Yang, R., Brice, B., Elloit, A. and Ryan, U. (2016) Morphological and molecular characterization of Eimeria labbeana-like (Apicomplexa:Eimeriidae) in a domestic pigeon (Columba livia domestica, Gmelin, 1789) in Australia. Experimental Parasitology, 166 . pp. 124-130.

Accepted Manuscript

Morphological and molecular characterization of *Eimeria labbeana-like* (Apicomplexa:Eimeriidae) in a domestic pigeon (*Columba livia domestica,* Gmelin, 1789) in Australia

Rongchang Yang, Belinda Brice, Aileen Elloit, Una Ryan

PII: S0014-4894(16)30071-6

DOI: 10.1016/j.exppara.2016.04.009

Reference: YEXPR 7234

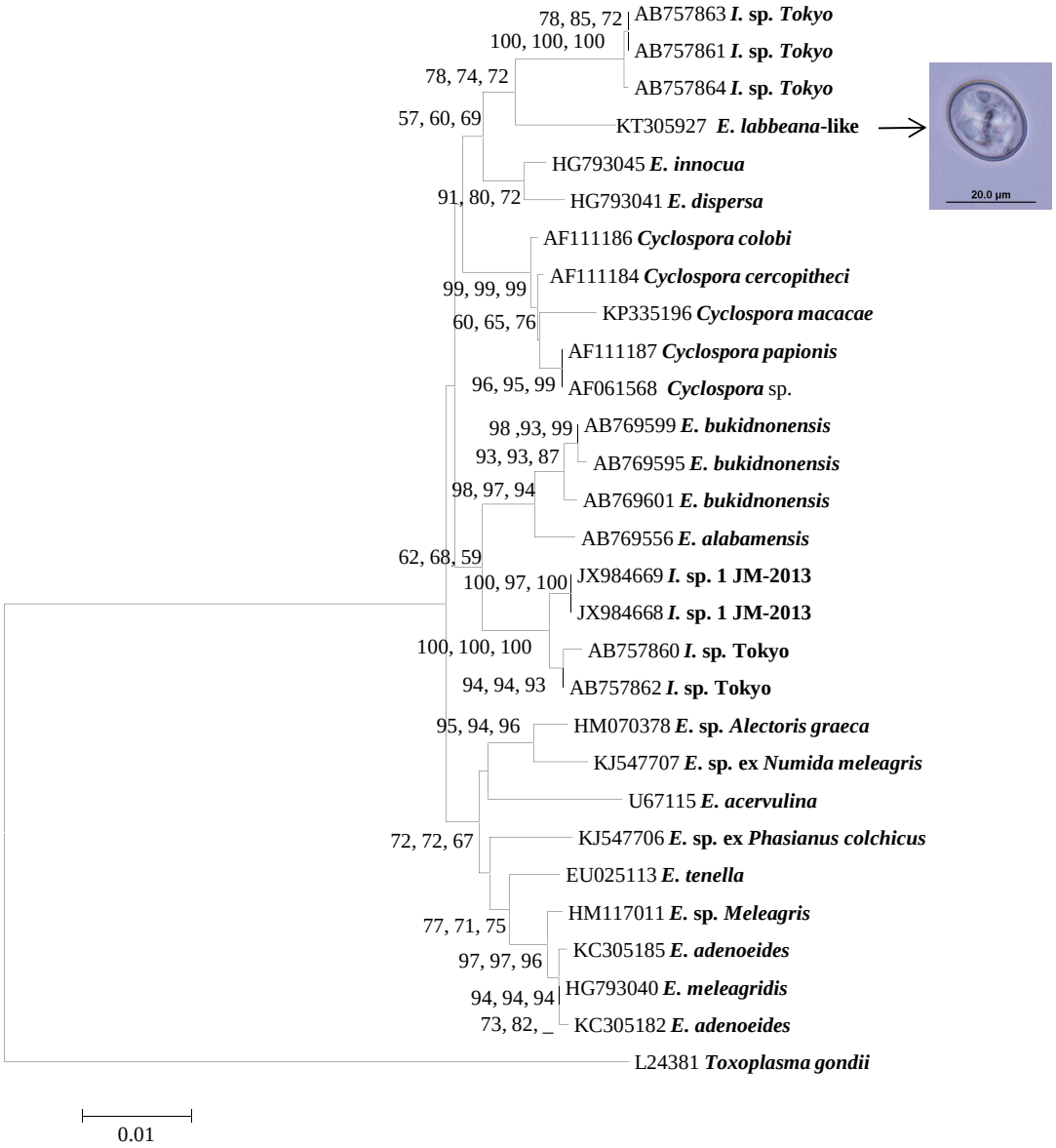
To appear in: *Experimental Parasitology*

Received Date: 7 August 2015

Revised Date: 7 January 2016

Accepted Date: 10 April 2016

Please cite this article as: Yang, R., Brice, B., Elloit, A., Ryan, U., Morphological and molecular characterization of (Apicomplexa:Eimeriidae) in a domestic pigeon (Gmelin, 1789) in Australia, *Experimental Parasitology* (2016), doi: 10.1016/j.exppara.2016.04.009.



Morphological and Molecular Characterization of *Eimeria labbeana-like* (Apicomplexa:Eimeriidae) in a domestic pigeon (*Columba livia domestica,* Gmelin, 1789) in Australia

Rongchang Yang[a*], Belinda Brice[b], Aileen Elloit[a] and Una Ryan[a]

[a]*School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150.*

[b]*Kanyana Wildlife Rehabilitation Centre, 120 Gilchrist Road, Lesmurdie, Western Australia 6076.*

_____

**Corresponding author. Mailing address: School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia, 6150. Phone: 61 89360 2495. Fax: 61 89310 4144.*

E-mail: R.Yang@murdoch.edu.au

ABSTRACT

An *Eimeria* species is described from a domestic pigeon (*Columba livia domestica*). Sporulated oocysts (n = 35) were subspherical, with a smooth bi-layered oocyst wall (1.0 µm thick). Oocysts measured 20.2 x 16.1 (22.0-18.9 x 15.7-18.9) µm, oocyst length/width (L/W) ratio, 1.38. Oocyst residuum and a polar granule were present. The micropyle was absent. Sporocysts are elongate-ovoid, 13.0 x 6.1 (14.5-12.5 x 5.5-7.0) µm, sporocyst L/W ratio, 2.13 (2.0-2.2), sporocyst residuum was present, composed of numerous granules in a spherical or ovoid mass. Each sporocyst contained 2 banana-shaped sporozoites, 12.3 x 3.5 (11.8-13.0 x 3.3-3.6) µm. A spherical-ellipsoid posterior refractile body was found in the sporozoites. A nucleus was located immediately anterior to the posterior refractile body. Molecular analysis was conducted at three loci; the 18S and 28S ribosomal RNA genes and the mitochondrial cytochrome oxidase gene (COI). At the18S locus, the new isolate shared 98.0% genetic similarity with three *Isospora* isolates from Japan from the domestic pigeon (*Columba livia domestica*). At the 28S locus, it grouped separately and shared 92.4% and 92.5% genetic similarity with *Isospora anthochaerae* (KF766053) from a red wattlebird (*Anthochaera carunculata*) from Australia and an *Isospora* sp. (MS-2003 - AY283845) from a Himalayan grey-headed bullfinch (*Pyrrhula erythaca*) respectively. At COI locus, this new isolate was in a separate clade and shared 95.6% and 90.0% similarity respectively with *E. tiliquae* n. sp. from a shingleback skink in Australia and an *Eimeria sp.* from a common pheasant (*Phasianus colchicus)* from America. Based on the morphological data, this isolate is most similar to *E. labbeana*. As no molecular data for *E. labbeana* is available and previous morphological data is incomplete, we refer to the current isolate as *E. labbeana*-like.

Keywords: 18S rRNA*; E. labbeana-like;* morphology; genetic characterization; mitochondrial cytochrome oxidase gene (COI); phylogeny.

1. Introduction

The domestic pigeon (*Columba livia domestica*) is derived from the rock pigeon (*Columba livia*), which is the world's oldest domesticated bird. The domestic pigeon was initially introduced into Australia by European settlers (Croome and Shields, 1992).

Coccidiosis is a widespread disease caused by protozoan parasites of the genus *Eimeria* (Coccidia: Eimeriidae), which is a complex and diverse group of protozoan parasites (Tenter, 2002). Over 1,700 *Eimeria* species have been identified worldwide (Duszynski et al., 2000). In birds, pathogenic *Eimeria* causes enteric disease and major economic losses in the global poultry industry (McDougald et al., 1997).

A total of 16 *Eimeria* species have been identified from the family Columbidae including *E. columbae* (Mitra and Das Gupta, 1937), *E. columbapalumbi* (Jamriška and Modrý, 2012), *E. columbarum* (Nieschulz, 1935), *E. curvata* (Adriano et al., 2000), *E. duculai* (Varghese, 1980), *E. gourai* (Varghese, 1980), *E. choudari* (Bhatia et al., 1973), *E. janovyi* (Bandyopadhyay et al., 2006), *E. kapotei* (Chatterjee and Ray 1969), *E. labbeana* (Pinto, 1928), *E. livialis* (Alyousif et al., 2009), *E. palumbi* (McQuistion, 1991), *E. sphenocercae* (Ray, 1952), *E. tropicalis* (Malhotra and Ray 1961), *E. turturi* (Golemansky, 1976), *E. waiganiensis* (Varghese, 1978) and *E. zenaidae* (Adriano et al., 2003). However, due to incomplete descriptions and lack of measurements for many *Eimeria* sp. from Columbidae in the past, it is difficult to validate existing species and Duszynski et al. (2000) have stated that it was likely only two species (*E. labbeana* and *E. columbarum*) occur in pigeons. As a result of these difficulties, molecular tools are essential to accurately delimit species and infer phylogenetic relationships among *Eimeria* species.

In the present study, we characterized an *Eimeria* isolate in a domestic pigeon (*Columba livia domestica*), using both morphological and molecular techniques. After extensive comparison of morphological data, we named this isolate as *E. labbeana-like.*

2. Materials and methods

2.1. Sample collection

An adult domestic pigeon came into care at the Kanyana Wildlife Rehabilitation Centre (KWRC), Perth in July, 2014. This bird had a leg band that identified it as having been banded in 2011 by the International Racing Pigeon Federation of Western Australia. The pigeon had a large old wound to the neck and another on a wing. It underwent surgery and made a full recovery. A faecal sample was taken soon after admission and microscopy revealed unsporulated coccidian oocysts.

Faecal flotation was conducted using a saturated sodium chloride and 50% sucrose (w/v) solution. A portion of faeces was placed in 2% (w/v) potassium dichromate solution ($K_2Cr_2O_7$), mixed well and poured into petri dishes to a depth of less than 1cm and kept at room temperature in the dark to facilitate sporulation. Sporulated oocysts were observed using an Olympus DP71 digital micro-imaging camera and images were taken using Nomarski contrast with a 100 x oil immersion objective. Faecal samples from another 19 domestic pigeons were collected (after midday) and also screened for coccidia.

2.2 Isolation of single Eimeria oocysts using a micromanipulator

A 3 axis hydraulic micromanipulator (MO-102, Nirashige, Japan) was used to isolate four separate single oocysts for DNA extraction and PCR.

2.3. DNA isolation

Oocyst DNA extraction was as described by Yang et al. (2014). Briefly, isolated single oocysts were placed on a slide and checked under the microscope (Olympus DP71 digital micro-imaging camera). Once the existence of a single oocyst on the cover slip was confirmed, photographs were recorded for morphological identification. The coverslip was then transferred into a PCR tube containing 10 µl of lysis buffer (0.005% SDS in TE solution). After a brief centrifugation, the tube was frozen in liquid nitrogen and thawed in a 95 °C water bath for four rounds to disrupt the oocyst wall. After the addition of 0.5 µl proteinase K (20 mM), the tube was incubated at 56 °C for 2 h and then at 95 °C for 15 min. The entire lysate from the single oocyst was used for three separate PCRs as described below.

2.4. PCR amplification and sequencing

A nested PCR with the primers EiGTF1 and EIGTR1 (Yang et al., 2015) was used for the external amplification of the 18S rRNA gene. The expected PCR product was ~1,510 bp. The primers EiGTF2 and EiGTR2 (Yang et al., 2015) were used for the internal reaction. The PCR reaction contained 2.5 µL of 10 × Kapa PCR buffer, 2 µl of 25 mM $MgCl_2$, 1.0 µL of 10 mM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 3.5 µL of DNA for the external reaction or 1 µL of external PCR product for the internal reaction, and 16.4 µL of H_2O. PCR cycling conditions both for the external and internal reactions were 1

cycle of 94ºC for 3 min, followed by 40 cycles of 94ºC for 30 sec, 55ºC for 30 sec and 72ºC for 2 min and a final extension of 72ºC for 5 min.

The PCR for the 28S rRNA locus was carried out using a nested PCR with the external primers: 28SExF and 28SExR as previously described (Schrenzel et al., 2005), which produced a PCR product size of ~1,362 bp. The internal primers, 28InF and 28SInR, produced an amplicon size of 1,420 bp (Yang et al., 2014). The PCR reaction contained 2.5 µL of 10 × Kapa PCR buffer, 2 µL of 25mM $MgCl^2$, 1 µL of 10 mM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 3.5 µL of DNA and 16.9 µL of H_2O. Both primary and secondary PCR's were conducted using the same cycling conditions; 1 cycle of 94ºC for 3 min, followed by 35 cycles of 94ºC for 30 sec, 60ºC for 30 sec and 72ºC for 90 sec and a final extension of 72ºC for 5 min.

A partial COI gene sequence (723 bp) was amplified using a nested PCR with the following primers COIF1 (Ogedengbe et al., 2011) and COXR1 (Dolnik et al., 2009) for the external reaction and COIF2 (Yang et al., 2013a) and COXR2 (Dolnik et al., 2009) for the internal reaction. The PCR reaction contained 2.5 µL of 10 × Kapa PCR buffer, 2 µL of 25 mM $MgCl_2$, 1.0 µL of 10 mM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 3.5 µL of DNA and 13.4 µL of H_2O. PCR cycling conditions were 1 cycle of 94 ºC for 3 min, followed by 40 cycles of 94 ºC for 30 sec, 58 ºC for 30 sec and 72 ºC for 1 min and a final extension of 72 ºC for 5 min. The external and internal PCR cycling conditions were identical.

The amplicons from the second round PCRs were gel purified using an in house filter tip method as previously described (Yang et al., 2013b). All the PCR products were sequenced using forward and reverse primers in duplicate using amplicons from different PCR runs. An ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems,

Foster City, California) was used for Sanger sequencing according to the manufacturer's instructions.

The results of the sequencing reactions were analysed and edited using FinchTV (Version 1.4), compared to existing *Eimeria* spp. 18S and 28S rRNA and COI sequences on GenBank using BLAST searches and aligned with reference genotypes from GenBank using Clustal W in BioEdit (V7.2.5).

2.5. Phylogenetic analysis

Phylogenetic trees were constructed for *Eimeria* spp. at the 18S, 28S and COI loci with additional isolates from GenBank. Parsimony analyses were conducted using MEGA (Molecular Evolutionary Genetics Analysis software, version 6, Arizona State University, Tempe, Arizona, USA). Neighbor-joining (NJ) and maximum likelihood (ML) analyses were conducted using Tamura-Nei based on the most appropriate model selection using ModelTest in MEGA 6. Bootstrap analyses were conducted using 1,000 replicates to assess the reliability of inferred tree topologies.

3. Results

3.4 Species description

3.4.1 E. labbeana-like isolate

Diagnosis: Sporulated oocysts are subspherical, with a smooth bi-layered oocyst wall (1.0 μm thick). Oocysts measured 20.2 x 16.1 (22.0-18.9 x 15.7-18.9) μm, oocyst length/width (L/W) ratio, 1.38. Oocyst residuum and a polar granule were present. The

micropyle was absent. Sporocysts are elongate-ovoid, 13.0 x 6.1 (14.5-12.5 x 5.5-7.0) μm, sporocyst L/W ratio, 2.13 (2.0-2.2), sporocyst residuum was present, composed of numerous granules in a spherical or ovoid mass. Each sporocyst contained 2 banana-shaped sporozoites, 12.3 x 3.5 (11.8-13.0 x 3.3-3.6) μm. A spherical-ellipsoid posterior refractile body was found in the sporozoites. A nucleus was located immediately anterior to the posterior refractile body.

Host: Domestic pigeon (*Columba livia domestica*).

Locality: Perth, Western Australia.

Prevalence: Ten out of twenty pigeons screened were found to be positive (50%).

Other hosts: Unknown.

Prepatent period: Unknown.

Patent period: Unknown.

Site of infection: Unknown.

Sporulation time: 72 - 96 hours.

Material deposited: DNA sequences have been deposited in GenBank under accession numbers KT305927, KT305928 KT305929 for the 18S, 28S and COI loci, respectively.

3.2 Phylogenetic analysis of the E. labbeana-like isolate *at the 18S locus*

Analysis of the five individual oocysts produced identical sequences at all loci analysed. At the 18S rRNA locus, a 1,229 bp PCR product of the *E. labbeana-like* isolate was successfully amplified and sequenced. Phylogenetic analyses of the *E. labbeana-like* isolate at this locus using Distance, Parsimony and ML analyses produced similar results (Fig. 2, ML tree shown). The *E. labbeana-like* isolate grouped in a clade with three *Isospora* isolates from Tokyo, Japan (AB757861, AB757863, AB757864) from the domestic pigeon (*Columba livia*

189 *domestica*) and shared 98.0% genetic similarity (Fig. 2).

190

191 *3.3. Phylogenetic analysis of the E. labbeana-like isolate at the 28S locus*

192

193 A 1,383 bp sequence of 28S DNA from *E. labbeana-like* was used for phylogenetic
194 analysis. There are few 28S rRNA sequences from *Eimeria* species available in GenBank and
195 no sequences from *Eimeria* derived from Columbiformes birds, therefore phylogenetic
196 analysis could only be conducted using available *Eimeria* 28S rRNA sequences and other
197 coccidian 28S sequences including *Isospora* spp. and *Goussi spp. Toxoplasma gondii* was
198 used as an outgroup. Phylogenetic analysis grouped the *E. labbeana-like* isolate in a separate
199 clade and shared 92.4% and 92.5% genetic similarity with *I. anthochaerae* (KF766053) from
200 a red wattlebird (*Anthochaera carunculata*) from Australia and an *Isospora* sp. (MS-2003 -
201 AY283845) from a Himalayan grey-headed bullfinch (*Pyrrhula erythaca)* respectively and
202 91% each genetic similarity with two chicken *Eimeria* species; *E. tenella* (AF026388) and *E.*
203 *acervulina* (GU593707) (Fig. 3).

204

205 *3.4. Phylogenetic analysis of the E. labbeana-like isolate at the COI locus*

206

207 Phylogenetic analysis of the 670 bp COI sequence placed the *E. labbeana-like* isolate in
208 a clade with *E. dispersa* (KJ608416) (95.6% similarity), a turkey-derived *Eimeria* (Fig. 4).

209

210 **4. Discussion**

211

212 Sporulated oocysts of the *E. labbeana-like* isolate are morphologically distinct from
213 other characterized *Eimeria* species (n=11) from columbiformes

(http://biology.unm.edu/biology/coccidia/ COLUMBIDAE.html (Accessed on 14th July 2015) and an additional 5 species which were not in the database (Table 1). The dimensions of the oocysts from the *E. labbeana-like* isolate overlap in size with oocysts from *E. columbapalumbi*, *E. choudari*, *E. labbeana* and *E. livialis.* However, an oocyst residuum is present in the *E. labbeana-like* isolate but is absent in *E. columbapalumbi* and *E. choudari*. Similarly, oocysts of the *E. labbeana-like* isolate have a polar granule, which was not present in *E. livialis*. For *E. labbeana*, which was originally described in 1928, only oocyst and sporocyst measurements were available, no other features were recorded in the original report and no molecular characterization was conducted (Pinto, 1928). We are therefore unable to fully compare this isolate with *E. labbeana* but have named it as *E. labbeana*-like due to the high similarity of the oocyst measurements (Table 1).

Molecular characterization of the oocysts of the *E. labbeana-like* isolate at the 18S rRNA locus showed that it was most closely related (98.0% genetic similarity) to *Isospora* isolates from the domestic pigeon (*Columba livia domestica*) from Japan. There were 5 cloned 18S sequences, all reportedly *Isospora* sequences from a domestic pigeon in Japan (AB757860 to AB757864) and two *Isospora* sequences from an Austral thrush (*Turdus falklandii*) from Spain (JX984668 and JX984669), available in GenBank. Three of the Japanese isolates (AB757861, AB757863 and AB757864) grouped with the *E. labbeana-like* isolate at the 18S locus (Fig. 2). However, the remaining two Japanese isolates (AB757860 and AB757862) grouped in a separate clade with the *Isospora* sequences from the Austral thrush (JX984668 and JX984669) (Fig. 2). This indicates that three of the Japanese isolates (AB757861, AB757863 and AB757864) were actually *Eimeria* isolates, while the remaining two sequences (AB757860 to AB757864) were *Isospora*, as they grouped with the *Isospora* sequences from the Austral trush. Phylogenetic analysis of COI gene sequences also grouped the *E. labbeana-like* isolate with *Eimeria*, where it exhibited 95.6% similarity with *E.*

dispersa. At the 28S rRNA locus, the *E. labbeana-like* isolate shared 92.4% and 92.5% genetic similarity with *I. anthochaerae* (KF766053) from a red wattlebird Australia and an *Isospora* sp. (MS-2003 -AY283845) from a Himalayan grey-headed bullfinch respectively and 91% each genetic similarity with two chicken *Eimeria* species; *E. tenella* (AF026388) and *E. acervulina* (GU593707) (Fig. 3).

Morphological analysis further confirmed that the *E. labbeana-like* isolate is an *Eimeria* as this parasite is differentiated from *Isospora* by the typical features for the *Eimeria* oocyst with 4 sporocysts per oocyst and 2 sporozoites per sporocyst (Becker, 1934) (Fig. 1a and 1b), whereas *Isospora* oocysts have 2 sporocysts per oocyst and 4 sporozoites per sporocyst (Becker, 1934).

In summary, this is the first report of the morphological and molecular characterization of an *Eimeria* species in the domestic pigeon from Australia. Future characterisation of *Eimeria* species in pigeons is necessary, using a combination of morphological, biological and molecular techniques.

Acknowledgements

The authors wish to thank June Butcher and the volunteers at the Kanyana Wildlife Rehabilitation Centre for their commitment and dedication in caring for all the animals admitted to the centre. We are also grateful to the staff at the Wattle Grove Veterinary Hospital, Perth for their expert treatment and care of the wildlife treated at their clinic.

References

Adriano, E.A., Thyssen, J.P., Cordeiro, N.S., 2000. *Eimeria curvata* n. sp. (Apicomplexa: Eimeriidae) in Columbina talpacoti and Scardafella squammata (Aves: Columbidae) from Brazil. Mem. Inst. Oswaldo Cruz. Rio de Janeiro. 95, 53–55.

Adriano, E.A., Thyssen, J.P., Cordeiro, N.S., 2003. A new species of *Eimeria* from the Eared Dove *Zenaida auriculata* (Aves: Columbidae) in Brazil. Acta Protozool. 42, 71–73.

Alyousif, S.M., Al-Shawa, R.Y., Al-Asiri, S.S. 2009. *Eimeria livialis* sp. n. (Apicomplexa: Eimeriidae) from the domestic pigeon, *Columba livia domestica* in Saudi Arabia. J. Egypt. Soc. Parasitol. 39, 383–388.

Bandyopadhyay, P.K., Bhakta, J.N., Shukla, R., 2006. A new *Eimeria* species (Protozoa: Apicomplexa: Sporozoea) from the blue rock pigeon *Columba livia* (Aves Columbidae). Zoos' Print Journal 21, 2386–2387.

Bathia, B.B., Pande, B.P., 1967. On *Eimeria*n oocysts from the purple moorhen (*Porphyrio porphyrio L.*) and the coot (*Fulica atra atra*): A preliminary study. Acta Vet. Acad. Sci. Hungar. 17, 347-349.

Becker, E.R., 1934. Coccidia and Coccidiosis of domesticated, game and laboratory animals and of man. Collegiate Press, Inc. Ames, Iowa.

Bhatia, B.B., Chauhan, P.P.S., Arora, G.S., Agrawal, R.D., 1973. Species composition of coccidia of some mammals and birds at the Zoological Gardens. Delhi and Luckow. Indian J. Anim. Sci. 43, 944–947.

BirdLife, 2007. "Bird Finder: Rock Dove". Birds in Backyards. Sydney: Australian Museum. Retrieved 15 July 2015.

Chatterjee, D.K., Ray, H.N., 1969. *Eimeria kapotei* n. sp., from the domestic pigeon, *Columba livia intermedia*. Proc. 56th Indian Sci. Congr. Abstr. 512.

Croome, F., Shields, J., 1992. Parrots and Pigeons of Australia. Angus and Robertson/National Photographic Index of Australian Wildlife. Sydney.

Duszynski, D.W., Couch, L. Upton, S.J., 2000. Coccidia of the world. Available at: http://www.k-state.edu/parasitology/worldcoccidia/COLUMBIFORMES. Accessed 10 Aug. 2015.

Golemansky, V., 1976. Three new coccidian species (Coccidia: Eimeriidae) found in wild birds from Bulgaria. Acta Protozool. 15, 399–404.

Jamriška, J., Modrý, D., 2012. A New Species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from the Common Wood Pigeon *Columba palumbus* Linnaeus, 1758 (Aves: Columbidae). Acta protozoolo. 51, 329-333.

Malhotra, M.N., Ray, H.N., 1961. On a new coccidium, *Eimeria tropicalis* n. sp. from the domestic pigeon, *Columba livia intermedia*. Proc. Ind. Sci. Cong. 48, 412.

Mitra, A. N., Das-Gupta, M. 1937. On a species of *Eimeria* (Coccidia-Sporozoa) from the intestine of a pigeon, *Columba intermedia*. Proc. Ind. Sci. Cong. Assoc.24, 291.

McAllister, C.T., Upton, S.J., 1990. Description of the oocysts of *Eimeria Columbae* (Apicomplexa: Eimeriidae) from *Fulica americana* (Aves: Gruiformes), with comments on synonyms of Eimerian species from related birds. J. Parasitol. 76, 27-29.

McDougald, L.R., Reid, W.M., 1997. Coccidiosis. In: Diseases of poultry (edited by Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R., Saif, M.Y.,) Iowa State University Press, Ames, IA, 865–883.

McQuistion, T E., 1991. *Eimeria palumbi*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the Galapagos dove (Zenaida galapagoensis). Trans. Am. Microsc Soc. 110,178–781.

Mitra, A.N., Das-Gupta, M., 1937. On a species of *Eimeria* (Coccidia-Sporozoa) from the intestine of a pigeon, *Columba intermedia*. Proc. Ind. Sci. Cong. Assoc. 24, 291.

Nieschulz, O., 1935 Ueber Kokziedien der Haustauben. Zentralbl. Bakteriol. I Abt. Orig. 134, 390–393.

Ogedengbe, J.D., Hanner, R.H., Barta, J.R., 2011. DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). Int. J. Parasitol. 41, 843-850.

Pinto, C., 1928. Synonymien de quelques species du genre *Eimeria* (Eimeriida, Sporozoa). C.R. Seance Soc. Biol. Paris. 98, 1564–1565.

Ray, D.K., 1952. On a new Coccidium, *Eimeria sphenocercaen*. sp., from Sphenocercus sphenurus (Kokla Green Pigeon). J. Parasitol. 38, 546–547.

Schrenzel, M.D., Maalouf, G.A., Gaffney, P.M., Tokarz, D., Keener, L.L., McClure, D., Griffey, S., McAloose, D., Rideout, B.A., 2005. Molecular characterization of isosporoid coccidia (*Isospora* and *Atoxoplasma* spp.) in passerine birds. J. Parasitol. 91, 635-647.

Tenter, A.M., Barta, J.R., Beveridge, I., Duszynski, D.W., Mehlhorn, H., Morrison, D.A., Thompson, R.C, Conrad, P.A., 2002. The conceptual basis for a new classification of the coccidia. Int. J. Parasitol. 32, 595-616.

Varghese, T., 1978. *Eimeria waiganiensis* sp. n. from the Greenwinged Ground dove (*Chalcophaps indica* Linnaeus) and the Magnificent Ground pigeon (*Otidiphaps nobilis* Gould) in Papua New Guinea. J. Parasitol. 64, 312–314.

Varghese, T., 1980. Coccidian parasites of birds of the avian order Columbiformes with a description of two new species of *Eimeria*. Parasitology 80, 183–187.

Yang, R., Brice, B., Bennett, M. D., Ryan, U., 2013a. Novel *Eimeria* sp. isolated from a King's skink (*Egernia kingii*) in Western Australia. Exp. Parasitol. 133, 162-165.

Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., Chalmers, R., Hadfield, S., Bath, A., Gordon C., Ryan, U.M., 2013b. Specific and quantitative

detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. Exp. Parasitol. 135, 142-147.

Yang, R., Brice, B., Ryan, U., 2014. Morphological and molecular characterization of *Eimeria paludosa* coccidian parasite (Apicomplexa:Eimeriidae) in a dusky moorhen (*Gallinula tenebrosa*, Gould, 1846) in Australia. Exp. Parasitol. 140, 1-7.

Yang, R., Belinda Brice, Ryan R., 2015. Morphological and molecular characterization of *Isospora neochmiae* n. sp. in a red-browed finch (*Neochmia temporalis*) (Latham, 1802) Exp. Parasitol. In press.

Fig. 1a. Nomarski interference-contrast photomicrographs of the *E. labbeana-like* isolate oocysts showing oocyst wall (OW), polar granule (PG), oocyst residuum (OR), sporozoites (SP), Stieda body (SB), and sporocyst residuum (SR).

Fig. 1b. Line Drawing of the sporulated oocyst of the *E. labbeana-like* isolate. Scale bar = 20 µm.

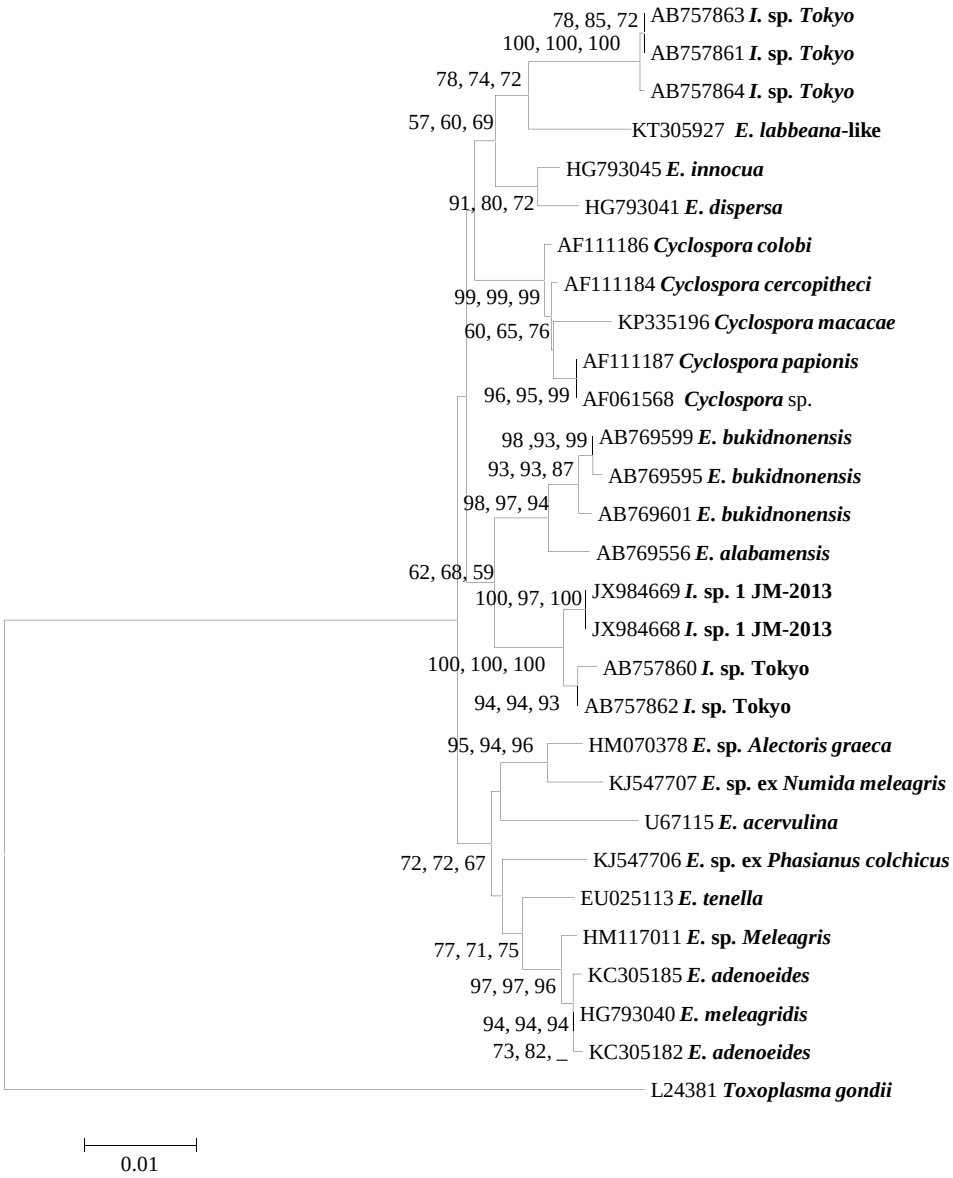
Fig. 2. Evolutionary relationships of the *E. labbeana-like* isolate inferred by distance analysis of 18S rRNA sequences (1,229 bp). Percentage support (>50%) from 1000 pseudoreplicates from distance, ML and parsimony analysis, respectively, is indicated at the left of the support node ('¬_' = Not available).

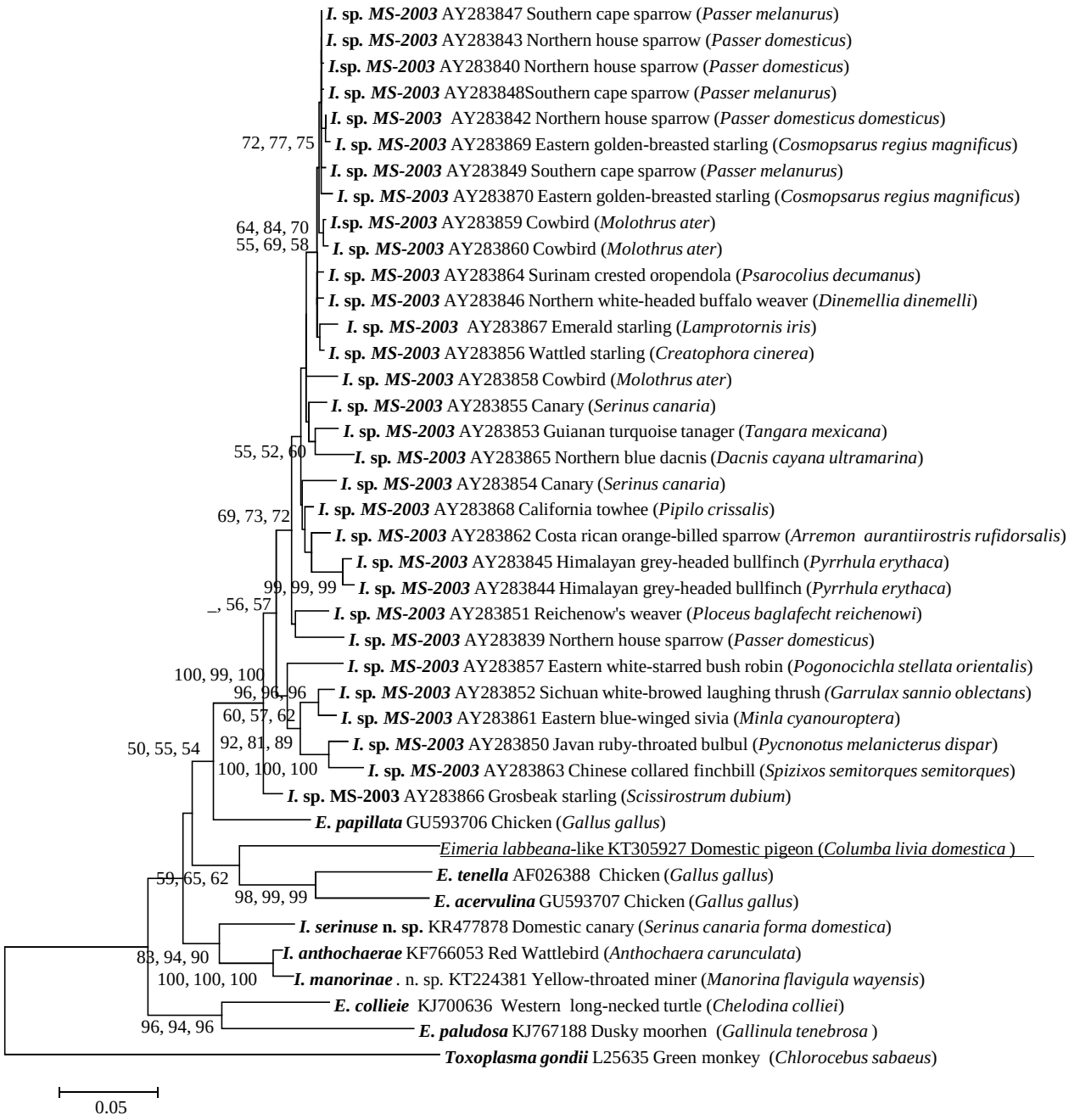
Fig. 3. Evolutionary relationships of the *E. labbeana-like* isolate inferred by distance analysis of 28S rRNA sequences (1,383 bp). Percentage support (>50%) from 1000 pseudoreplicates from distance, ML and parsimony analysis, respectively, is indicated at the left of the support node ('¬_' = Not available).

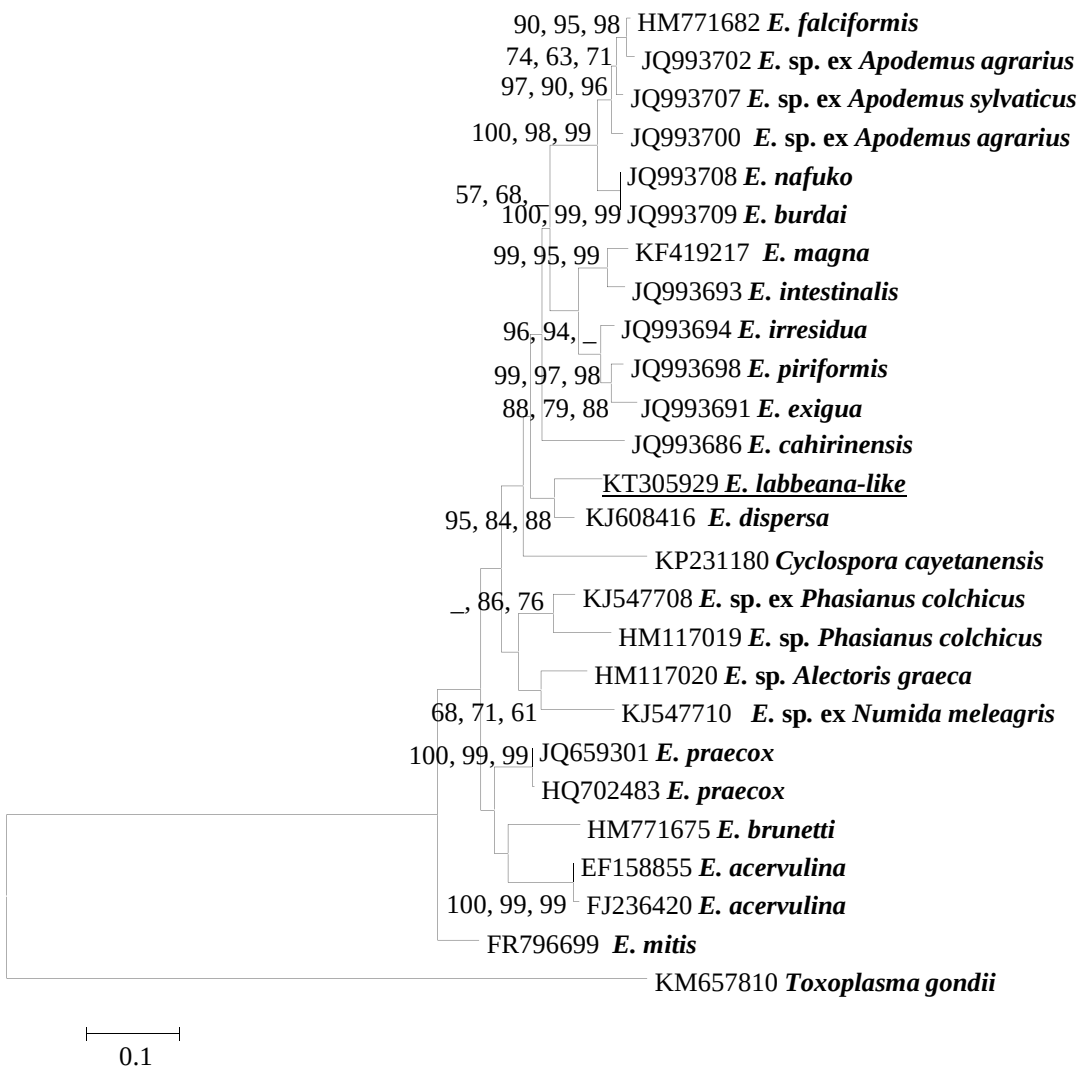
Fig. 4. Evolutionary relationships of the *E. labbeana-like* isolate inferred by distance analysis of COI sequences (670 bp). Percentage support (>50%) from 1000 pseudoreplicates from distance, ML and parsimony analysis, respectively, is indicated at the left of the support node ('¬_' = Not available).

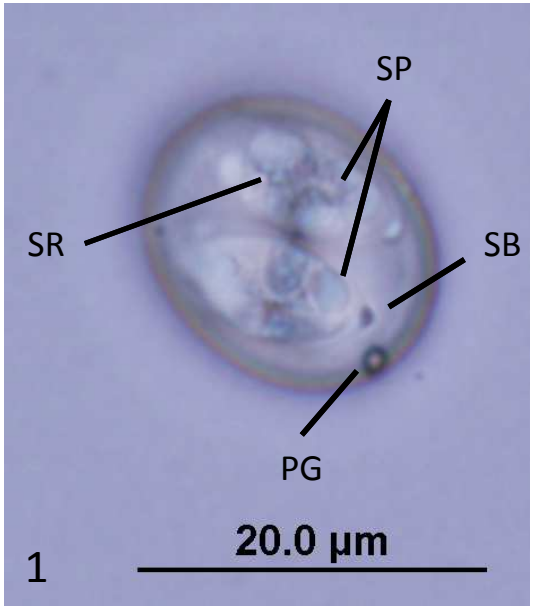
Table 1. Comparison of the morphology of *Eimeria* spp. derived from Columbidae family

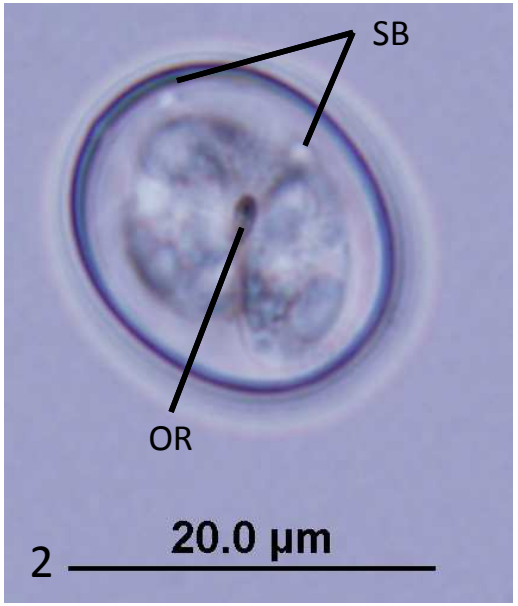
Species	Host	Oocyst	Oocyst shape index	Oocyst residuum	Micropyle	Polar granule	Sporocyst	Sporocyst shape index	Sporocyst residuum	Stieda body	Substieda body	References
E. columbae	*C. intermedia*	16.4 × 14.35	-	-	-	no	7.2 × 4.8	-	-	-	-	Mitra and Das, 1937
E. columbapalumbi	*C. palumbus*	17–24 × 15–18 1	1-1.44	no	no	yes (2)	11–16 × 6–7	1.69–2.17	scattered	yes	no	Jamriška and Modrý, 2012
E. columbarum	*C. livia*	19–21 × 17.5–20	0.92–0.95	no	no	-	-	-	yes	-	-	Nieschulz, 1935
E. curvata	*Columbina talpacoti, squammata*	17–19 × 15–17	1.1–1.3	no	no	yes (1)	11.5–13 × 5.5–6	2.1	compact	yes	no	Adriano et al., 2000
E. duculai	*Ducula spilorrhoa*	26–31 × 23–27	1.1	no	inconspicuous	yes	14–16 × 6.5–8	-	granular	yes	no	Varghese, 1980
E. gourai	*Goura victoria*	19–22 × 18–21	1	no	no	yes	10–13 × 4–6	-	granular	yes	no	Varghese, 1980
E. choudari	*Streptopelia decaocto*	16.9–22.1 × 13–18.2	-	no	no	yes	13.6 × 7.2	-	no	yes	no	Bhatia et al., 1973
E. janovyi	*C. livia*	24.3 × 19.8	1.2	no	no	yes (1)	12.06 × 10.1	1.2	granular	yes	-	Bandyopadhyay et al., 2006
E. kapotei	*C. intermedia*	24–30 × 21.6–26.4	-	-	yes	-	-	-	scattered	yes	-	Chatterjee and Ray, 1969
E. labbeana	*C. domestica; C. livia; S. decaocto; S. orientalis; S. senegalensis*	20–21 × 16–18	-	-	-	-	12.4 × 6.4	-	yes	-	-	Pinto, 1928
Eimeria labbeana-like	*C. livia domestica*	22.0-18.9 x 15.7-18.9	1.38	yes	no	yes (1)	14.5-12.5 x 5.5-7.0	2.0-2.2	granular	yes	no	Present study
E. livialis	*C. domestica*	19.5–23.2 × 14.3–16.5	1.35–1.49	yes	No	no	9.5–11.7 × 6.2–8.1	-	scattered	yes	no	Alyousif et al., 2009
E. palumbi	*Zenaida galapagoensis*	22–27 × 19–24	1.05–1.21	yes	no	no	15–17 × 8–8.5	2.12–1.76	-	yes	no	McQuistion, 1991
E. sphenocercae	*Sphenocercus sphenurus*	17.5–25.0 × 12.5–15	-	no	yes	-	17.5–18.75 ×12.5–13.75	-	no	-	-	Ray, 1952
E. tropicalis	*C. intermedia*	19–24 × 18–23	-	yes	-	-	10 × 6	-	yes	yes	-	Ray, 1961
E. turturi	*Streptopelia turtur*	22.8–29.2 × 17.8–25.4	-	no	no	no	11.5–13 × 6–7.5	-	dispersed	no	-	Golemansky, 1976
E. waiganiensis	*Chalocphaps indica, Otidiphaps nobilis*	22–25 × 19–23	1.08–1.2	no	yes	yes (2-4)	9–10.5 × 6–7.5	-	yes	yes	yes	Varghese, 1978
E. zenaidae	*Zenaida auriculata*	22.1–26.4 × 19.2–22.1	1.2	no	no	yes	12.0–14.4 × 7.2–7.7	1.8	scattered	yes	no	Adriano et al., 2003

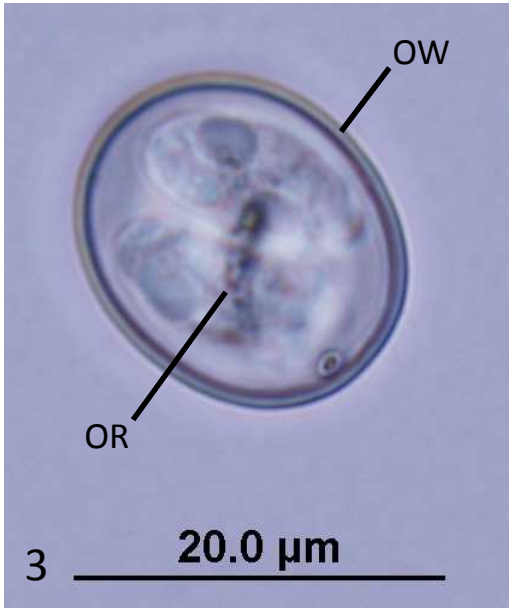




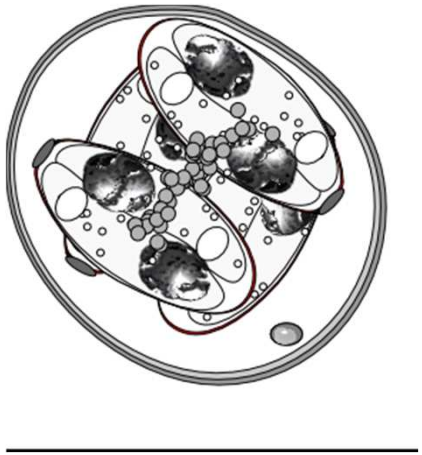








1a



1b

Highlights

- A new *Eimeria* isolate identified in domestic pigeon.
- Morphology study: ***Eimeria labbeana***-like.
- Genetic study: 98% similar to *Isospora* spp. from domestic pigeon at 18S locus.