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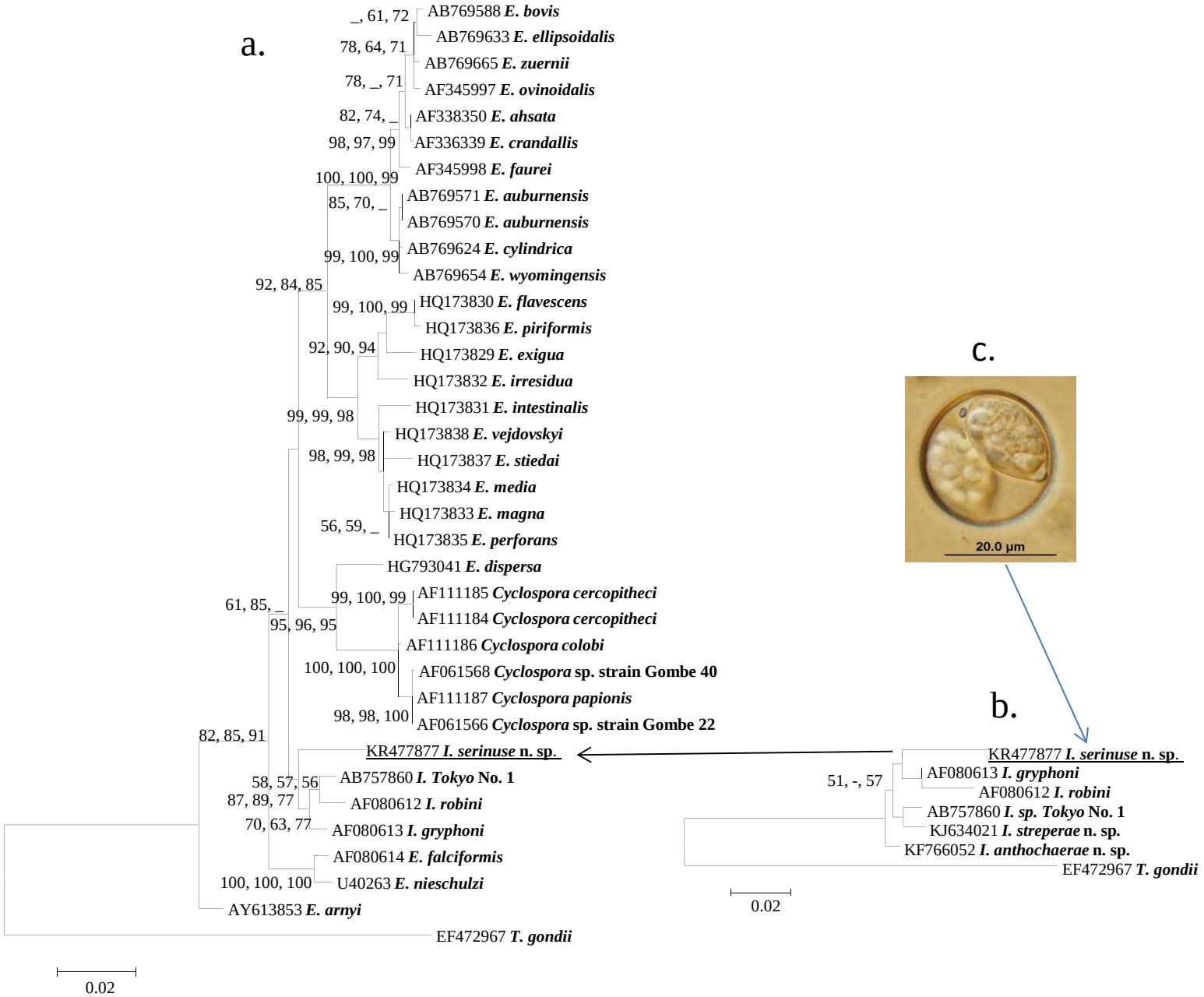

Evolutionary relationships of *Isospora serinuse* n. sp. inferred by distance analysis of 18S rRNA sequences.

Isospora serinuse n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria forma domestica*) (Passeriformes: Fringillidae) in Western Australia

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Abstract

A new species, *Isospora serinuse* n. sp., (Apicomplexa:Eimeriidae) is described from a single domestic canary (*Serinus canaria forma domestica*) (subspecies *S. c. domestica*) in Western Australia. Sporulated oocysts of *Isospora serinuse* n. sp. are spherical or subspherical, 25.5 (24.4-27.0) × 23.5 (22.0-24.8) µm, with a shape index (length/width) of 1.09; and a smooth bilayered oocyst wall, 1.2 µm thick (outer layer 0.9 µm, inner 0.3 µm). A polar granule is present, but a micropyle and oocyst residuum are absent. The sporocysts are lemon-shaped, 18.9 (17.8 – 20.2) × 11.8 (10.6 -13.0) µm, with a shape index of 1.6. Stieda and substieda bodies are present, the Stieda body being a small crescent shape and the substieda being indistinct. Each sporocyst with four vermiform sporozoites arranged head to tail. A sporocyst residuum is present and composed of numerous granules of different sizes that are scattered among the sporozoites. Morphologically, the oocysts of *Isospora serinuse* n. sp. were different from those of all known valid *Isospora* spp. Molecular analysis was conducted at 3 loci: the 18S and 28S ribosomal RNA and two separate regions of subunit I of the mitochondrial cytochrome oxidase (COI) gene (designated COIa and COIb). At the 18S locus, *Isospora serinuse* n. sp. exhibited 97.5% similarity to *Isospora* sp. Tokyo from a domestic pigeon (*Columba livia domestica*) in Japan. At the 28S locus, *I. serinuse* n. sp. exhibited 94.9% similarity to *I. anthochaerae* n. sp. from a red wattlebird (*Anthochaera carunculata*) in Australia. At the COIa locus, *I. serinuse* n. sp. exhibited 95.7% similarity to *I. sospora* sp. ex *Apodemus flavicollis* from a yellow-necked mouse and *I. gryphoni* from an American goldfinch (*Carduelis tristis*) respectively. At the COIb locus, *I. serinuse* n. sp. exhibited 96.7% similarity to an *Isospora* (iSAT4) from a European pied flycatcher (*Ficedula hypoleuca*). Based on morphological and molecular data, this isolate is a new species of *Isospora*, which is named *Isospora serinuse* n. sp. after its host, the domestic canary (*Serinus canaria* forma *domestica*).

Keywords: *Isospora*; domestic canary; morphology; phylogeny; 18S rRNA; 28S rRNA; COIa; COIb.

1. Introduction

Canary is the common name for the small passerine bird *Serinus canaria* in the finch family, Fringillidae (Clement et al. 1993). The domestic canary is a domesticated form of the wild canary. Little is known about the origin of canaries as cage birds but it is thought that captive canaries were first collected from the Canary Islands (Wetmore, 1923; Snow and Perrins 1998). Domestic canaries are generally divided into three main groups: Colorbred canaries, Type canaries (including the Australian Plainhead canary) and the Song canaries. The Australian Plainhead canary has its roots in the old style Norwich canaries that originated in Norwich, England and became a popular show variety in Australia (Frank, 2000).

Isospora are the most common coccidian parasites infecting passerine birds (Duszynski et al., 1999). To date, numerous species of *Isospora* has been characterised from passerine birds worldwide (Schrenzel et al., 2005; Berto, et al., 2011; Schoener et al., 2013). In Australia, two species of *Isospora* from honeyeaters (Meliphagidae) have been described; *I. lesouefi* from the endangered regent honeyeater (*Xanthomyza phrygia*), which is endemic to south-eastern Australia (Morin-Adeline et al., 2011) and *I. anthochaerae* from a red wattlebird (*Anthochaera carunculata*) (Yang et al., 2014). Another *Isospora* species, *I. streperae* has been described from a grey currawong (*Strepera versicolour plumbea*) in Western Australia (Yang et al., 2015). Two *Isospora* species (*I. cf serini* and *I. canaria*) have been morphologically characterized in the canary (*Serinus canaries* Linnaeus) (Box, 1975; Speer and Duszynski, 1975; Berto et al., 2013). Recently, the complete mitochondrial genome sequence of *I. cf. serini* from a domestic canary (*Serinus canaria*) was published (Ogedengbe et al., 2015). To date, no species of *Isospora* has been characterized from the domestic canary in Australia. In the present study, we characterized a new species of *Isospora* from a domestic canary in Western Australia, both morphologically and molecularly, and propose the species name *Isospora serinuse* n. sp.

2. Materials and methods

2.1 Sample collection

An adult domestic canary was admitted to the Kanyana Wildlife Rehabilitation Centre (KWRC) in July 2014. The bird appeared to be healthy. It was assumed to be an escaped pet. Faecal samples were collected on the day of admittance to KWRC. Microscopy was performed on a wet mount and this was found to be positive for coccidian oocysts as well as avian gastric yeast (AGY). A portion of collected faeces was also stored at 4°C until DNA extraction was performed. The canary was treated with Toltrazuril (50 mg/ml) at a dose rate of 15 mg/kg, in a single daily dose for a period of 3 days. Amphotericin B (a quarter of a 10mg tablet, crushed) was added to the birds' drinking water for 20 days. The bird was successfully re-homed 3 weeks later after a clear faecal test. A further faecal sample was collected and tested 6 months later and was found to be negative for both coccidia and AGY.

2.2 Morphological analysis

The presence of oocysts was determined by direct microscopic examination of a faecal suspension in saline. Unsporulated coccidian oocysts were allowed to sporulate in a petri dish containing a thin layer of 2% (w/v) potassium dichromate solution ($K_2Cr_2O_7$). The petri dish was kept at room temperature (20-22°C) and in the dark. Sporulated oocysts were observed using the 100 × oil immersion objective of an Olympus CH-2 binocular microscope, in combination with an ocular micrometre.

2.3 DNA isolation

Total DNA was extracted from 200 mg of each faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California) with some modifications. Briefly, samples were subjected to four

cycles of freeze/thaw in liquid nitrogen and boiling water to ensure efficient lysis of oocysts before being processed using the manufacturer's protocol. A negative control (no faecal sample) was included.

2.4 PCR amplification of 18S and 28S ribosomal sequences and the COI gene

Generic apicomplexan primers (CRYPTOF 5'-AAC CTG GTT GAT CCT GCC AGT and CRYPTOR 5'-GCT TGA TCC TTC TGC AGG TTC ACC TAC) were used to amplify the almost full length 18S rRNA gene as described by Eberhard et al., (1999). The expected PCR product was ~1,702 bp. The PCR reaction contained 2.5 µL of 10 × Kapa PCR buffer, 3 µl of 25 mM $MgCl_2$, 1.5 µL of 10nM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 1 µL of DNA (~50 ng) and 14.9 µL of H_2O. PCR cycling conditions were 1 cycle of 94ºC for 3 min, followed by 45 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: 5'-TAC CCG CTG AAC TTA AGC and 28SExR: 5'- CMA CCA AGA TCT GCA CTA G as previously described (Schrenzel et al., 2005), which produced a PCR product size of ~1,362 bp. The internal primers (28InF: 5' – ACT ATG TTC CCT AGT AAC G and 28SInR 5'-AAC GCT TCG CCA CGA TCC) were designed for the present study using Primer 3 (http://frodo.wi.mit.edu/) and produced an amplicon size of 1,420 bp. The PCR reaction contained 2.5 µL of 10 × Kapa PCR buffer, 2 µL of 25mM $MgCl_2$, 1 µL of 10mM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 1 µL of DNA (~50 ng) 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.

Two separate regions of the subunit I of the mitochondrial cytochrome oxidase (COI) gene (designated COIa and COIb) were amplified (Table 1). For the COIa region, PCR reactions contained 2.5 µL of 10 × Kapa PCR buffer, 1 µL of 25mM $MgCl_2$, 1 µL of 10mM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 1 µL of DNA (for primary reaction) or 1 µL primary PCR product (for secondary reaction) 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, 55ºC for 30 sec and 72ºC for 1 min and a final extension of 72ºC for 5 min. For the COIb region, PCR reaction conditions were the same as for the COIa PCR. 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, 57ºC for 30 sec and 72ºC for 1 min and a final extension of 72ºC for 5 min.

2.5 Sequence analysis

The amplicons from the second round PCRs were gel purified using an in house filter tip method as previously described (Yang et al., 2013). All the PCR products were sequenced using forward and reverse primers in duplicate, using amplicons from different PCR runs. Any mismatches were confirmed by re-amplifying with *pfu* (Promega, Sydney, Australia) and repeating the sequencing. An ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) was used for Sanger sequencing according to the manufacturer's instructions (with the exception that the annealing temperature was at 58ºC).

The results of the sequencing reactions were analysed and edited using Finch *TV*® v1.4.0. (http://www.geospiza.com/Products/finchtv.shtml). Sequences were compared to existing *Isospora* and other coccidian parasite sequences available on GenBank using BLAST searches and aligned with reference sequences with BioEditor (http://bioeditor.sdsc.edu/download.shtml).

2.5 Phylogenetic analysis

Phylogenetic trees were constructed for *Isospora* spp. at the 18S, 28S, COIa and COIb 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 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.

2.6 Line drawing

Oocyst line drawings were conducted using Inkscape (http://www.inkscape.org/en/).

3. Results

3.1 Description

3.1.1 *Isospora serinuse* n. sp. (Alveolata Cavalier-Smith, 1991, Apicomplexa Levine, 1970, Eimeriidae Minchin, 1903).

Diagnosis:
Sporulated oocysts of *Isospora serinuse* n. sp. are spherical or subspherical, 25.5 (24.4-27.0) × 23.5 (22.0-24.8) µm, with a shape index (length/width) of 1.09; and a smooth and bilayered oocyst wall, 1.2 µm thick (outer layer 0.9 µm, inner 0.3 µm). A polar granule is present, but a micropyle and oocyst residuum are absent. The sporocysts are lemon-shaped, 18.9 (17.8 – 20.2) × 11.8 (10.6 -13.0) µm, with a shape index of 1.6. Stieda and substieda bodies are present, the Stieda body being small and crescent-shaped and the substieda being indistinct. Each sporocyst with four vermiform sporozoites arranged head to tail. A sporocyst residuum is present and composed of numerous granules of different sizes scattered among the sporozoites. (Fig. 1a and 1b and Table 2).
Type hosts: domestic canary (*Serinus canaria* forma *domestica*)

Type locality: Perth (31.9522°S, 115.8589°E), Western Australia.

Prevalence: Unknown

Other hosts: Unknown.

Prepatent period: Unknown.

Patent period: Unknown.

Site of infection: Unknown

Sporulation time: 48 hours.

Material deposited: DNA sequences have been deposited in GenBank under the accession numbers KR477877 and KR477878, for the 18S and 28S respectively, KR477879 for COIa and COIb sequences.

Etymology: This species is named *Isospora serinuse* n. sp. after its host (*Serinus canaria*) (domestic canary).

3.2 Phylogenetic analysis of I. serinuse n. sp. *at the 18S locus*

A 1,702 bp sequence of 18S rRNA from *of I. serinuse* n. sp was aligned with two other *Isospora* spp. sequences from passerine birds; *I. gryphoni* (AF080613) (Olson et al., 1998) and *I. robini* (AF080612) (Carreno and Barta, 1999), one *Isospora* spp. sequence from a domestic pigeon (*Isospora* sp. Tokyo - AB757860), three mammalian *Cyclospora* spp.; *Cyclospora cercopitheci* (AF111184, AF111185), *Cyclospora colobi* (AF061566, AF061568) and *Cyclospora papionis* (AF111187), as well as 25 Eimerian 18S rRNA sequences from GenBank. *Toxoplasma gondii* was used as the outgroup.

Phylogenetic analysis using distance, parsimony and ML revealed that *I. serinuse* n. sp. exhibited 97.5%, 97.4% and 97.0%, similarity with *Isospora* sp. *Tokyo* from a domestic pigeon (*Columba livia domestica*), *I. gryphoni* from an American goldfinch (*Carduelis tristis*) and *I. robini* from the American robin (*Turdus migratorius*) respectively (Fig. 2a). *Isospora anthochaerae* and *I. streperae* were not included in the 18S tree because only shorter sequences (300 bp) were available. However, a separate phylogenetic analysis that included these two species revealed that the genetic similarity between *I. serinuse* n. sp. and *I. anthochaerae* and *I. streperae* were 98.3% and 91.7%, respectively (Fig. 2b).

3.4 Phylogenetic analysis of I. serinuse n. sp. *at the 28S locus*

A 1,362 bp amplicon from *I. serinuse* n. sp. was obtained at the 28S rRNA locus. Phylogenetic analysis included thirty-one *Isospora* sequences from passerine birds from a single report by Schrenzel et al., (2005) and *I. anthochaerae* (Yang et al., 2014). A 28S sequence from *I. streperae* was also available, but was only 904 bp in length and therefore was not included in the phylogenetic analysis. Analysis revealed that *I. serinuse* n. sp. grouped with *I. anthochaerae* (Fig. 3) with a similarity of 95.0%. Although not included in the tree, the genetic similarity between *I. serinuse* n. sp. and *I. streperae* was 93.7%

3.5 Phylogenetic analysis of I. serinuse n. sp. *at the COIa locus*

A 461 bp amplicon at the COIa locus from *I. serinuse* n. sp. was obtained. Phylogenetic analysis included five sequences from various *Isospora* sp. available in GenBank and forty-one *Eimeria* COI gene sequences. *Toxoplasma gondii* was used as the outgroup. *Isospora serinuse* n. sp. grouped with *Isospora* sp. ex *Apodemus flavicollis* isolate B13 (JQ993711) from a yellow-necked mouse in the Czech Republic with 95.7% similarity. *Isospora serinuse* n. sp. also exhibited 95.9% similarity with *Eimeria tiliquae* (JX839284) from a shingleback skink (*Tiliqua rugosa rugosa*) in Western Australia, 95.7% similarity with *I. gryphoni* (KC346355) from an American goldfinch (*Carduelis tristis*) in Canada and 95.5 % similarity with *I. cf. serini* Ogedengbe et al., 2015 from a domestic canary (Fig. 4). A sequence for *I. anthochaerae* was not available for this locus.

3.5 Phylogenetic analysis of I. serinuse n. sp. *at the COIb locus*

A 215 bp amplicon at the COIb locus from *I. serinuse* n. sp. was also obtained. Phylogenetic analysis included COIb sequences from thirteen *Isospora* sp. and forty-one *Eimeria* species.

Toxoplasma gondii was used as the outgroup. *Isospora serinuse* n. sp. formed a separate clade and exhibited 96.7% and 96.6% similarity with *Isospora* sp. iSAT4 (FJ269360) and *Isospora* sp. iSAT1 (FJ269357) respectively, both of which were from a Eurasian blackcap (*Sylvia atricapilla*) in Germany. *Isospora serinuse* n. sp. also showed a high similarity (96.2%) with *I. hypoleucae* (FJ269363) from a European pied flycatcher (*Ficedula hypoleuca*) in Germany. *Isospora serinuse* n. sp. exhibited 95.7% similarity with *I. anthochaerae* and 91.6% with *I. streperae*, both from Western Australia and 95.3 % similarity with *I. cf. serini* (Ogedengbe et al., 2015) from a domestic canary at this locus (Fig. 5).

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4. Discussion

Sporulated oocysts of *I. serinuse* n. sp. are morphologically different from other *Isospora* species from birds and did not match any other existing documented *Isospora* species from Passeriformes (http://biology.unm.edu/biology/coccidia/passer.html (Accessed on 8 Apr. 2015). For example, oocysts of *I. serinuse* n. sp. are spherical to subspherical and measured 25.5 (24.4-27.0) × 23.5 (22.0-24.8) µm in size with a L/W ratio = 1.09. Oocysts contained a polar granule and an oocyst residuum. Oocysts of *I. canaria* are also spherical and measured 24.4 (21-27. 9) × 22.2 (19.0-25.0) µm, with L/W ratio = 1.1, but the oocyst residuum was absent. Oocysts of *I. serini* are smaller (20.1 × 19.2 µm) and the oocyst residuum was also absent. Oocysts of *I. gryphoni* are larger than those from *I. serinuse* n. sp. They measure 30.7 (28.0-34.0) × 29.2 (25.0 -33.0) µm with a L/W ratio of 1.05 and contain 2-4 rice-grain-shaped polar bodies (Olson et al., 1998) (Table 2). All five species have two ovoid shaped sporocysts but the sporocysts of *I. serinuse* n. sp. measured 18.9 (17.8 - 20.2) × 11.8 (10.6 -13.0) µm, whereas sporocysts of *I. canaria* measured 17.6 (16.0-20.0) × 10.6 (10.0-12.0). Sporocysts of *I. serini* measured 15.2 × 9.4, *I. gryphoni* measured 22.2 (15.0-25.0) × 13.4 (12.0-14.5) µm and sporocysts of *I. anthochaerae* measured 14.5 (11.0-17.0) ×10.1 (9.0-11.0) µm (Table 2). Each sporocyst of *I. serinuse* n. sp. had four vermiform sporozoites arranged head to tail which is a distinguishing feature of *Isospora* (Barta et al., 2005).

Traditionally, characterization of avian species of *Isospora* has mainly been based on morphological features and host specificity, however this is problematic due to morphological ambiguities and unknown host specificity (Grulet et al., 1982; Levine, 1982). Molecular data are therefore essential to accurately delimit species. In the present study, molecular characterization of *I. serinuse* n. sp. was conducted at three loci (18S, 28S rRNA and COI). Due to the limited availability of sequences for avian *Isospora* species at these loci, the phylogenetic trees were generated with different data sets. The most common locus used for molecular characterization of coccidian parasites is the 18S rRNA gene as evidenced by the large number of coccidian18S RNA sequences in GenBank, followed by the COI gene, which is highly conserved (Barta, 2001). The COI gene has been shown to have a higher resolving power than the 18S rRNA gene in delineating recent speciation events (Ogedengbe et al., 2011). Two sets of nested PCR primers were used in the present study targeting two different regions of the gene (COIa and COIb), at the COI locus (Table 1).

Phylogenetic analysis of 18S rRNA sequences revealed that *I. serinuse* n. sp. exhibited the highest similarity (98.3%) with *I. anthochaerae* from an Australian red wattlebird. At the 28S rRNA locus, *I. serinuse* n. sp. was also most closely related (95.0% similarity) to *I. anthochaerae.* At the COIa locus, a sequence for *I. anthochaerae* was not available and *I. serinuse* n. sp. exhibited the highest similarity (95.9%) with *E. tiliquae.* At the COIb locus, *I. serinuse* n. sp. exhibited 96.7% and 96.6% similarity with two *Isospora* sp. from a Eurasian blackcap and 95.7% similarity with *I. anthochaerae*. For the COIa tree (Fig. 4), *Isospora* sequences were randomly distributed along the tree among other eimerian sequences, whereas for the COIb tree (Fig. 5), all the *Isospora* sequences grouped separately from *Eimeria* sequences with the exception of *E. callospermophili*, which grouped with the *Isospora* sequences. This suggests that the COIb PCR primers may be more reliable for species delimitation of *Isospora* species in future studies.

In the present study, morphological and molecular data were used to describe *I. serinuse* n. sp. found in the faeces of a domestic canary in Western Australia. Future studies are needed to examine if this species has any pathogenic effects and more isolates need to be genetically characterized at multiple loci to better understand the epidemiology of *Isospora* sp. infecting the domestic canary.

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359

Fig. 1a. Nomarski interference-contrast photomicrographs of *Isospora serinuse* n. sp. showing two spheroidal to subspheroidal sporocysts. Scale bar = 20 µm. **Fig. 1b**. Composite line drawing of *Isospora serinuse* n. sp. sporulated oocyst. Scale bar = 10 µm.

Fig. 2.a. Evolutionary relationships of *Isospora serinuse* n. sp. inferred by distance analysis of 18S rRNA sequences. Percentage support (>50%) from 1000 pseudoreplicates from distance, ML and parsimony analysis, respectively, is indicated at the left of the support node ('_' = Not available). **b.** Phylogenetic relationships of *I. serinuse* n. sp., *I. gryphoni*, *I. robini*, *I. sp. Tokyo* No. 1, *I. streperae* n. sp and *I. anthochaerae n. sp.* (300 bp 18S rRNA sequence only).

Fig. 3. Evolutionary relationships of *Isospora serinuse* n. sp. inferred by distance analysis of 28S rRNA sequences. 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 *Isospora serinuse* n. sp. inferred by distance analysis of COIa sequences. Percentage support (>50%) from 1000 pseudoreplicates from distance, ML and parsimony analysis, respectively, is indicated at the left of the support node ('_' = Not available).

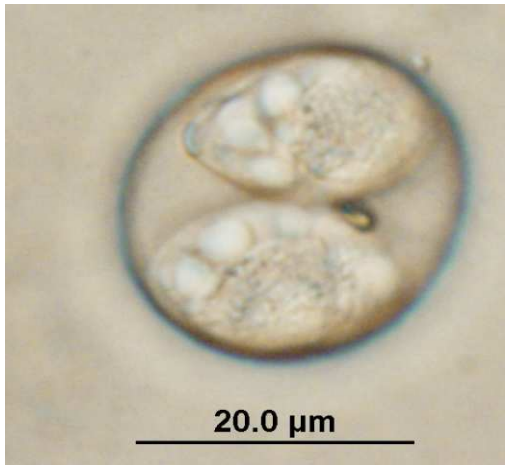
Fig. 5. Evolutionary relationships of *Isospora serinuse* n. sp. inferred by distance analysis of COIb sequences. Percentage support (>50%) from 1000 pseudoreplicates from distance, ML and parsimony analysis, respectively, is indicated at the left of the support node ('_' = Not available).

Table 1. Primers and PCR conditions used for amplification of two regions of the mitochondrial cytochrome oxidase (COI) gene.

Target region	Position on COI gene	Primers	Sequences	Size (bp)	Annealing	References
COIa	367-386	COIF1	5'-GGTTCAGGTGTTGGTTGGAC	805	55	Ogedengbe et al., 2011
	1153-1172	COIR1	5'-AATCCAATAACCGCACCAAG			
	401-418	COIF2	5'-TAAGTACATCCCTAATGTC	461	55	Yang et al., 2013a
	865-884	COIR2	5'-GTCATCATATGRTGTGCCCA			
COIb	854-875	COIbF1	5'-GWTCATTAGTATGGGCACATCA	302	57	Dolnik et al., 2009
	1135-1157	COIbR1	5-CCAAGAGATAATACRAARTGGAA			
	886-884	COIbF2	5-GGGCACATCATATGATGAC	257	57	
	1100-1121	COIbR2	5-ATAGTATGTATCATGTARWGCAA			

Table 2. Morphological comparison of *I. serinuse* n. sp. with other *Isospora* species.

Coccidia	Hosts	References	Oocysts						Sporocysts				
			Shape	Measurements (um)	Shape index	Wall (um)	Polar granule	oocyst residuum	Shape	Measurements	Stieda body	Substieda body	Residuum
I. anthochaerae	red wattlebird (*Anthochaera carunculata*)	Yang et al., 2014	subspherical	23.4 x 20.7 (20.0-26.0 x 19.0-22.0)	1.12	bi-layered c. 0.8	absent	absent	ovoid	14.5 x 10.1 (11.0-17.0 x 9.0-11.0)	hemi-dome	rectangular-shaped	compact
I. canaria	canary (*Serinus canaria Linnaeus*)	Box 1975; Berto et al., 2013	subspherical to ellipsoidal	24.6 x 21.8 (17-30 x 17-30)	1.13	tri-layered c. 1.2	present	absent	lemon	18.1 x 11.5 (17.0-22.0 x 1.00-13.0)	nipple-like	2.0 x3.0	compact
I. gryphoni	American goldfinch (*Carduelis tristis L.*)	Olson et al., 1998	spherical	29.2 x 30.7 (25.0-33.0 x 28.0-34.0)	1.05	bi-layered c. 0.8	present	absent	ovoid	22.2 x 13.4 (15-25.0 x 12.0-14.5)	small	indistinct	prominent
I. serini	canary (*Serinus canaria* Linnaeus)	Box 1975; Speer and Duszynski, 1975	spherical to subspherical	20.1 x 19.2 (13.0-23.0 x13.0-23.0)	1.05	tri-layered c. 1.2	absent	absent	ellipsoidal	15.2 x 9.4 (13.0-16.0 x 8.0-11.0)	2.0x0.6	5.0 x3.0	scattered granules
I. serinuse n. sp.	canary (*Serinus canaria* forma *domestica*)	This study	spherical to subspherical	25.5 x 23.5 (24.4-27.0 x 22.0-24.8)	1.09	bi-layered c. 1.2	present	absent	lemon	18.9 x 11.8 (17.8-20.2 x 10.6-13.0)	small	indistinct	compact
I. streperae	Grey currawong (*Strepera versicolor)*	Yang et al., 2015	spherical	23.8 x 22.5 (22-24.5 x 21.8 x 24.5)	1.06	bi-layered c. 1.0	absent	present	ovoid	14.4 x 11.2 (11.5-15.8 x (10.4-12.5)	hemi-dome	rectangular-shaped	compact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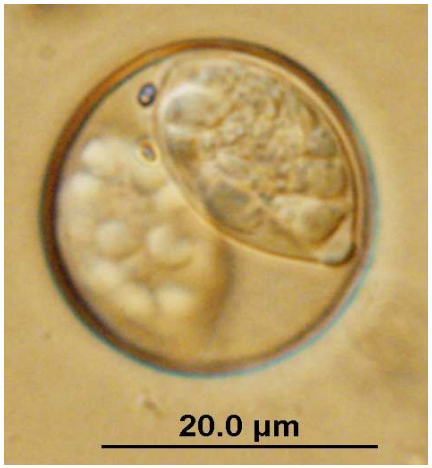
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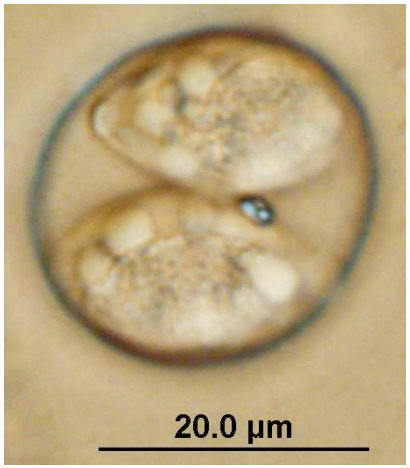
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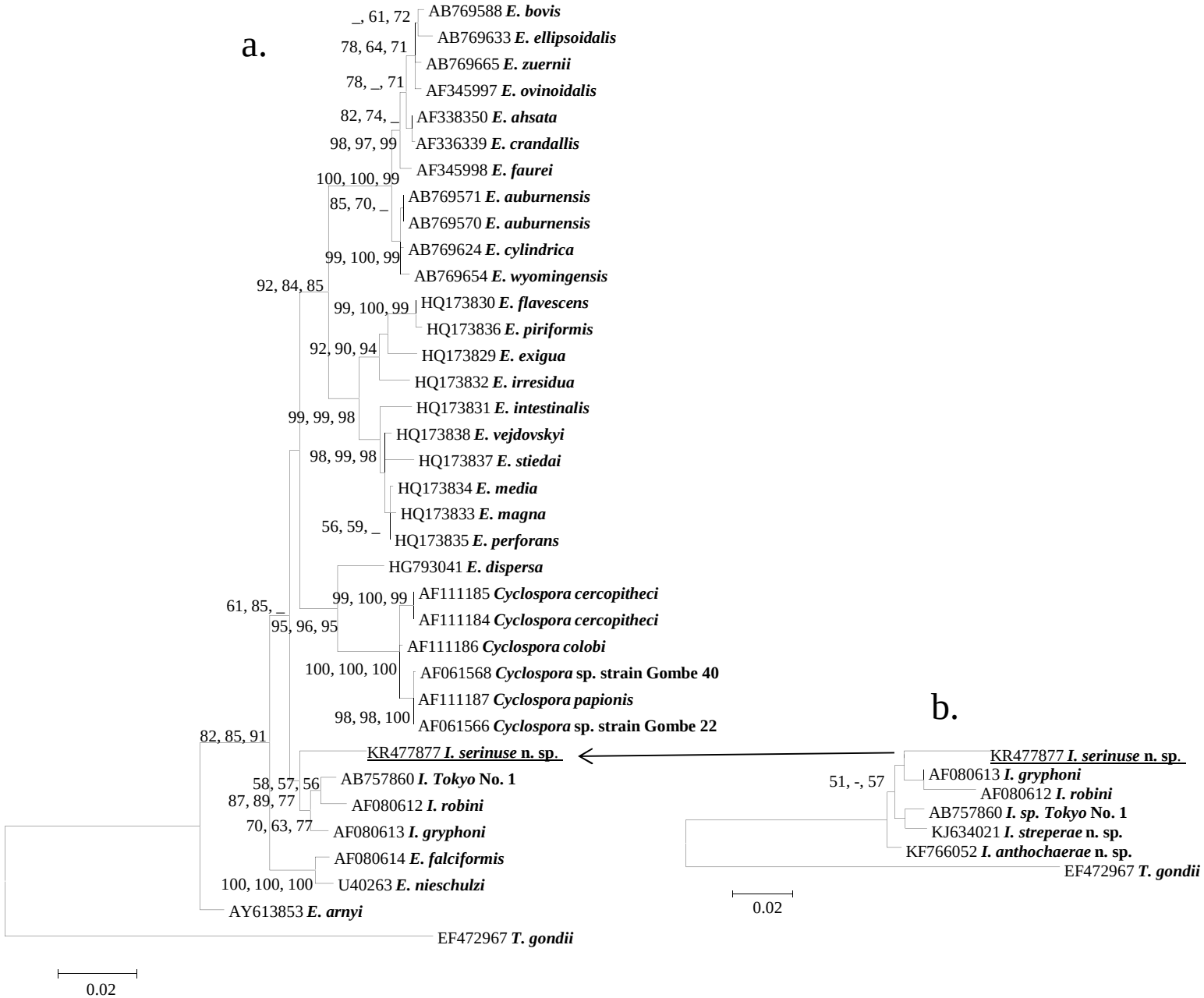
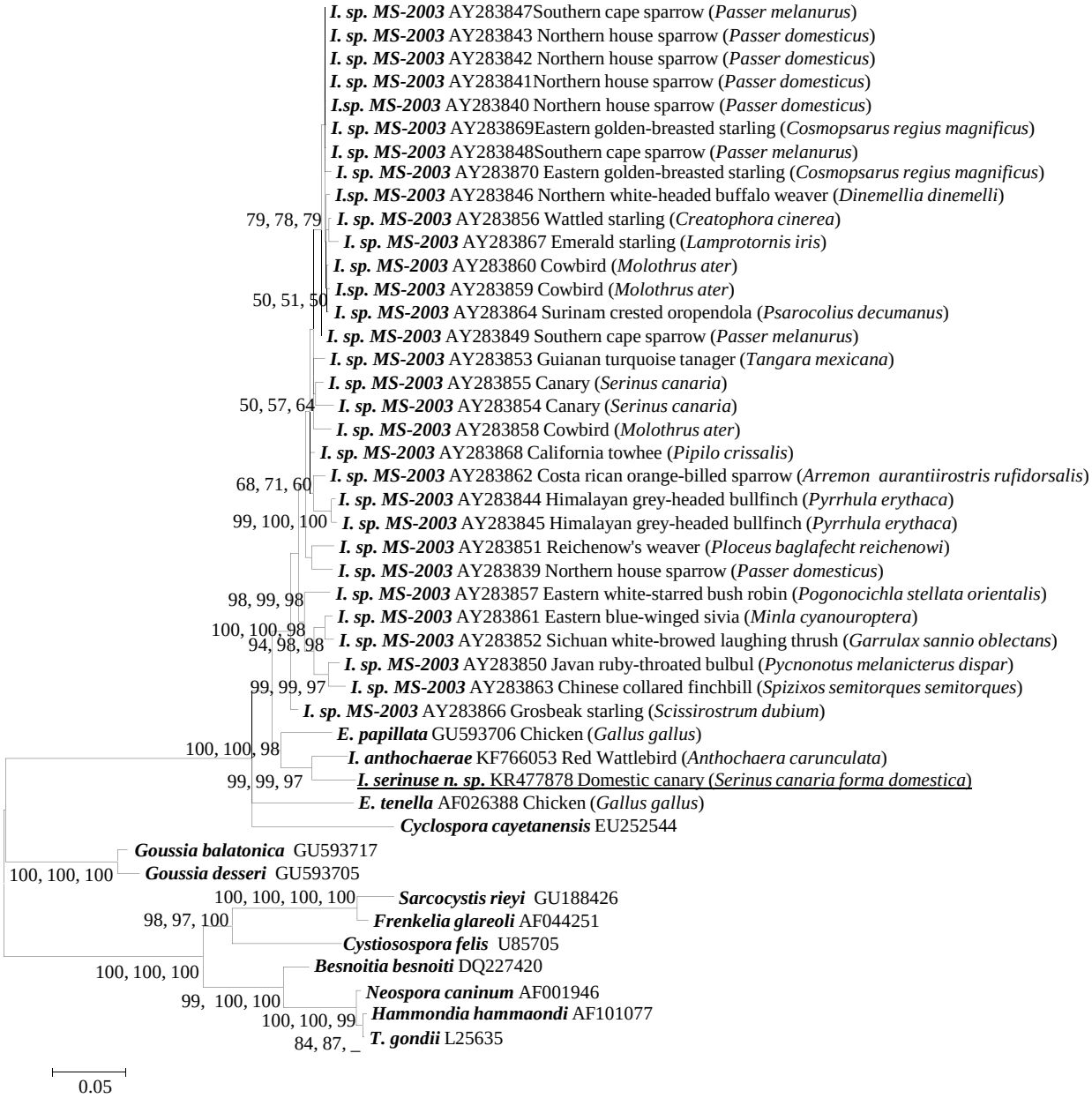
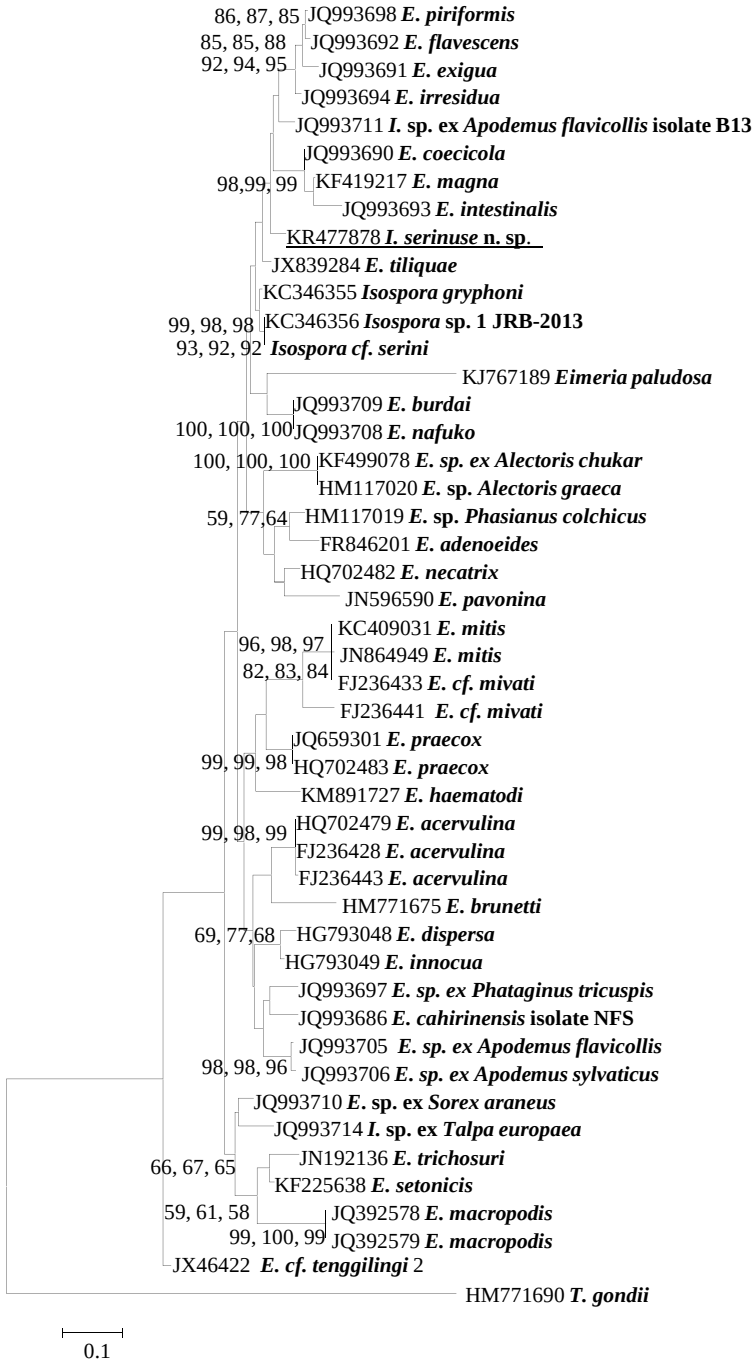
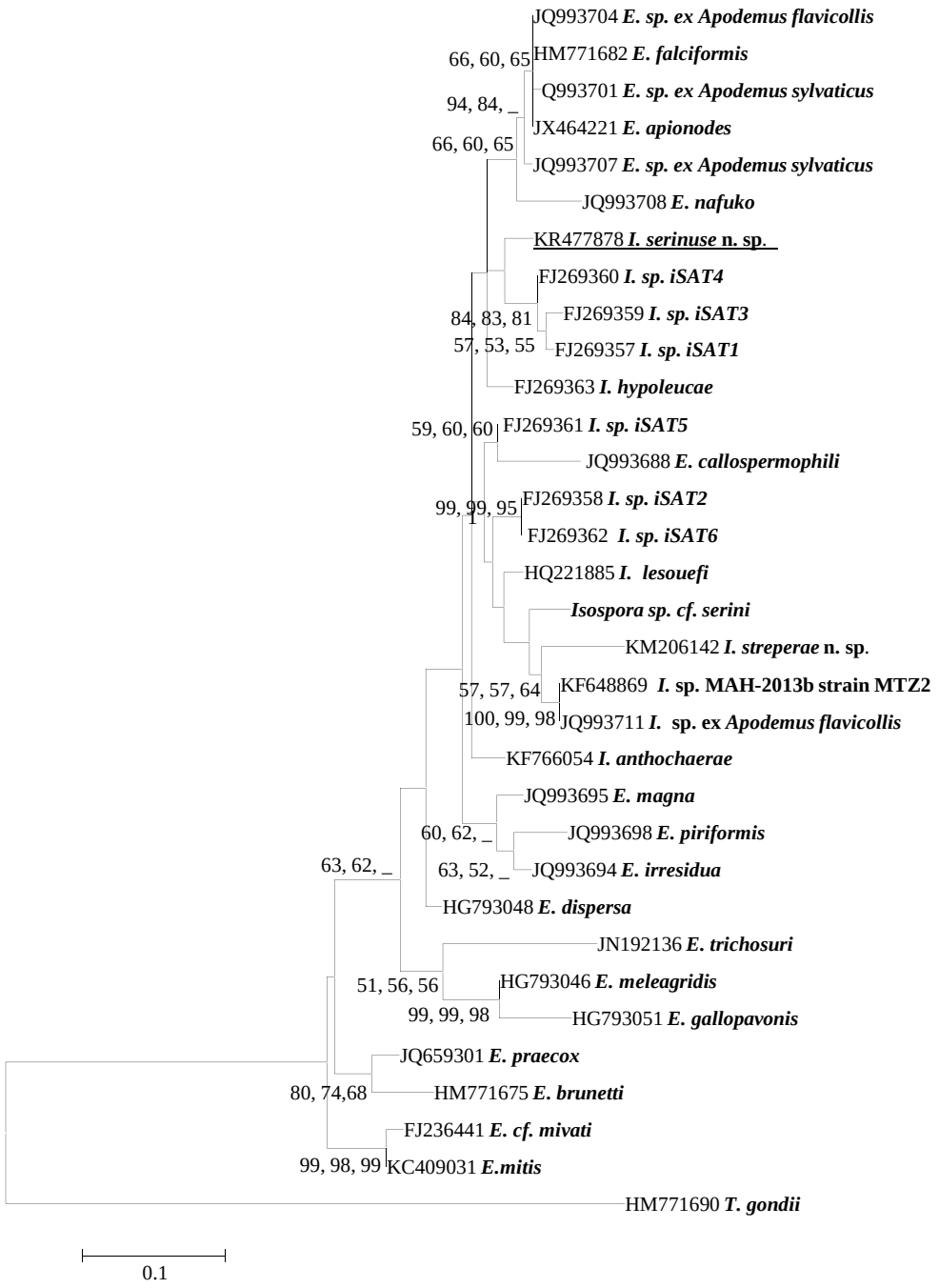

a.
_, 61, 72
AB769588 E. bovis
AB769633 E. ellipsoidalis
78, 64, 71
AB769665 E. zuernii
78, _, 71
AF345997 E. ovinoidalis
82, 74, _
AF338350 E. ahsata
98, 97, 99
AF336339 E. crandallis
AF345998 E. faurei
100, 100, 99
AB769571 E. auburnensis
85, 70, _
AB769570 E. auburnensis
AB769624 E. cylindrica
99, 100, 99
AB769654 E. wyomingensis
92, 84, 85
HQ173830 E. flavescens
99, 100, 99
HQ173836 E. piriformis
92, 90, 94
HQ173829 E. exigua
HQ173832 E. irresidua
HQ173831 E. intestinalis
99, 99, 98
HQ173838 E. vejdovskyi
98, 99, 98
HQ173837 E. stiedai
HQ173834 E. media
HQ173833 E. magna
56, 59, _
HQ173835 E. perforans
HG793041 E. dispersa
99, 100, 99
AF111185 Cyclospora cercopitheci
61, 85, _
AF111184 Cyclospora cercopitheci
95, 96, 95
AF111186 Cyclospora colobi
100, 100, 100
AF061568 Cyclospora sp. strain Gombe 40
AF111187 Cyclospora papionis
98, 98, 100
AF061566 Cyclospora sp. strain Gombe 22
82, 85, 91
KR477877 I. serinuse n. sp.
58, 57, 56
AB757860 I. Tokyo No. 1
87, 89, 77
AF080612 I. robini
70, 63, 77
AF080613 I. gryphoni
AF080614 E. falciformis
100, 100, 100
U40263 E. nieschulzi
AY613853 E. arnyi
EF472967 T. gondii
0.02
b.
KR477877 I. serinuse n. sp.
51, -, 57
AF080613 I. gryphoni
AF080612 I. robini
AB757860 I. sp. Tokyo No. 1
KJ634021 I. streperae n. sp.
KF766052 I. anthochaerae n. sp.
EF472967 T. gondii
0.02







Highlights

- Description of a new *Isospora* species(*I. serinuse* n. sp.) in a domestic canary
- Morphology study distinct to other *Isospora* species from birds.
- Genetic study: 98.3% similar to *I. anthochaerae* (KF766052) at 18S locus.