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RESEARCH ARTICLE

CONFORMATIONAL STUDIES FOR THE PRESENCE OF COCCIDIAN PARASITES ON WHITE SPOTTED GECKO TARENTOLA ANNULARIS (CHORDATA: GEKKONIDAE)

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ABSTRACT

The white spotted gecko *Tarentola annularis* (Family Gekkonidae) is a reptilian species found in the Middle East, Africa, and some states in the United States mainly Florida. A total of 40 specimens of this species were collected during the period of February –November 2016 from Abu-Rawash, Giza Governorate, Egypt; they were dissected and examined for the presence of parasitic infection. Only 35 (87.50%) specimens were found to be naturally infected with coccidian parasites. Seasonally, the prevalence of infection was reached its maximum value of 80.0% during summer and minimum value of 50.0%, 40.0%, 20.0% during spring, autumn, and winter, respectively. Prevalence and mean intensity of parasitic infection were negatively correlated with the host size as smaller geckos (<5cm in length and <30gm in weight) are more infected than larger ones (5-10 cm in length and >60gm in weight). The numbers of parasites of male and female *T.annularis* were compared, and no significant differences were observed. Morphology of the recovered parasites was studied by using light microscopy and revealed the presence of two new coccidian species identified as *Eimeria tarentoli* and *Eimeria ghaffari* belonged to the family Eimeriidae. Oocysts of *Eimeria tarentoli* n. sp. were spherical to sub-spherical with single-layered, measured 21.6-31.4 x 15.3-25.2 (26.5 x 19.2) µm, with shape-index (length/width) was 2.01 (1.58-2.34). Both micropyle and oocyst residuum were absent, but a fragmented polar granules were present. Oocysts of *Eimeria ghaffari* n. sp. were elongated to cylindroids with single-layered wall, measured 29.6-31.3 x 14.7-23.2 (34.5 x 19.3) µm, shape-index (length/width) was 2.01 (1.98-2.65). Micropyle and oocyst residuums were absent, but single polar granule was present. Combining morphological characteristics, host specificity and geographical distribution, tissue tropism, in addition to molecular analysis of partial sequence of SSU ribosomal DNA gene, revealed that the recovered parasite species described herein were genetically distinct from other coccidian species, but had 95.7-95.1% sequence similarity to *E. collieie* and *E. arnyi*. Also, Phylogenetic analysis placed the present coccidian species in the gekkonid *Eimeria* clade, which is a sister group of bovids *Eimeria* species. In addition, the present study was considered as the first report for occurrence of eimerian species from the white spotted gecko in Egypt.

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INTRODUCTION

Phylum Apicomplexa Levine, 1970 is a large protist group composed by a diverse array of obligatory parasitic organisms (Upton and Oppert 1991; Gillis *et al*., 2003). However, regardless of its high medical and veterinary importance, it is estimated that only 0.1% for the diversity of this phylum has been described (Katayama *et al*., 2003; Morrison 2009). Reptiles are hosts to a wide variety of apicomplexan parasites, including families with human medical importance as Sarcocystidae (e.g., *Toxoplasma* spp.), Eimeriidae (e.g., *Cryptosporidium parvum*), and Haemosporidae (e.g., *Plasmodium* spp.) (El–Toukhy *et al*., 2013). Geckos are common reptiles of houses belonging to the family Gekkonidae (Miska *et al*., 2010). Five major genera of Eimeriidae Minchin, 1903 had been found to infect Squamata (Reptilia) (Sulieman *et al*., 2014). These genera are distinguished by the structure of their sporulated oocysts and their life cycles. Specifically, Squamata host eimeriids with dizoic, tetrasporocyst oocysts that develop on the epithelial surface of the gall bladder or in microvillous zone of the intestine (i.e. genera *Choeleoeimeria*, *Acroeimeria* and *Eimeria* (i.s.) sensu Paperna and Landsberg 1989); parasites with single, octozoicsporocyst oocysts with known extra-

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intestinal development, including the formation of fully sporulated oocysts (i.e. genus *Caryospora* Léger 1904); and parasites with tetrasporozoic, diplosporocystic oocysts (i.e. genus *Isospora* Schneider 1881). About 51 species of *Eimeria* causing coccidiosis have been described from lizards (Rusiev and Davronov 1984; Davronov 1985, MatuSchka and Bannert 1986, 1987; McAllister *et al*., 1988; Upton and Barnard, 1987; Upton *et al*., 1988; Barta *et al*., 1997; Beck *et al*., 2009). In the last two decades, several studies concerning intestinal coccidia infecting reptiles in Egypt have been carried out (El–Toukhy 1994; Sakran *et al*., 1994; Abdel–Gawad *et al*., 1995; El–Toukhy *et al*., 1997; Fayed 2003; Abou El–Nour 2005). However, the phylogenetic relationships among these groups of parasites remained unknown (Slapeta *et al*., 2001, 2003; Honma *et al*., 2007; Xiang *et al*., 2010). Although molecular techniques have now become established as standard tools for monitoring parasite populations (Beck *et al*., 2009), this is heavily biased to certain groups within Apicomplexa, such as *Plasmodium* spp., and is also directed primarily towards humans or commercially important animal groups (Dunn 2009; Kutkiene *et al*., 2011).

Therefore, the present study aimed to:(1) determine the prevalence and rate of natural infection of two Eimeriid species in relation to the host age and gender. (2) describe different stages of the recovered parasite species by using light microscopic studies. (3) assess the existence of *Eimeria tarentoli* sp. nov. and *Eimeria ghaffari* sp. nov. infecting the intestinal wall of the white spotted gecko *Tarentola annularis* by applying molecular tools and using 18S rDNA primers.

MATERIALS AND METHODS

Experimental animals: A total of 40 white spotted gecko *Tarentol annularis* (Family Gekkonidae) were collected during the period of February–November 2016, utilizing a hand net from Abu-Rawash, Giza Governorate, Egypt. Geckos were brought alive to Laboratory of Parasitology Research, Zoology Department, Faculty of Science, Cairo University, Egypt. The collected geckos were identified according to Marx (1968) and grouped into two age classes on the basis of their snout-vent length (SVL) (juveniles: <5cm, and adults: 5-10cm).

Parasitological examination: Geckos were kept overnight in separate containers, fecal samples being removed in the morning prior to release and screened for coccidian parasites by using flotation technique as following: 2 g of each sample was weighed, transferred into a plastic beaker and soaked in approximately 10 ml of distilled water overnight. The soaked samples were then homogenized thorough stirring using a glass rod and filtered through a metal sieve of small size. The filtrate from each sample was allowed to sediment for one hour on laboratory bench, after which the supernatant fluid was discarded into a clean beaker. The presence of oocysts was confirmed microscopically by transferring the equivalent of 3 ml of sediment into a centrifuge tube and testing for the presence of coccidial oocysts using saturated saline flotation technique. Oocysts were assigned putative species identity basedupon microscopic morphology (Haug *et al*., 2008). For each positive sample, oocysts were recovered from the remaining sediment using the centrifugal flotation technique (Soulsby 1986). The harvested oocysts were re-suspended in distilled water and washed by centrifugation three to four times to remove flotation solution. Sediment containing oocysts was transferred into petri-dishes, re-suspended in 2.5% (w/v) potassium dichromate solution and allowed to sporulate at room temperature for 6-10 hr with regular stirring. After sporulation, oocysts within each sample were cleaned from the residual fecal debris by treatment with sodium hypochlorite (4 % active chlorine) and three successive washes in distilled water as described by Eckert *et al*. (1995). After cleaning, sporulated oocysts and sporocysts were carefully examined and photographed by using a Zeiss photomicroscope equipped with a Canon digital camera and measured using an elaborated ocular micrometer and then photographed. Measurements were reported in micrometers (μm) with ranges followed by means±SD in parentheses. Prevalence of infections was calculated according to the age and gender of the host as number of the infected host/number of the examined host×100. Approximately $10x10^8$ oocysts were combined to form an oocyst pool from each positive sample. Isolated oocysts were suspended in 2% (w/v) potassium dichromate solution and stored at 4 °C until DNA extraction.

***In vivo* propagation of *Eimeria* oocysts:** All samples contained sporulated coccidial oocysts were used for *in vivo* propagation as a consequence of overall low oocyst recovery. Individually caged 2 week old specific- pathogen free (SPF) white spotted gecko were inoculated orally with 1.000 sporulated mixed oocysts from single field parasite populations. Progeny oocysts were recovered from cecal tissue and the contents were collected during post-mortem 3 days post infection to be sporulated and purified.

Determination of phylogenetic relationship

Extraction of genomic DNA from recovered coccidian species. DNA was isolated from approximately 100.000 oocysts from each propagated sample. The potassium dichromate was removed by repeated centrifugation and re-suspension in distilled water. The washed oocysts were then sterilized and prepared for isolation by sodium hypochlorite treatment (4% available chlorine, 1 h, 4 °C). Oocysts were subjected to 3 freeze–thaw cycles of 2 min each in a dry ice/ethanol bath and a 100 °C water bath. Total genomic DNA (gDNA) from the lysed oocysts was extracted using a QIAamp® DNA Mini Kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions.

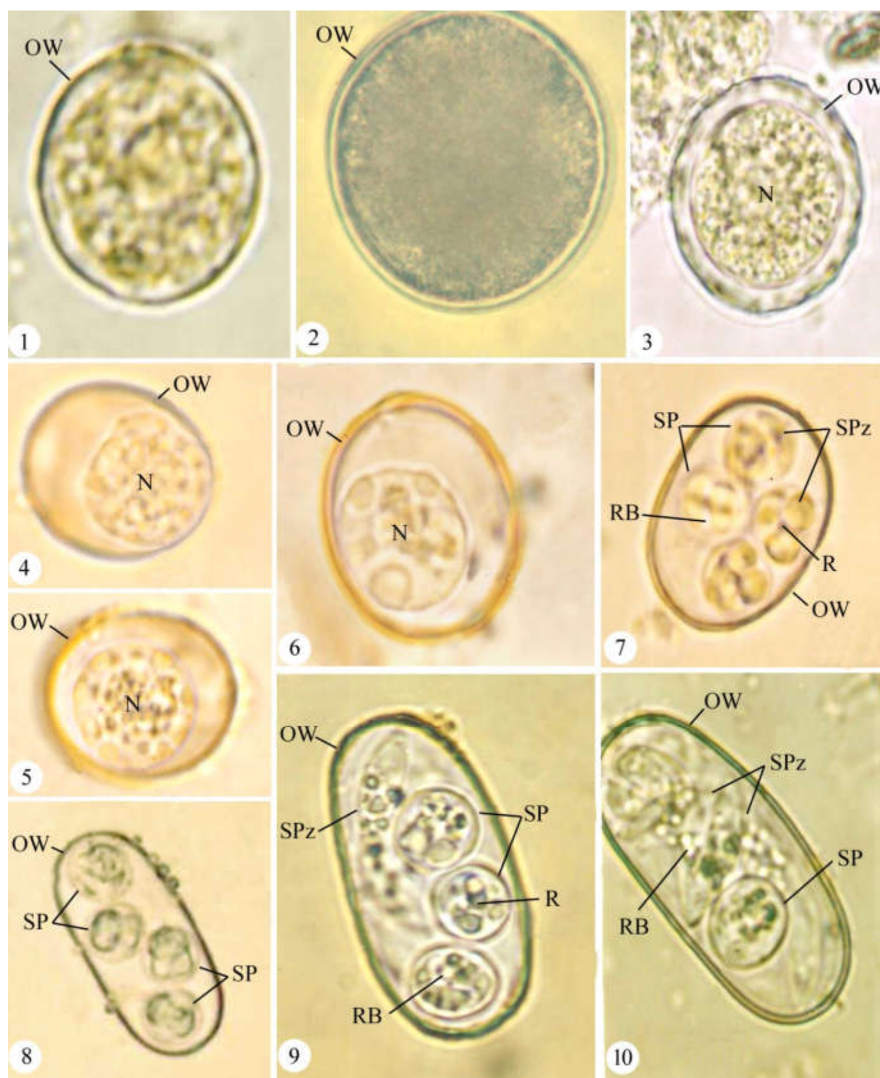
Amplification of small subunit rDNA of coccidian species. The SSU rDNA gene clusters were targeted for amplification by using PCR. Procedures for avoiding contamination were strictly followed, and negative (no-DNA) controls were included in every experiment. SSU rDNA genes from positive samples were amplified with the universal primer-pairs coded 'BTF' was 5'-GGT TGA TCC TGC CAG TAG T-3', and the reverse primers coded 'EimIsoR1' was 5'-AGG CAT TCC TCG TTG AAG ATT-3' and 'EimIsoR3' was 5'-GCA TAC TCACAA GAT TAC CTA G-3'; in 25-μl reaction mixture consisting of the following: 1 μl of extracted genomic DNA, 5 μl of 1mM deoxyribonucleotide triphosphates (dNTPs, MBI Fermentase), 0.25 μl of each primer (50 pmolμl^{-1}), 2.5 μl of 10x Taq polymerase buffer (MBI Fermentase), 2 μl of 25 mM $Mgcl_2$, 1 μl Taq DNA polymerase (2 U) (MBI Fermentase), and 13 μl of distilled water. The PCR reaction condition comprised initial denaturation of DNA at94°C for 3 min, followed by 35 cycles of 94° for 1 min, each annealing temperature for 30 s, and 72° for 1 min, and a final 10 min 72°C extension step. This was followed by a second round of nested PCR was performed by using the universal primer-pair

coded 'BSEF' was 5'-CTG TGA ATT CAT CGG A-3', and the reverse primer coded 'BSER' was 5'-ATC GCA TTT CGC TGC GTC CT-3'. A similar PCR reaction mix, as described above, was used for the nested PCR except that the PCR program comprised an initial denaturation step for 5 min at 95 °C, followed by 40 cycles, each consisting of 15 sec denaturation at 95 °C, 30 sec annealing at 45 °C and 30 sec extension step at 72 °C with the final extension continued for 10 min. *Cloning*. PCR products were analyzed by electrophoresis in 1.0% (w/v) agarose gel, visualized after ethidium bromide staining, purified using QIAquick Gel Extraction Kit (Qiagen, GmbH, Germany) and cloned into the pDrive Cloning Vector using a Qiagen PCR Cloning Kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. The isolation of plasmid DNAs was performed by Gene Jet Plasmid Miniprep kit (Fermentas) and detection of plasmids containing a cloned PCR product was determined by digestion of plasmid DNA with restriction end nuclease EcoRI (Fermentas) followed by agarose gel electrophoresis. Plasmids containing a PCR insert were sequenced using T7 promoter primer at the sequencing unit of the Molecular Biology Facility in VACSERA.

DNA sequencing and phylogenetic analysis. Bands with predicted size were purified using a QIAquick® PCR Purification kit (Qiagen, CA) and sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with 310 Automated DNA Sequencer (Applied Biosystems, USA) using the same set of primers pair used in amplification. The various forward and reverse sequence segments were aligned using CLUSTAL-X v1.83 software implemented in the program Bio Edit (Hall, 1999). The partial 18S rDNA gene sequences generated in the present study submitted to Gen Bank under Accession KY419096 and KY419097. Phylogenetic calculations were performed with PAUP 4.0b10 (Swofford 2000). Maximum likelihood (ML) and neighbor-joining (NJ) analyses were conducted using Tamura-Neimodel and pairwise deletion for gapsbased on the most appropriate model selection using Model Test in MEGA 6(Tamura *et al*., 2007). Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

RESULTS

The adult specimens of the white spotted gecko *Tarentola annularis* (Family Gekkonidae) were found to be parasitized by two new coccidian parasites were *Eimeria tarentoli* and



Figs. 1-10. Photomicrographs of the coccidian parasites infecting the white spotted geckos *Tarentola annularis*. 1-5Different stages of unsporulatedoocysts of: 1-3*E. tarentoli* n. sp. with nucleus (N) and covered by oocyst wall (OW). 4,5 *E. ghaffari* n. sp.6 Early stage of sporulated oocyst of *E. tarentoli* n. sp. 7 Sporulated oocyst of *E. tarentoli* n. sp. with four sporocysts (SP) each one has two sporozoites (SPz), residuum (R) and refractile body (RB). 8-10 Sporulated oocysts of *E. ghaffari* n. sp with four sporocysts (SP) each one has two sporozoites (SPz), residuum (R) and refractile body (RB)

Eimeria ghaffari belonged to the family Eimeriidae and reaching a prevalence rate of 87.50 % (35/40). The highest percentage of parasitic infection was recorded in summer season to be 80.0% (8 specimens out of 10). The rate decreased gradually to 50.0 % (5 out of 10) and 40.0 % (4 out of 10) in spring and autumn, respectively. The lowest value of parasitic infection was detected in winter when only 20.0 % (2 out of 10) geckos were infected. Prevalence and mean intensity of parasitic infection were negatively correlated with the host size as smaller geckos (<5cm in length and <30gm in weight) are more infected than larger ones (5-10 cm in length and >60gm in weight). The number of parasites of male and female *T.annularis* were compared, and no significant differences were observed.

***Eimeria tarentoli* sp. nov. (Figs. 1-3,6,7,11)**

Description: Oocysts were spherical to sub-spherical in shape and measured 21.6-31.4 x 15.3-25.2 (26.5 x 19.2) µm (n=40); shape-index (length/width) was 2.01 (1.58-2.34). Wall was single-layered and reached approximately 0.75 µm thick. Single polar granule was present; micropyle and oocyst residuum were absent. Sporocysts were spherical to sub-spherical in shape and measured 6.8-9.1 x 5.2-6.4 (7.8 x 5.9) µm (n=10); shape index (length/width) was 1.4 (1.23-1.62). Wall was single-layered and stieda body was absent. Curved sporozoites contained noticeable refractile body and lie around compact sporocyst residuum.

Taxonomic summary

Parasite name: *Eimeria tarentoli* sp. nov.

Type of host: White spotted gecko *Tarentola annularis* (Geoffroy, 1827) (Family Gekkonidae)

Type locality: Abu Rawash, Egypt

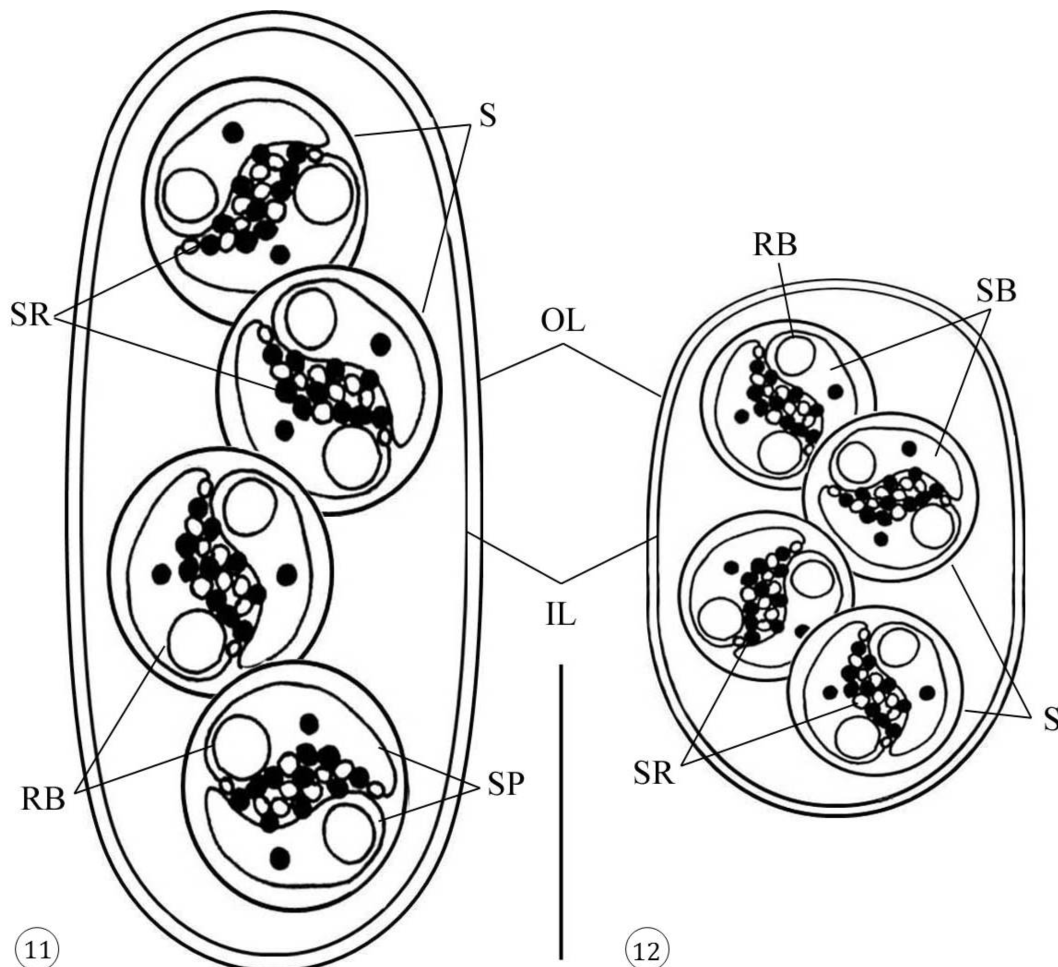
Infection site: Probably intestine of the infected gecko and oocysts found in faces

Material deposition: Specimens were deposited in Zoology Department, Faculty of Science, Cairo University, Egypt

Etymology: The specific name reflects the generic name of the host.

***Eimeria ghaffari* sp. nov. (Figs. 4,5, 8-10,12)**

Description: Oocysts were elongated to cylindroids and measured 29.6-31.3 x 14.7-23.2 (34.5 x 19.3) µm (n=40); shape-index (length/width) was 2.01 (1.98-2.65). Wall was single-layered and reached approximately 0.75 µm thick. Single polar granule was present; micropyle and oocyst residuum were absent. Sporocysts were ellipsoidal and measured 7.9-14.6 x 6.2-7.5 (10.6 x 7.2) µm (n=10); shape index (length/width) 1.4 (1.32-1.59).



Figs. 11,12. Line drawings of sporulated oocysts of two coccidian parasites collected from faces of *Tarentola annularis*. 11*E. tarentoli* n. sp.12*E. ghaffari* n. sp. each oocyst surrounded with outer layer (OL) and inner layer (IL) membrane and containing four sporocysts (S), each one with sporocyst residuum (SR) and two sporozoites (SP); each sporozoite have one refractile bodies (RB). Scale bar = 10 µm

Table 1. Morphometric comparison between the present *Eimeria* species and others previously recorded

Eimerianspp.	Host (s)	Locality	Shape and Measurements of				Reference
			Oocyst		Sporocyst		
E. gekkonis	Gekko japonicus	Japan	Ovoid	17.0-20.0 × 13.0 – 15.0	No data	No data	Tanabe (1928)
E. flaviviridis	Hemidactylus flaviviridis	India	Ellipsoid- cylindroid	25.0- 34.0 × 11.0-14.0	Ovoid	7.0-9.0 × 5.0 – 7.0	Setna and Bana (1935)
E. Knowlesi	Hemidactylus flaviviridis	India	Spherical-ovoid	15.3- 21.2 x 13.6- 20.4	No data	No data	Bhatia (1936)
E. koidzumii	Gekko japonicus	Japan	Elongate-ellipsoid	30.0 x 14.0	No data	13.0 x 9.0	Matubayasi (1941)
E. gehyrae	Gehyra vartegata	Australia	Cylindroid	29.6- 34.6 x 19.7- 21.8	No data	13.3- 14.0 x 7.4- 8.3	Cannon (1967)
E. michikoa	Gekko japonicus	Japan	Subspherical	20.0- 29.0 x 19.0- 26.0	Ellipsoid	7.0- 9.0 x 10.0- 12.0	Bovee (1971)
E. scinci	Hemidactylus flaviviridis	Tunisia	Ellipsoid	36.0 x 25.0	Ellipsoid	14.0 x 10.0	Pellerdy (1964)
E. cicaki	Gekko mutilate	Malaysia	Ellipsoid	20.0- 26.0 x 18.0- 23.0	Ellipsoid	11.0- 13.0 x 8.0- 10.0	Else and Collet (1975)
E. helenae	Hemidactylus brookei	Gamia	Ellipsoid	20.3-23.2 x 13.9- 16.2	No data	7.0- 9.3 x 6.4- 7.5	Bray (1984)
E. tarentolae	Tarentola mauritanica	Minorca	Ellipsoid	17.6- 18.7 x 12.9- 14.0	Round	6.4- 7.0	Matuschka and Bannert (1986a)
E. delalandii	Tarentola delalandii	Canary Islands	Cylindroid	42.3- 47.9 x 19.9- 26.0	No data	12.3- 15.3 x 9.4- 11.2	Matuschka and Bannert (1986b)
E. brygooi	Phelsuma madagascriensis	Madagascar	Spherical	18.8- 25.2 x 16.4- 23.2	Ovoid	8.0- 10.0 x 7.2- 8.8	Upton and Barnard (1987)
E. gallotiae	Gallotia galloti	Canary Islands	Elongate- ellipsoid	29.1- 32.6 x 14.0- 17.9	Ellipsoid	12.2- 17.3 x 8.2- 11.2	Matuschka and Bannert (1987)
E. turcicus	Hemidactylus turcicus	USA	Elongate- cylindroid	35.2- 40.8 x 16.8- 20.0	Ovoid	10.0- 12.0 x 8.0- 9.4	Upton *et al*., (1988)
E. lineri	Hemidactylus turcicus	USA	Ellipsoid	21.6- 28.0 x 18.4- 21.6	Ellipsoid	8.2- 9.6 x 7.2- 8.8	McAllister *et al*., (1988)
E. boveroi	Hemidactylus mabouia	Mexico	Spherical	16.0 – 21.6 x 16.0- 20.8	Ovoid	7.6- 9.6 x 7.2- 8.0	McAllister and Upton (1989)
E. dixoni	Hemidactylus frenatus	USA	Spherical	17.0- 22.0 x 17.0- 21.0	Ovoid	8.0- 11.0 x 7.0- 8.0	McAllister *et al*., (1990)
E. rangei	Palmatogecko rangei	Namibia	Ellipsoid	25.0- 29.0 x 18.0- 19.5	Spherical	9.0- 10.5 x 8.0- 9.0	Upton *et al*., (1991)
E. barnadi	Rhoptropus barnardi	Namibia	Ellipsoid	21.0- 26.5 x 16.0- 22.0	Subspherical	8.0- 11.0 x 7.5- 9.0	Upton *et al*., (1992)
E. stenodactyl	Stenodactylus	Egypt	Subspherical	26.0- 32.0 x 22.0- 27.0	Ovoid	9.0- 11.0 x 7.5-8.5	El-Toukhy (1994)
E. vittati	Gekko vittatus	UK	Elongate- ellipsoid	32.5- 36.5 x 16.5- 17.5	Ovoid	10.0- 12.5 x 5.7- 5.0	Ball and Daszak (1995)
E. lineri	Hemidactylus turcicus	Egypt	Ellipsoidal	25.5- 28.5 x 18.5- 21.0	Ellipsoid	9.0- 11.0 x 7.5- 8.5	El-Toukhy *et al*., (1997)
E. tripolitani	Tropiocolotes tripolitanus	Egypt	Ellipsoid- ovoid	20.5- 28.3 x 16.6- 18.6	Subspherical	6.8- 9.8 x 6.8- 8.8	Abdel-Aziz (2001)
E. ptyodactyli	Ptyodactus hasselquistii	Egypt	Spherical	20.9- 24.0	Ovoid	10.4- 11.5 x 8.0- 8.8	Abdel-Aziz (2001)
E. gizaensis	Ptyodactus hasselquistii	Egypt	Oval	29.0- 30.0 x 22.0- 24.0	Subspherical	9.4- 10.4 x 7.3-9.4	Abdel-Aziz (2001)
E. hailensis	Ptyodactus hasselquistii	Saudi Arabia	Cylindroid	35.7- 38.4 x 15.5- 20.0	Subspherical	8.1- 12.1 x 7.4- 8.8	Abdel-Aziz (2001)
E. dahabensis	Tropiocolotes nattereri	Egypt	Ellipsoid-ovoid	24.4-33.0 x 17.6- 23.8	Ellipsoid	13.8- 16.6 x 6.7- 10.4	Abou El-Nour (2005)
E. raleighi	Phelsuma rosagularis	Mauritius	Spherical	16.0- 19.2 x 14.4- 16.8	Subspherical	7.2- 8.0 x 6.4- 7.2	Daszak *et al*., (2009)
E. alexandriensis	Tarentola mauritanica	Egypt	Ellipsoid	22.7- 29.6 x 14.4- 19.5	Ellipsoid	9.6- 16.7 x 5.6- 8.4	El-Toukhy *et al*., (2013)
E. tarentoli sp. nov.	Tarentola annularis	Egypt	Spherical	21.6-31.4 x 15.3- 25.2	Subspherical	6.8- 9.1 x 5.2- 6.4	Present study
E. ghaffari sp. nov.	Tarentola annularis	Egypt	Elongate- cylindroid	29.6- 31.3 x 14.7- 23.2	Ellipsoid	7.9- 14.6 x 6.2- 7.5	Present study

Wall was single-layered and stieda body was absent. Sporozoites contained prominent refractile body, lie curved to one side of large and distinctly globular sporocyst residuum.

Taxonomic summary

Parasite name: *Eimeria ghaffari* sp. nov.

Type of host: White spotted gecko *Tarentola annularis* (Geoffroy 1827) (Family: Gekkonidae)

Type locality: Abu Rawash, Egypt

Infection site: Probably intestine of the infected gecko andoocysts found in faces

Material deposition: Specimens were deposited in Zoology Department, Faculty of Science, Cairo University, Egypt

Etymology: The species is named for Prof Fathy Abdel-Ghaffar in order to acknowledge his assistance in this work.

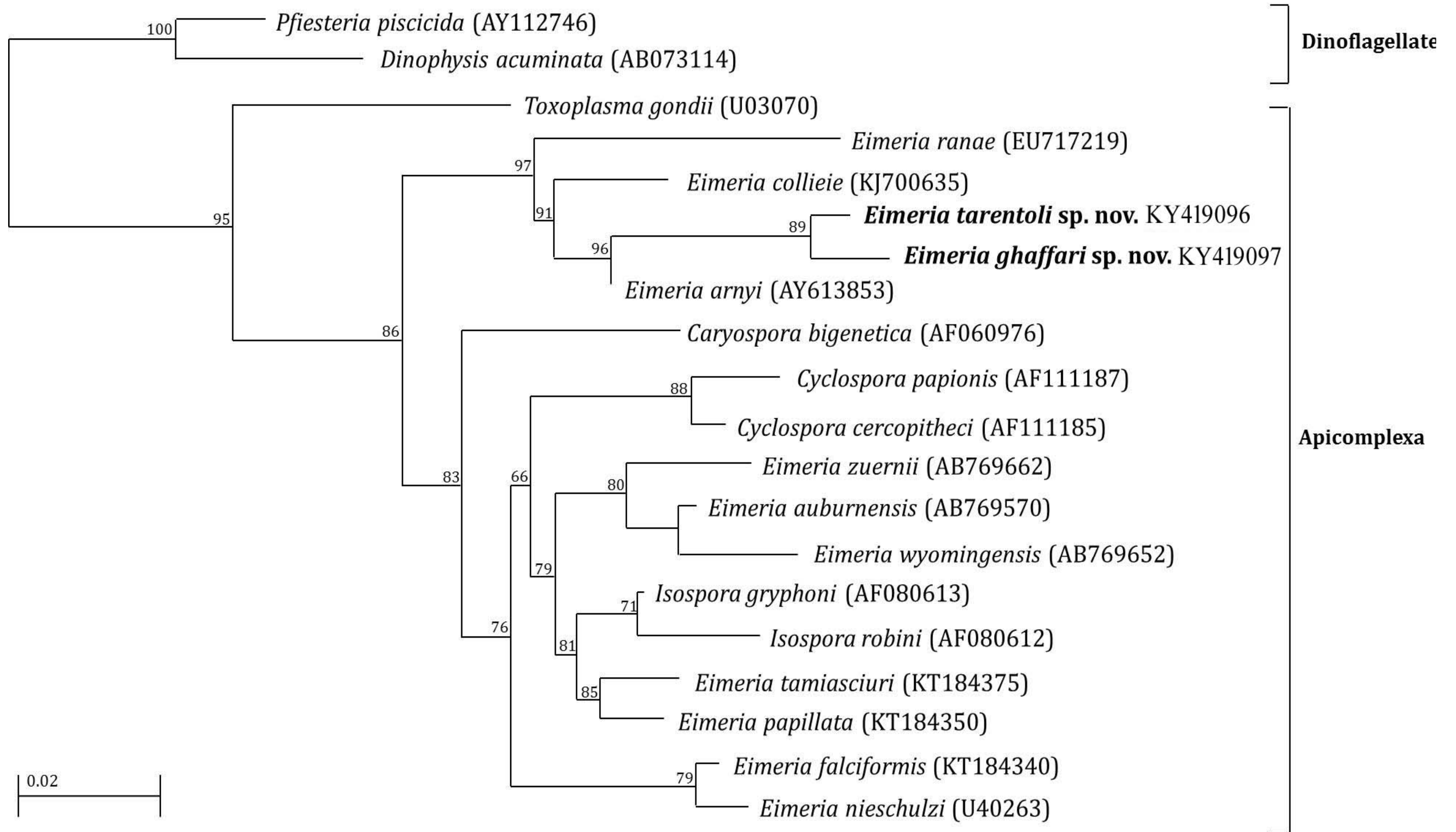


Fig. 13. Phylogenetic tree generated by maximum parsimony analyses of the 18SrDNA sequences. Numbers atnodes indicate bootstrap confidence values (100 replications). GenBank accession numbers are given in parentheses. *Eimeria* species examined in the present study are in bold

Phylogenetic analysis

Analyses of 18SSUrDNA gene sequences for 18 taxa by neighbor-joining, maximum parsimony and maximum likelihood tree inference methods recovered similar topologies (Fig. 13). Sequencing of 910 and 840 nucleotides of 18S rDNA for two coccidian species under study were successfully amplified, sequenced and placed them among members of Conoidasida species belonging to phylum Apicomplexa with 45.5% and 44.5% GC content, respectively. Comparison of the nucleotide sequences and divergence showed that the 18S rDNA of these species revealed sequence identities ≥90% with intra-specific differences varied from 0.1%-1.5%. The obtained results revealed that the recovered coccidian species exhibited 95.7% similarity to *E. collieie* (acc. no. KJ700635), 9 5.1% similarity to *E. arnyi* (acc. no. AY613853), 94.7% similarity to *E. ranae* (acc. no.EU717219), 94.5% similarity to *Caryospora bigenetica* (acc. no.AF060976), 94.5% similarity to *Cyclospora papionis* (acc. no. AF111187), 94.2% similarity to *Cyclospora cercopitheci* (acc. no.AF111185), 94.1% similarity to *E. zuernii* (acc. no.AB76662), 93.5% similarity to *E. auburnensis* (acc. no.AB769570), 92.9% similarity to *E. wyomingensis* (acc. no.AB769652), 92.6% similarity to *Isospora gryphon i*(acc. no.AF080613), 92.5% similarity to *Isospora robini* (acc. no.AF080612), 92.3% similarity to *E. tamiasciuri* (acc. no.KT184375), 92.8% similarity to *E. papillata* (acc. no.KT184350), 92.6% similarity to *E. falciformis* (acc. no.KT184340), 91.4% similarity to *E. nieschulzi* (acc. no.U40263), and 91% similarity to *Toxoplasma gondii* (acc. no.U03070) with a high bootstrap values with paraphyletic origin. The phylogenetic tree showed that recovered coccidian species were deeply embedded within the genus *Eimeria* with close relationship to other *Eimeria* species infecting gekkonid group of *E. collieie* and *E. arnyi* as a more related sister taxon. Three species of bovids *Eimeria* (*E. auburnensis*, *E. zuernii* and *E. auburnesis*) tend to cluster together into separate clade and other *Eimeria* species that infect ruminants (*E. tamiasciuri*, *E. papillata*, *E. falciformis* and *E. nieschulzi*) form a distinct clade. The constructed phylogenetic tree showed that din flagellates were present as outgroup.

DISCUSSION

Reptiles are hosts of different coccidian parasites including *Eimeria, Isospora*, *Caryospora*, *Cyclospora*, *Cryptosporidium*, *Sarcocystis, Haemogregarina* and *Hepatozoon* species (Abou El–Nour, 2005). Eimeriid coccidians generally inhabit the intestinal tract, although extra-intestinal development has been recorded. In the last two decades, several studies concerning intestinal coccidia infecting reptiles in Egypt have been carried out (El–Toukhy, 1994; Sakran *et al.,* 1994; El-Toukhy *et al.,* 1997; Fayed, 2003). To identify the present eimerian species, a comparative data with the previously described *Eimeria* spp. infecting gekkonid hosts was given in Table (1). The comparison was based on certain significant criteria such as host species, its geographical distribution and characteristics of oocyst and sporocyst. It is known that no eimerian from lizards has ever been shown to cross generic boundaries, although this has not been tested (Aquino–Shuster *et al.,* 1990). Pellerdy and Durr (1969) and McLoughlin (1969) concluded also that "although, there were only few acceptable records of the transmission of *Eimeria* spp. from one host genus to another, the host specificity of an *Eimeria* species is strong and it is rare for such parasite to occur naturally or to complete the endogenous development in more than one host genus".

So, the description of *Eimeria* from different lizard hosts as a new species has been only based on the differences in hosts and their geographical distribution. Considering the above mentioned reasons and according to the available data given in Table (1), it was found that shape of oocysts as well as sporocysts of the present *Eimeria tarentoli* sp. nov. was similar to *E. knowlesi* Bhatia (1936) from *Hemidactylus flaviviridis* in India, *E. boveroi* McAllister and Upton (1989) from *Hemidactylus mabouia* in Mexico, *E. dixoni* McAllister *et al*., (1990) from *Hemidactylus frenatus* in USA, and *E. raleighi* Daszak *et al*. (2009) from *Phelsuma rosagularis* in Mauritius. While, the shape of oocysts as well as sporocysts of the present *E. ghaffari* sp. nov. was similar to *E. gehyrae* Cannon (1967) from *Gehyra vartegata* in Australia, *E. delalandii* Matuschka and Bannert (1986b) from *Tarentola delalandii* in Canary Islands, *E. turcicus* Upton *et al*., (1988) from *Hemidactylus turcicus* in USA, *E. hailensis* Abdel-Aziz (2001) from *Ptyodactus hasselquistii* in Saudi Arabia. Both recovered species have the same geographical host location with *E. stenodactyl* El-Toukhy (1994) from *Stenodactylus*, *E. lineri* El-Toukhy *et al*., (1997) from *Hemidactylus turcicus*, *E. tripolitani* Abdel-Aziz (2001) from *Tropiocolotes tripolitanus*, *E. ptyodactyli* Abdel-Aziz (2001) from *Ptyodactus hasselquistii*, *E. gizaensis* Abdel-Aziz (2001) from *Ptyodactus hasselquistii*, *E. dahabensis* Abou El-Nour (2005) from *Tropiocolotes nattereri*, and *E. alexandriensis* El-Toukhy *et al*. (2013) from *Tarentola mauritanica*. Further, the present sporocysts were the only among those of the above comparable eimerian species in having a stieda body and sporocyst residuum. However, the present oocysts as well as sporocysts differ from the comparable species in size and host species. It seems to be justified to consider the present *Eimeria* species as new ones. It is suggested to be named as *E. tarentoli* sp. nov. and *E. ghaffari* sp. nov.

Recently, identification of different coccidian parasites, especially those of *Eimeria* species, based on morphological and morphometric basis, is rather difficult due to qualitative and quantitative features of oocyst morphology often overlap among and vary within *Eimeria* species (Khodakaram-Tafti and Mansourian 2008, Hill *et al*., 2012, Yang *et al*., 2013).Thus, molecular techniques have recently been proven useful for the identification or classification of these parasites to overcome the limitations of these traditional approaches (Matsubayashi *et al*., 2005; Kawahara *et al*., 2010). In the present study, the establishment of two new *Eimeria* species was supported by the molecular phylogenetic analyses of the 20 taxon SSU rDNA sequence dataset consisting of din flagellates, and apicomplexans. 18S rDNA gene were employed as molecular genetic approaches to investigate the phylogenetic analysis and DNA sequence variations of *E. tarentoli* sp. nov. and *E. ghaffari* sp. nov. compared with other coccidian species that exist in Gen Bank. Comparison of the nucleotide sequences and divergence showed that the 18S rDNA revealed sequence identities ≥90% with low intra-specific variations in the DNA sequences. These results agreed with data obtained by Kawahara *et al*., (2010) followed by Khodakaram-Tafti *et al*., (2013) whom stated that the presence of high degree of sequence similarity, low intra-specific and high inter-specific variations in the DNA sequence help in primers design in order to minimizing the risk of cross-reactions with different species. Phylogenetic inferences of the

genus *Eimeria* show a high relatedness between species from closely related hosts, and in mostcases, *Eimeria* from single host groups are placed in monophyletic lades.

However, host groups of *Eimeria* were under-represented with phylogenetic inferences based only on *Eimeria* from rodents, bats, rabbits and birds (Kvicerova *et al*., 2008; Slapeta *et al*., 2001; Yabsley and Gibbs, 2006; Zhao and Duszynski, 2001).Inclusion of *Eimeria* from a diversity of host groups, particularly from hosts representing ancient evolutionary lineages, would provide more informative and reliable inferences. Based on the results of the present investigation, the 18S rDNA sequence derived from *E. tarentoli* sp. nov. and *E. ghaffari* sp. nov. showed a high degree of similarity with *E. collieie, E. arnyi* and *E. ranae* with few differences in nucleotides and formed one cluster. However, the phylogram based on the 18S rDNA sequence showed that *E. collieie* and *E. arnyi* were the closest taxon to the recovered *Eimeria* species. The phylogram based on the 18S rDNA sequences showed that bovids group included *E. auburnensis*, *E. zuernii* and *E. auburnesis* formed a distinct group separate from the other remaining *Eimeria* spp. with monophyletic in origin. Similarly, Barta *et al*., (2001) reported a tendency provided by phylogenetic analysis of avian Eimeria for *E. necatrix* and *E. tenella*, the most pathogenic *Eimeria* in chicken, followed by Khodakaram-Tafti *et al*., (2013) demonstrated that *E. arloingi* in goats and *E. bovis* and *E. zuernii* in cattle are highly pathogenic and formed a monophyletic group in the position away from other members in spite of many different biological characteristics and the pathological lesions. The other two rodent species, *E. nieschulzi* and *E. falciformis*, formed a separate group in a distinct clade, this results agreed with Zhao *et al*., (2001) who stated that, within *Eimeria*, morphological similarity of sporulated oocystsmay be more significant in reflecting parasite–host phylogenetic/evolutionary relationships than is host specificity. The level of confidence in the branching topology was highly significant. Bootstrapping indicated that the 5 bovids *Eimeria* species and 2 rodent *Eimeria* species are monophyletic in 100% of 1,000 sampled trees in 83% in the nuclear 18S rDNA tree. Our results demonstrated that two of the human-associated *Cyclospora* spp. were closely related to the genus *Eimeria*. This data agreed with the suggestion of Megia-Palma *et al*., (2016) followed by Relman *et al*., (2016) stated that the structural and sporulation characteristics have led to the classification of *Isospora* as a member of the family Eimeriidae, one might speculate that *Isospora* and *Cyclospora* are also closely related.

Conclusion

This is the first report of morphological and molecular characterizations of two coccidian parasites belonged to family Eimeridae and identified as *E. tarentoli* sp. nov. and *E. ghaffari* sp. nov. in the white spotted gecko from Egypt. Future research should be also focused on the analyses of other genes to confirm whether SSU rDNA phylogeny corresponded to the organismal phylogeny. The combined analyses of appropriated genes could also increased the resolution of phylogenetic trees and could help to clarify the phylogenetic relationships of apicomplexans.

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Compliance with ethical standards

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by Institutional Animal Care and Use Committee (IACUC) at Zoology Department in Faculty of Science, Cairo University, Egypt.

Conflict of Interest

Authors declared that they neither have conflict of interest nor received financial support.

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