7.0–8.4	van de Berghe and Chardome (1953)
<i>Eimeria dorsalis</i>	<i>Dendrohyrax dorsalis</i>	spherical 19.0 M, OR, PG: all –	Spherical 7.9	van de Berghe and Chardome (1962)

M – micropyle; OR – oocyst residuum; PG – polar granule; OW – oocyst wall; SB – stieda body; SSB – substieda body; PSB – parastieda body; SR – sporocyst residuum.

The prepatent and patent periods of *E. tamimi* was also determined for the first time in the type host of the parasite. The infected animals shed the oocysts for a short period of time which was 5 days. The prepatent period of 5 days was probably enough time for the parasite to produce unsporulated oocysts of *E. tamimi* (see Long 1990). This could possibly be the case in the wild unless animals concentrate in large densities in a small area as the case of *Eimeria impalae* Prasad et Narayan, 1963 in South Africa (Bigalke 1966).

As reported previously (Morrison et al 2004, Yabsley and Gibbs 2006, Kvičerová and Hypša 2013) *Eimeria* is clearly a paraphyletic group. In this study, *E. tamimi* falls in a clade including species of the Eimeriidae assigned to as different genera (e.g., *Caryospora* Leger, 1904, *Cyclospora* Schneider, 1881, *Isospora* Schneider, 1881). This result confirmed the conflict between phenotypic traits, used widely in the classification of eimerians, and phylogenetic relationships.

Although major clades within *Eimeria* support their host specificity clustering, it does not stem from a cophylogenetic process. Kvičerová and Hypša (2013) found that 11 eimerian species isolated from the genus *Apodemus* Kaup cluster in four different clades. They suggested an adaptation (ecology, physiology, etc.) as explanation to the observed patterns rather than host-parasite co-speciation. Comparison of the accuracy of ML and BI is problematic because these methods are implemented in different programs. However, several study estimated that the BI generally fares better than the ML approach in accuracy (Beerli 2006).

It is probably that eimerian parasites of the rock hyrax evolved with their host and therefore it is different to any related eimerian parasites and even eimerian parasites from related hosts. Sirenia and Proboscidea are phylogenetically related to the Hyracoidea (Rohland et al. 2007, Kuntner et al. 2011, Meredith et al. 2011). However, there

is no named eimerian parasite described from the Proboscidea and only three species have been described from the Sirenia, namely, *Eimeria manatus* Upton, Odell, Bossart et Walsh, 1989, *Eimeria nodulosa* Upton, Odell, Bossart et Walsh, 1989 (from the Florida manatee, *Trichechus manatus latirostris* Linnaeus) and *Eimeria trichechi* Lainson, Naiff, Best et Shaw, 1983 (from the Amazonian manatee, *Trichechus inunguis*) (Lainson et al. 1983, Upton et al. 1989). These coccidia are far much smaller in size compared to the eimerian species reported in the present study. *Eimeria manatus* measures 10.5–13.5 × 9.0–13.5 µm (11.8 × 10.5 µm), whereas *Eimeria nodulosa* measures 14.5–17.5 × 13.0–16.0 µm (15.6 × 14.7 µm), and *E. trichechi* measures 13.4 × 13.3 µm. All of them lack the micropyle, oocysts residuum, and polar body. All has sporocyst residuum and Stieda body with the exception of *E. trichechi*. It is likely that the lack of the Stieda body could be an artefact and it is a feature of mammalian eimerian species. *Eimeria dorcadis* Mantovani, 1966 was first described by Mantovani (1966) and the original description was devoid of Stieda body. Mohammed et al. (2012) redescribed the species with a visible Stieda body which goes well with the findings from all mammalian eimerian parasites which possess Stieda body. Therefore, it may be likely that reinvestigating of *E. trichechi* may result in demonstrating the presence of Stieda body and also investigating their phylogenetic relationship with relevant eimerian parasites.

Eimeria tamimi is the first coccidia reported from the rock hyrax (*P. capensis jayakari*). The prepatent and patent periods of this eimerian parasite have also been determined for the first time. Molecular data support the identity validity of *E. tamimi* as a new species and indicate its relationship with some other eimerian species.

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