Parasitology Research (2019) 118:2399–2408
https://doi.org/10.1007/s00436-019-06378-8

PROTOZOOLOGY - ORIGINAL PAPER

Isospora coronoideae n. sp. (Apicomplexa: Eimeriidae) from the Australian raven (*Corvus coronoides*) (Passeriformes: Corvidae) (Linnaeus, 1758) in Western Australia

Dandan Liu[1,2] · Belinda Brice[3] · Aileen Elliot[1] · Una Ryan[1] · Rongchang Yang[1]

Received: 5 March 2019 / Accepted: 10 June 2019 / Published online: 20 June 2019

Abstract

A new *Isospora* (Apicomplexa: Eimeriidae) species is described from an Australian raven (*Corvus coronoides*) in Western Australia. Sporulated oocysts ($n = 21$) are ovoid, 21.2 (18.4–23.9) μm in length and 18.8 (16.9–20.6) μm in width, with a shape index of 1.13. The bi-layered oocyst wall is smooth and colourless, 1.2 μm thick. A polar granule and oocyst residuum is present, but the micropyle is absent. The sporocysts are ovoid-shaped, 16.3 (13.7–18.9) × 10.7 (8.4–12.9) μm, with a shape index (length/width) of 1.52. Stieda and substieda bodies are present, the Stieda body being small and hemidome-shaped and the substieda being indistinct. Each sporocyst with four vermiform sporozoites arranged head to tail. The sporozoites are crescent-shaped, 9.0 (8.9–9.2) × 2.7 (2.3–3.0) μm, with a shape index (length/width) of 3.33. The sporocyst residuum is present. The isolated oocysts had different morphological characteristics when compared with all known *Isospora* spp. The coccidian parasite was analysed at the 18S and 28S ribosomal RNA and the mitochondrial cytochrome oxidase (COI) loci. At the 18S locus, *I. coronoideae* n. sp. exhibited 98.9% similarity to *I. neochmiae* from a captive-bred red-browed finch (KT224380) and *Isospora* sp. from domestic pigeons (*Columba livia domestica*) (AB757860), 98.5% similarity to *I. gryphoni* (AF080613) from an American goldfinch and 98.3% similarity to *I. manorinae* (KT224379) from a yellow-throated miner. At the 28S locus, it exhibited 95.4% and 94.8% similarity to *I. manorinae* (KT224381) and *I. anthochaerae* (KF766053), respectively. At the COI locus, it exhibited 99.8% and 99.7% similarity to *I. butcherae* (KY801687) and *I. neochmiae* (KT224378), respectively. Based on morphological and molecular data, this isolate is a new species of *Isospora*, which is named *Isospora coronoideae* n. sp. after its host, the Australian raven (*Corvus coronoides*) (Passeriformes: Corvidae) (Linnaeus, 1758).

Keywords *Isospora* · Australian raven · Morphology · Phylogeny · 18S rRNA · 28S rRNA · COI

Introduction

The Australian raven (*Corvus coronoides*) is a member of the order Passeriformes and belongs to the Corvidae family. Horsfield and Vigors described the Australian raven (*Corvus coronoides*) in 1827 (Vigors and Horsfield 1827), which is native to much of northeastern and southern Australia. The Australian raven has all black plumage and is a largish bird (48–52 cm) with white eyes and a longish, strong beak. It has prominent throat hackle feathers which fan out when the bird is calling and the throat expands. In Australia, they inhabit farmlands, dunes, beaches, mangroves, islands, urban areas, and alpine regions up to 1500 m (Pizzey et al. 2007). Ravens can live up to 21 years in the wild (Wasser and Sherman 2010) and are unusually intelligent. Part of their success as a species is due to their omnivorous diet. They are also extremely versatile and opportunistic in finding sources of nutrition, feeding on carrion, insects, cereal grains, berries, fruit, small animals and food waste. Australian ravens are often located in coastal regions because these areas provide easy access to water and a

Handling Editor: Julia Walochnik

✉ Rongchang Yang
R.Yang@murdoch.edu.au

[1] College of Science, Health, Engineering and Education, Murdoch University, Murdoch, Western Australia 6150, Australia

[2] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou 225009, Jiangsu, People's Republic of China

[3] Kanyana Wildlife Rehabilitation Centre, 120 Gilchrist Road, Lesmurdie, Western Australia 6076, Australia

variety of food sources as well as stable weather patterns without extreme cold or hot temperatures (Ewins et al. 1986).

To date, 12 *Isospora* species have been reported in the Corvidae family (Order: Passeriformes) worldwide (Upton et al. 1985; Poon and Chew 1991; Upton et al. 1995), with the exception of the Australian continent. In the present study, we describe a new species of *Isospora* from an Australian raven (*Corvus coronoides*) in Western Australia, both morphologically and molecularly, and propose the name *Isospora coronoideae* n. sp.

Materials and methods

Sample collection

A juvenile Australian raven was admitted to the Kanyana Wildlife Rehabilitation Centre (KWRC), Perth, in November 2018. The bird was in poor body condition and had a dirty vent. A faecal sample was collected on the day of admittance to KWRC and parasitological examination showed large numbers of unsporulated coccidian oocysts. Treatment was implemented and the bird was released back at the found location 2 weeks later. Faecal samples were also collected and screened from an additional 33 Australian ravens that were admitted to KWRC over the next 8 months.

Morphological analysis

The presence of coccidian oocysts was determined by direct microscopic examination of a faecal suspension in saline, as well as by faecal float. Faecal flotation was performed by suspending a portion of faeces in a saturated sodium chloride and 50% sucrose (*w*/*v*) solution. Faeces was also mixed with 2% (*w*/*v*) potassium dichromate solution ($K_2Cr_2O_7$), and the mixture was poured into a petri dish to a depth of less than 1 cm and kept in the dark at room temperature to aid sporulation. The morphological characteristics of sporulated oocysts were observed using an Olympus CH-2 binocular microscope (Olympus Corp., Tokyo, Japan).

DNA extraction

Two hundred milligrams of faecal sample was used to extract DNA with a Power Soil DNA Kit (MolBio, Carlsbad, CA) following the manufacturers' instruction with some modifications including subjecting the sample to five freeze/thaw cycles in liquid nitrogen and boiling water prior to DNA extraction, to lyse the oocysts. A negative control with no faecal sample was included.

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

Nested PCR for 18S, 28S rRNA and partial COI loci was conducted as described by Yang et al. (2016b).

Sequence analysis

PCR amplicons from the 18S, 28S rRNA and COI loci were purified using in-house filter tip method previously described (Yang et al. 2013). All PCR products were sequenced using the specific primers used to amplify the target loci respectively. PCR products were sequenced in triplicate by using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions.

The results of Sanger sequencing were analysed and edited by Finch TV® v1.4.0. (http://seq.mc.vanderbilt.edu/dna/html/SoftDetail.html). Sequences were compared with 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).

Phylogenetic analysis

Phylogenetic trees for *Isospora* spp. were constructed at the 18S, 28S and COI loci with additional sequences reported in GenBank. Parsimony analyses were

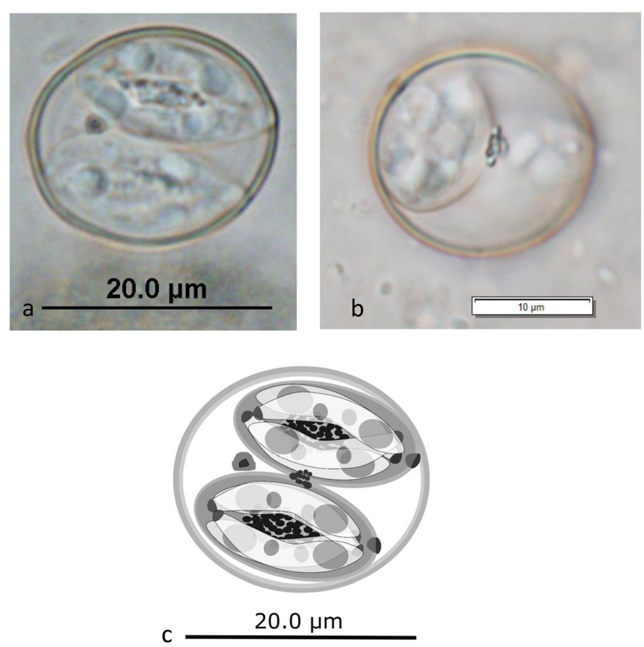

Fig. 1 **a** Nomarski interference-contrast photomicrographs of *Isospora coronoideae* n. sp. Scale bar = 20 µm. **b** Composite line drawing of *I. coronoideae* n. sp. sporulated oocyst. Scale bar = 20 µm

Table 1 Morphological comparison of *I. coronoideae* n. sp. with other *Isospora* species

Coccidia	Hosts	References	Oocysts				
			Shape	Measurements (µm)	Shape index	Wall (µm)	Polar granule
*I. anthochaerae**	Red wattlebird (*Anthochaera carunculata*)	Yang et al. 2014	Subspherical	23.4 × 20.7 (20.0–26.0 × 19.0–22.0)	1.12	Bi-layered, c. 0.8	Absent
*I. bengalensis***	*Corvus splendens*	Mandal and Chakravarty, 1964	Spherical	18.7–23.3 (range not given)	1.25	Bi-layered, c. 1.1	
*I. brachyrhynchi**	*Corvus brachyrhynchos*	Wobester and Cawthorn 1985	Subspherical	20.4 × 18.9 (15.0–25.0 × 14.0–23.0)	1.10	*c.*1.0	Present
*I. butcherae**	silvereye (*Zosterops lateralis*)	Yang et al. 2018	Spherical to subspherical	24.2 × 23.3 (23.1–25.2 × 22.8–23.9)	1.02	Bi-layered, c. 1.2	Present
*I. calocitta**	*Calocitta Formosa*	Upton et al. 1995	Subspherical	28.8 × 27.7 (26–31 × 25–29)	1.00	Bi-layered, c.2.0	Present, 1 to 3
I. coronoideae	Australian raven (*Corvus coronoideae*)	This study	Subspherical	21.2 × 18.8 (18.4–23.9 × 16.9–20.6)	1.13	Bi-layered, c. 1.2	Present
*I. corvi**	*Corvus splendens*	Poon and Chew 1991	Spherical to subspherical	18.5 × 17.1 (15.5–21.4 × 13.6–20.0)	1.08		
*I. cyanocoracis**	*Cyanocorax chrysops*	Upton et al. 1985	Subspherical	28.7 × 26.8 (25–30 × 24–29)	1.10	Bi-layered, c.2.0	Present, 1 or 2
*I. garruli****	*Garrulus glandarius*	Ray et al., 1952		25.2 × 21.2 (25.0–27.5× 20.0–25.0)			
*I. gryphoni**	American goldfinch (*Carduelis tristis* L.)	Olson et al. 1998	spherical	29.2 × 30.7 (25.0–33.0 × 28.0–34.0)	1.05	Bi-layered, c. 0.8	Present
*I. manorinae**	Yellow-throated miner (*Manorina flavigula obscura*)	Yang et al. 2016a	Spherical to subspherical	22.8 × 18.3 (20.3–23.8 × 17.7–18.7)	1.25	Bi-layered, c. 1.3	Present
*I. monedulae***	*Corvus monedula*	Yakimoff and Matschoulsky, 1936	Spherical to oval	Spherical, 16.0–20.0 (aver. 18.0); oval, 16.0–22.0 × 14.0–18.0	1.00 or 1.19		
*I. nankinovi****	*Garrulus glandarius*	Golemansky, 1976; Scholtyseck, 1956		32.5 × 25.5 (30.0–35.0 × 22.5–28.5)			
*I. neochmiae**	Red-browed finch (*Neochmia temporalis*)	Yang et al. 2016b	Spherical	18.3 × 18.2 (18.2–18.9 × 18.2–18.6)	1.01	Bi-layered, c. 1.2	Present
*I. nucifragae****	*Nucifraga caryocatactes*	Galli-Valerio, 1933		24.0 × 21.0			
*I. rochalimai****	*Pica pica*	Golemansky, 1976; Nemeseri,1949; Yakimoff, 1940; Yakimoff and Matschoulsky, 1936		23.9 × 19.9 (23.0–24.0 × 18.0–23.0)			
*I. rodhani***	*Corvus corone*	Yakimoff and Matschoulsky, 1938	Spherical to subspherical and oval	Round, 17.0–25.0 (aver. 22.9); subspherical, 19.0–27.0 × 21.0–25.0 (aver. 24.9 × 22.9)	1.00 or 1.09		
*I. schwetzi***	*Corvus corone*	Yakimoff and Matschoulsky, 1940	Round to oval	Round, 16.0–26.0 (aver. 23.0); oval, 15.0–28.0 × 13.0–25.0 (aver. 24.7 × 22.6)	1.00 or 1.09		

Table 1 (continued)

Coccidia	Oocysts	Sporocysts				
	Oocyst residuum	Shape	Measurements (μm)	Stieda body	Substieda body	Residuum
*I. anthochaerae**	Absent	Ovoid	14.5 × 10.1 (11.0–17.0 × 9.0–11.0)	Hemi-dome	Rectangular-shaped	Compact
*I. bengalensis***	Absent	Pyriform	14.4 × 16.4 (range not given)	Present		Present
*I. brachyrhynchi**		elongate	16.2 × 10.6 (14–20 × 8–13)	Present		Diffuse
*I. butcherae**	Absent	Ovoid	16.1 × 10.5(17.3–15.7 × 11.5–9.8)	Hemi-dome	Rectangular-shaped	Scattered granules
*I. calocitta**		Ovoid	20.1 × 12.6 (19–22 × 11–14)	Present	Prominent	Diffuse
I. coronoideae	Present	Ovoid	16.3 × 10.7 (13.7–18.9 × 8.4–12.9)	Knob-like	Round	Scattered granule
*I. corvi**	Present	Elongate ovoid	14.3 × 8.8 (11.8–16.8 × 6.8–10.2)	Present	Present	Present
*I. cyanocoracis**		Ovoid	19.3 × 11.4 (17–21 × 10–12)	Prominent	Homogeneous	Compact
*I. garruli****						
*I. gryphoni**	Absent	Ovoid	22.2 × 13.4 (15–25.0 × 12.0–14.5)	Small	Indistinct	Prominent
*I. manorinae**	Absent	Lemon	15.5 × 9.7 (14.6–15.7 × 9.5–9.7)	Hemi-dome	Rectangular-shaped	Compact
*I. monedulae***	Absent	Pyriform	12.0–16.0 × 6.0–8.0	Present		Present
*I. nankinovi****						
*I. neochmiae**	Absent	Ovoid	13.3 × 8.6 (9.5–16.4 × 6.8–10.0)	Indistinct	Absent	Compact
*I. nucifragae****						
*I. rochalimai****						
*I. rodhani***	Absent	Pyriform	10.5–14.7 × 8.4–10.5	Present		Present
*I. schwetzi***	Absent					Present

*Source of data from original author; **Source of data from Poon and Chew (1991); ***Source of data from Upton et al. (1995)

Table 2 Pairwise comparison of 18S rRNA between *I. coronoideae* n. sp. and other *Isospora* and *Eimeria* species

Name of species	Identity (%)	Name of species	Identity (%)	Name of species	Identity (%)
HQ173835 ***E. perforans***	98.06	JQ993646 ***E. cahirinensis***	98.23	KT224379 ***I. manorinae* n. sp**.	98.66
HQ173834 ***E. media***	97.98	AF307880 ***E. albigulae***	98.32	AB757862 ***I.* sp. Tokyo2**	99.00
HQ173833 ***E. magna***	97.89	AB757860 ***I.* sp. Tokyo**	98.83	KR477877 ***I. serinuse* n. sp.**	97.55
HQ173837 ***E. stiedai***	97.98	AF080612 ***I. robini***	98.40	KT224380 ***I. neochmiae***	98.91
HQ173838 ***E. vejdovskyi***	98.06	JX984668 ***I.* sp. 1 JM-2013 haplotype JFI1**	98.74	AY331569 ***I.* sp. MS-2003**	98.41
HQ173831 ***E. intestinalis***	97.81	KF648871 ***I.* sp. MAH-2013b**	98.40	KF648870 ***I.* sp. MAH-2013a**	98.83
HQ173836 ***E. piriformis***	98.06	AF080613 ***I. gryphoni***	98.57	KY801685 ***I. butcherae*. n. sp**.	97.80
HQ173830 ***E. flavescens***	97.98	AY331571 ***I.* sp. MS-2003**	98.83	L24381 ***Toxoplasma gondii***	89.87
AF291427 ***E. alabamensis***	98.15	U40263 ***E. nieschulzi***	97.63		
AB769635 ***E. subspherica***	98.06	AF080614 ***E. falciformis***	97.54		

conducted using MEGA version 7 (MEGA 7: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, AZ, USA). Maximum likelihood (ML) and neighbour-joining (NJ) analyses were conducted using the Tamura-Nei algorithm based on model selection using ModelTest in MEGA 7. Bootstrap analyses (1000 replicates) were performed to assess reliability of the inferred tree topologies.

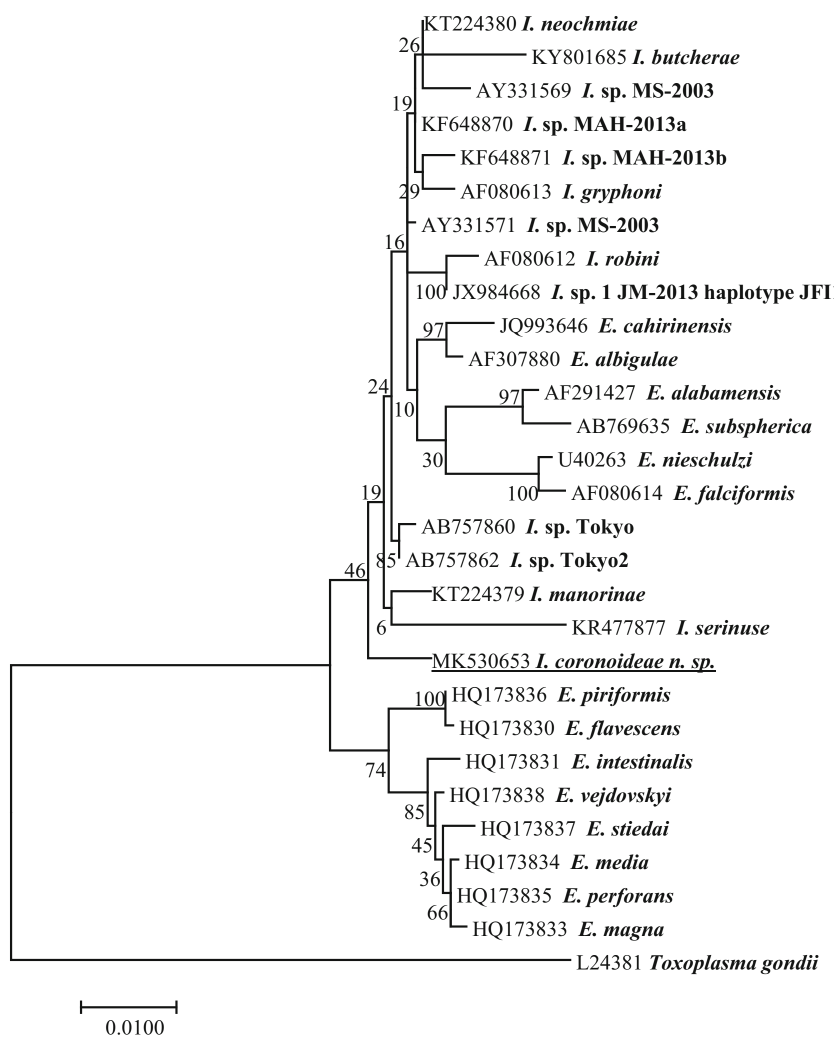

Fig. 2 Evolutionary relationships of *Isospora coronoideae* n. sp. inferred by distance analysis of 18S rRNA sequences. Percentage support (> 50%) from 1000 pseudoreplicates from ML analysis

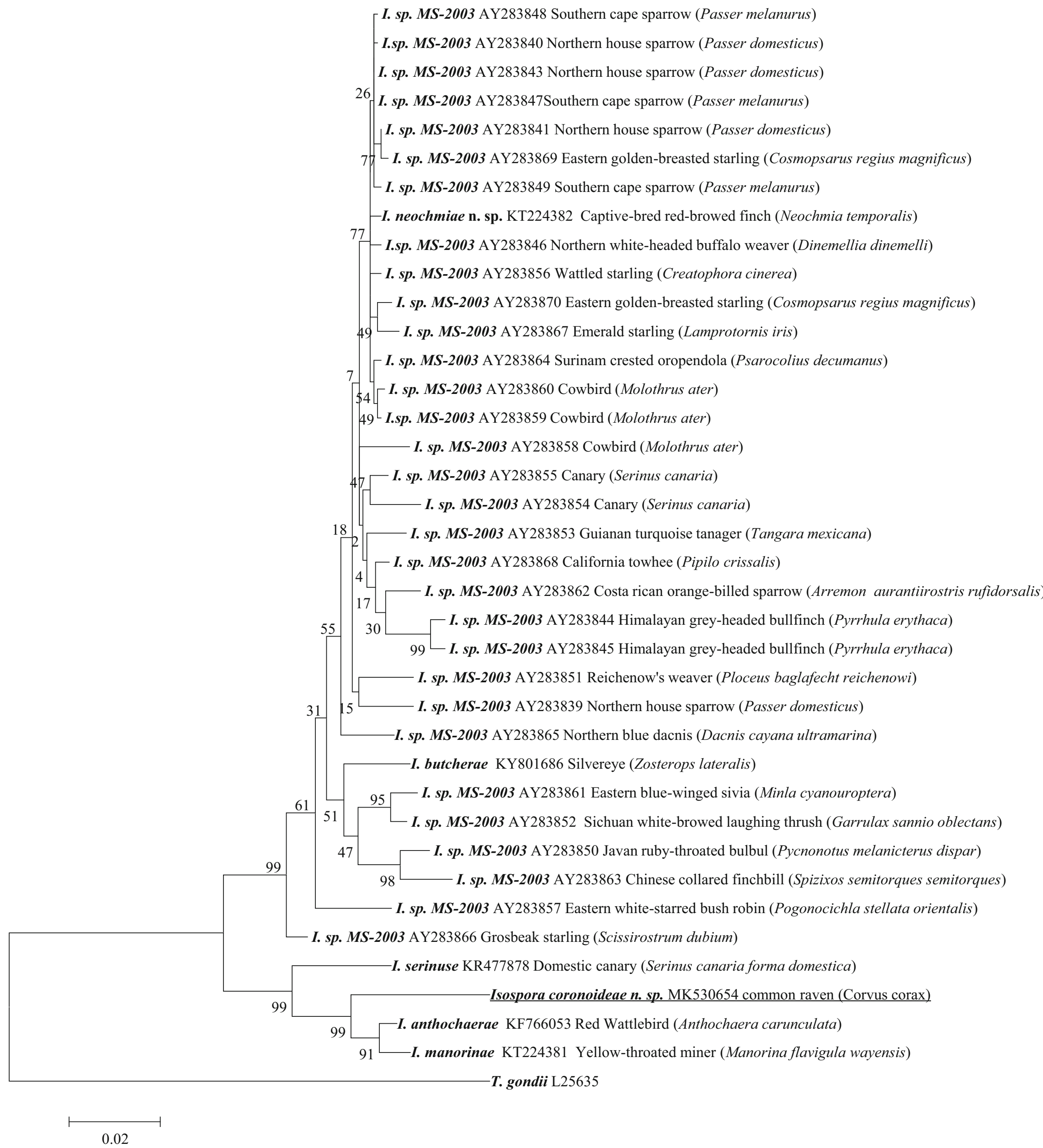

Fig. 3 Evolutionary relationships of *Isospora coronoideae* n. sp. inferred by distance analysis of 28S rRNA sequences. Percentage support (> 50%) from 1000 pseudoreplicates from ML analysis

Line drawing

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

Results

Fifteen out of 34 Australian ravens were shedding coccidian oocysts (single isosporan morphotype) when screened by wet mount and faecal float (44.1% prevalence).

Description

Sporulated oocysts of *I. coronoideae* n. sp. are ovoid, 21.2 (18.4–23.9) × 18.8 (16.9–20.6) μm, with a shape index (length/width) of 1.13, and a smooth and bi-layered oocyst wall, 1.2 μm thick (outer layer 0.9 μm, inner 0.3 μm). A polar granule and oocyst residuum is present, but a micropyle is absent. The sporocysts are ovoid-shaped, 16.3 (13.7–18.9) × 10.7 (8.4–12.9) μm, with a shape index (length/width) of 1.52. Stieda and substieda bodies are present, the Stieda body being small and knob-like and the substieda being round. Each sporocyst has four vermiform sporozoites arranged head to tail. A sporocyst residuum is present. The sporozoite is crescent-shaped, 9.0 (8.9–9.2) × 2.7 (2.3–3.0) μm, with a shape index (length/width) of 3.33 (Fig. 1a–c and Table 1).

Type hosts: Australian raven, *Corvus coronoides* (Aves: Passeriformes).

Type locality: Greenfield road bridge over Canning river-road reserve, Perth (31.953512 S, 115.857048 E), Western Australia.

Prevalence: 44.1% (15/34).

Other hosts: Unknown.

Prepatent period: Unknown.

Patent period: Unknown.

Site of infection: Unknown.

Sporulation time: 72–96 h at room temperature.

Material deposited: DNA sequences have been submitted to GenBank under the accession numbers MK530653, MK530654 and MK867778, for the 18S, 28S and COI loci respectively.

Etymology: This species is named *Isospora coronoideae* n. sp. after its host.

Phylogenetic analysis of *I. coronoideae* n. sp. at the 18S rRNA locus

A 1223-bp unique 18S rRNA sequence from *I. coronoideae* n. sp. ($n = 15$) was aligned with 13 other *Isospora* spp. sequences: *I. neochmiae* (KT224380) (Yang et al. 2016b), *I. butcherae* (KY801685) (Yang et al. 2018), *I.* sp. MS-2003 (AY331569), *I.* sp. MS-2003 (AY331571) (Schrenzel et al. 2005), *I.* sp. MAH-2013a (KF648870), *I.* sp. MAH-2013b (KF648871), *I. gryphoni* (AF080613) from an American goldfinch (Olson et al. 1998), *I. sp.* 1JM-2013 (JX984668) and *I. robini* (AF080612) (Carreno and Barta 1999), *I. manorinae* (KT224379) from a yellow-throated miner (Yang et al. 2016a), *I. serinuse* (KR477877) (Yang et al. 2015), two *Isospora* spp. sequences from domestic pigeons (*Isospora* sp. Tokyo - AB757860 and AB757862), as well as 14 *Eimeria* 18S rRNA sequences from GenBank. *Toxoplasma gondii* was used as outgroup.

Phylogenetic analysis using distance, parsimony and ML revealed that *I. coronoideae* n. sp. exhibited 98.9% similarity to *I. neochmiae* (KT224380) from a captive-bred red-browed finch and *Isospora* sp. (AB757860) from domestic pigeons (*Columba livia domestica*), 98.5% similarity to *I. gryphoni* (AF080613) and 98.3% similarity to *I. manorinae* (KT224379) (Table 2). As *I. coronoideae* n. sp. 18S sequence was the only species available from the avian Corvidae family, in the present phylogenetic analysis, it sat in an independent clade from the *Isospora* spp. from other passerine birds (Fig. 2).

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

A 1373-bp unique 28S rRNA sequence from *I. coronoideae* n. sp. ($n = 15$) was compared with 36 *Isospora* 28S sequences in GenBank (Fig. 3), 31 of which were *Isospora* sequences from North American passerine birds characterized by Schrenzel et al. (2005) and 5 *Isospora* sequences from Western Australian passerine birds: *I. anthochaerae* (KF766053) from a red wattlebird, *I. butcherae* (KY801686) from a silvereye, *I. manorinae* (KT224381) from a yellow-throated miner, *I. neochmiae* (KT224382) from a captive-bred red-browed finch and *I. serinuse* (KR477878) from a domestic canary (Yang et al. 2014; Yang et al. 2015; Yang et al. 2016a, b; Yang et al. 2018).

Table 3 Pairwise comparison of 28S rRNA between *I. coronoideae* n. sp. and other *Isospora* and *Eimeria* species

Name of species	Identity (%)	Name of species	Identity (%)	Name of species	Identity (%)
KF766053 ***I. anthochaerae***	96.10	AY283864 ***I. sp. MS-2003***	92.05	AY283851 ***I. sp. MS-2003***	92.23
KR477878 ***I. serinuse* n. sp.**	94.08	AY283846 ***I. sp. MS-2003***	92.06	AY283839 ***I. sp. MS-2003***	91.78
AY283843 ***I. sp. MS-2003***	92.06	AY283856 ***I. sp. MS-2003***	92.06	AY283866 ***I. sp. MS-2003***	92.83
AY283870 ***I. sp. MS-2003***	91.72	AY283867 ***I. sp. MS-2003***	91.70	AY283861 ***I. sp. MS-2003***	92.23
AY283841 ***I. sp. MS-2003***	92.06	AY283858 ***I. sp. MS-2003***	91.87	AY283852 ***I. sp. MS-2003***	92.15
AY283869 ***I. sp. MS-2003***	91.97	AY283855 ***I. sp. MS-2003***	91.69	AY283850 ***I. sp. MS-2003***	91.96
AY283848 ***I. sp. MS-2003***	91.98	AY283854 ***I. sp. MS-2003***	91.51	AY283863 ***I. sp. MS-2003***	91.69
AY283847 ***I. sp. MS-2003***	92.06	AY283853 ***I. sp. MS-2003***	91.41	KY801686 ***I. butcherae* n. sp.**	92.40
AY283840 ***I. sp. MS-2003***	91.97	AY283865 ***I. sp. MS-2003***	92.13	AY283857 ***I. sp. MS-2003***	91.63
AY283849 ***I. sp. MS-2003***	92.06	AY283868 ***I. sp. MS-2003***	92.23	KT224381 ***I. manorinae* n. sp.**	96.09
KT224382 ***I. neochmiae* n. sp.**	91.97	AY283862 ***I. sp. MS-2003***	92.04	L25635 ***T. gondii***	81.40
AY283860 ***I. sp. MS-2003***	92.05	AY283844 ***I. sp. MS-2003***	91.60		
AY283859 ***I. sp. MS-2003***	92.06	AY283845 ***I. sp. MS-2003***	91.69		

Analysis revealed that *I. coronoideae* n. sp. had 95.4% similarity with *I. manorinae* (KT224381) and 94.8% similarity with *I. anthochaerae* (KF766053) (Table 3). As with the 18S sequence, there was no other 28S sequence from the Corvidae family available. The 28S sequence from *I. coronoideae* n. sp. sat on a separate branch and formed a clade with *I. anthochaerae* (KF766053) and *I. manorinae* (KT224381) (Fig. 3).

Phylogenetic analysis of *I. coronoideae* n. sp. at the COI locus

A 725-bp unique fragment of the COI gene of *I. coronoideae* n. sp. ($n = 15$) was compared with 22 sequences from various *Isospora* spp. and an additional 18 *Eimeria* sequences available at this locus in GenBank. *Toxoplasma gondii* was used as outgroup (Fig. 4). *I. coronoideae* n. sp. showed 99.8%

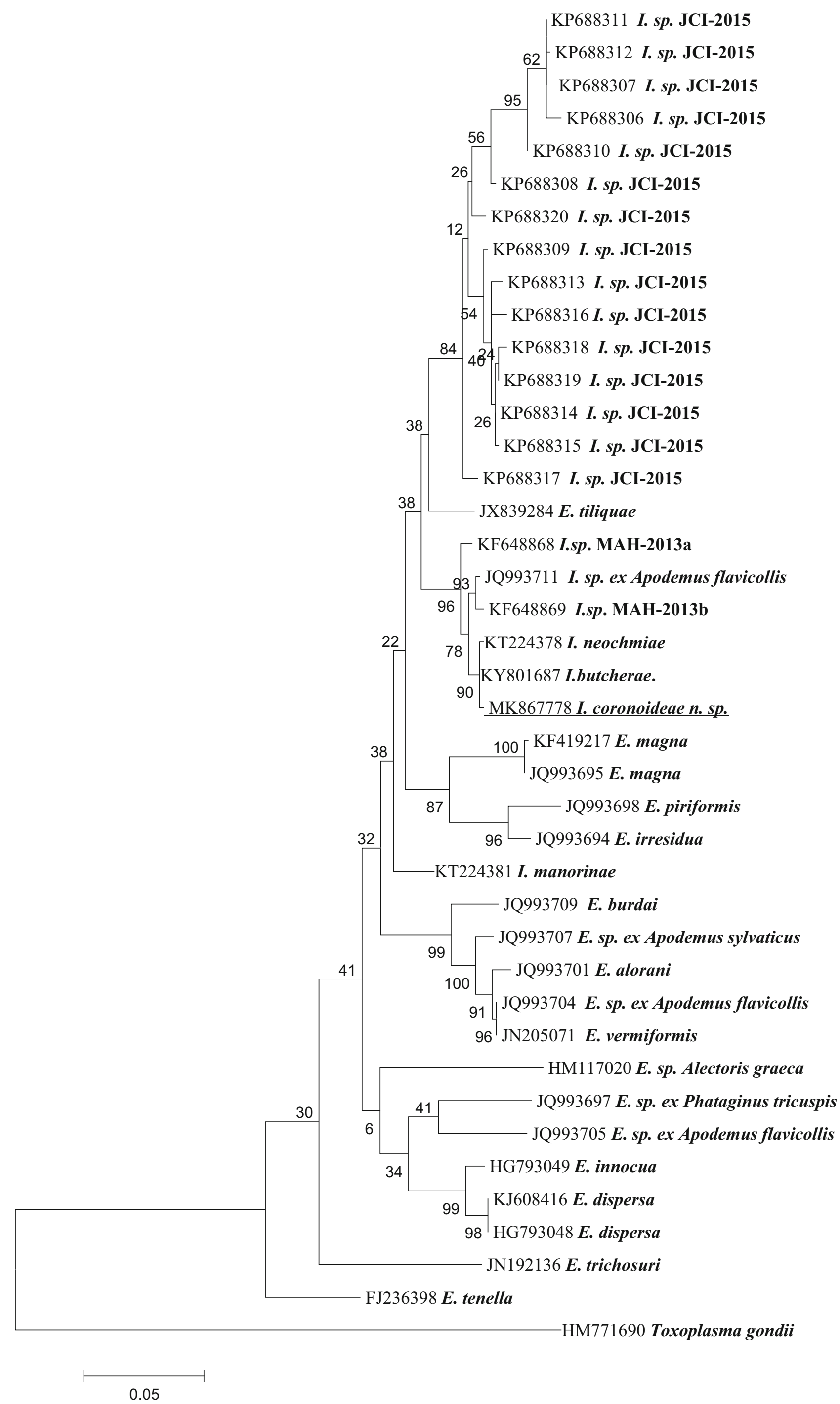

Fig. 4 Evolutionary relationships of *Isospora coronoideae* n. sp. inferred by distance analysis of COI sequences. Percentage support (> 50%) from 1000 pseudoreplicates from ML analysis

Table 4 Pairwise comparison of COI gene between *I. coronoideae* n. sp. and other *Isospora* and *Eimeria* species

Name of species	Identity (%)	Name of species	Identity (%)	Name of species	Identity (%)
KT224381 ***I. manorinae.* n. sp.**	95.51	JQ993694 ***E. irresidua***	93.99	KP688317 ***I. sp.* JCI-2015**	95.35
KF648868 ***I.sp.* MAH-2013a**	98.75	JQ993707 ***E. sp.***	91.88	KP688319 ***I. sp.* JCI-2015**	94.84
JQ993711 ***I. sp.***	98.91	JQ993709 ***E. burdai***	91.28	KP688314 ***I. sp.* JCI-201**	95.01
KF648869 ***I. sp.* MAH-2013b**	98.75	JQ993701 ***E. alorani***	91.15	KP688313 ***I. sp.* JCI-2015**	94.68
KF419217 ***E. magna***	93.43	KP688307 ***I. sp.* JCI-2015**	94.34	KP688309 ***I. sp.* JCI-2015**	95.01
JQ993697 ***E. sp. ex***	91.57	HM117020 ***E. sp.***	89.67	KP688308 ***I. sp.* JCI-2015**	94.69
JQ993698 ***E. piriformis***	93.11	KP688311 ***I. sp.* JCI-2015**	94.34	KP688315 ***I. sp.* JCI-2015**	94.84
JX839284 ***E. tiliquae* n. sp.**	95.15	KP688312 ***I. sp.* JCI-2015**	94.17	JN192136 ***E. trichosuri***	88.85
JQ993695 ***E. magna***	93.43	KP688310 ***I. sp.* JCI-2015**	94.84	FJ236398 ***E. tenella***	89.26
KJ608416 ***E. dispersa***	92.42	KP688306 ***I. sp.* JCI-2015**	94.34	KT224378 ***I. neochmiae* n. sp.**	99.69
HG793049 ***E. innocua***	92.41	KP688318 ***I. sp.* JCI-2015**	94.84	KY801687 ***I. butcherae.* n. sp.**	99.85
HG793048 ***E. dispers***	92.42	KP688316 ***I. sp.* JCI-2015**	94.83	HM771690 ***Toxoplasma gondii***	60.72
JQ993704 ***E. sp.***	92.03	JQ993705 ***E. sp.***	91.12		
JN205071 ***E. vermiformis***	92.03	KP688320 ***I. sp.* JCI-2015**	95.01		

similarity with *I. butcherae* (KY801687) from a silvereye and 99.7% similarity with *I. neochmiae* (KT224378) from a captive-bred red-browed finch (Table 4). Phylogenetic analysis revealed that *I. coronoideae* n. sp. grouped together with *I. butcherae* and *I. neochmiae* to form a sub-clade in the COI phylogenetic tree (Fig. 4).

Discussion

In the present study, coccidian oocysts were identified in the faeces of Australian ravens ($n = 15$). Morphological and molecular analysis showed that it was a novel species of *Isospora* which we have named *I. coronoideae* n. sp. The sporulated oocysts of *I. coronoideae* n. sp. are morphologically different from other *Isospora* species in the Corvidae family and are also morphologically distinct from all *Isospora* species identified from the other Passeriformes (http://biology.unm.edu/biology/coccidia/passer1.html) (accessed on 01 May 2019). Similar to *I. corvi* from the Corvidae family (Poon and Chew 1991), an oocyst residuum is present; however, the different oocyst dimensions exclude the possibility of them being the same species (Table 1).

As with the morphological analysis, sequence analysis at three loci (18S rRNA, 28S rRNA and COI) revealed that *I. coronoideae* n. sp. exhibited the highest similarity with other *Isospora* species from Passeriformes in Western Australia. At the 18S rRNA loci, *I. coronoideae* n. sp. exhibited the highest similarity with *I. neochmiae* (98.9%) from a captive-bred red-browed finch (Yang et al. 2016b) and 98.3% similarity to *I. manorinae* (KT224379) from a yellow-throated miner (Yang et al. 2016a). At the 28S rRNA locus, it exhibited the highest similarity with *I. manorinae* (KT224381) from a yellow-throated miner (95.4%) (Yang et al. 2016a) and *I. anthochaerae* (KF766053) from a red wattlebird (94.8%) (Yang et al. 2014). At the COI locus, it had a higher similarity with *I. butcherae* (KY801687) from a silvereye (99.8%) (Yang et al. 2018) and *I. neochmiae* (KT224378) (99.7%) (Yang et al. 2016b). Interestingly, all these three bird species have overlapping home ranges on the east coast of Australia. They all have very different feeding styles however, with finches eating grass seeds, ravens eating mainly on the ground and silvereyes feeding mainly in trees (Pizzey et al. 2007). As there were no 18S, 28S rRNA and COI sequences from other *Isospora* species from the Corvidae family available in GenBank, we only compared our sequence data with *Isospora* species from Passeriformes found in Western Australia.

In the present study, morphology and molecular analysis were used to characterize the oocysts in the faeces from an Australian raven as a new species of *Isospora*, named *Isospora coronoideae* n. sp. after its host. This finding highlights the diversity of *Isospora* species in Australian birds. Further analysis is required to determine the clinical impact (if any) of *Isospora coronoideae* n. sp. on its host and the host range of this new species.

Acknowledgements The authors thank all the volunteers at KWRC for their commitment and dedication in caring for all the animals admitted to the centre. We are also grateful to the veterinarians and staff at the Wattle Grove and Kalamunda Veterinary Hospitals for their expert treatment and care of the wildlife treated at their clinics.

Funding This work was partially supported by the Postdoctoral Research Funding Plan in Jiangsu Province, China (Grant No. 1601054C) and Jiangsu Government Scholarship for Overseas studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Murdoch University Animal Ethics Permit (Protocol No. 569).

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