

## A New Species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the Blue-crowned Laughingthrush *Dryonastes courtoisi* (Passeriformes: Timaliidae)

Ján JAMRIŠKA<sup>1</sup>, Alberto Rodríguez BARBÓN<sup>2</sup> and David MODRÝ<sup>1,3</sup>

<sup>1</sup>Dept. of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic; <sup>2</sup>Durrell Wildlife Conservation Trust, Les Augrés Manor, Trinity, Jersey; <sup>3</sup>Institute of Parasitology, Biology Centre AS CR, České Budějovice, Czech Republic

**Abstract.** A new species of *Isospora* Schneider (Apicomplexa: Eimeriidae) is described from captive Blue-crowned Laughingthrush *Dryonastes courtoisi* (Ménégaux, 1923) (Passeriformes: Timaliidae). Sporulated oocysts of *Isospora courtoisi* n. sp. are ellipsoidal 24.5 × 14.5 µm. Micropyle and oocyst residuum are absent. Sporocysts are broadly ellipsoidal, 15.9 × 8.5 µm, with Stieda and substieda bodies. Sporocyst residuum is scattered composed of hundreds of small granules. Sporozoites are elongate and slightly curved, each with two refractile bodies. The nuclei of the sporozoites were not well discernible. Considering the critically endangered status of *Dryonastes courtoisi* and assumed high host specificity of described coccidium, also *I. courtoisi* can be classified as critically endangered organism.

**Key words:** Coccidia, *Isospora courtoisi*, Blue-crowned Laughingthrush, passeriformes.

### INTRODUCTION

Coccidia (Apicomplexa: Eimeriidae) are, together with haemosporidians among the most diversified intracellular endoparasites of birds (Levine 1985). Traditionally, based on morphological concept, the isosporoid coccidia are defined by production of an oocyst, which when sporulated, contain 2 sporocyst each with 4 spo-

rozoites (Levine 1970, 1988). Recent phylogenetic studies demonstrated the polyphyletic character of the genus *Isospora* Schneider, 1881, when the presence of Stieda bodies and character of the merogonic development in avian isosporoid coccidia distinguishes these taxa from the isosporoid coccidia of mammals (Carreno and Barta 1999, Barta *et al.* 2005). Extraintestinal development was demonstrated in several avian isosporoid coccidia, with merogonial stages detectable in circulating mononuclear leucocytes and in various host tissues. Species with stages presented in blood cells were historically classified as members of the genus *Atoxoplasma* Garnham, 1950, however, the genus was recently synonymized with *Isospora* (Upton *et al.* 2001, Barta *et al.* 2005).

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Address for correspondence: Ján Jamriška, Dept. of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1–3, 612 42 Brno, Czech Republic. E-mail: jan.jamriska5@gmail.com

In general, eimeriid coccidia are considered as highly host specific, which is reflected also in enormous diversity within individual coccidian genera. The Passeriformes is the largest and most diverse avian clade, including almost 6,000 bird species. Nevertheless, there are just over 140 species of isosporoid coccidia described from passerines till now, using the traditional, morphological species concept (Pellérdy 1974, Duszynski *et al.* 1999, Upton *et al.* 2001, Berto *et al.* 2011). The babblers (Timaliidae Vigors and Horsfield, 1872) is a large assemblage of tropical passerines consisting of more than 310 species in 84 genera distributed in the Asian and African region (del Hoyo *et al.* 2005). The genus *Dryonastes* Sharpe, 1883 represents a relatively homogenous group of species containing those Laughingthrushes which have the nostrils almost completely hidden by bristles. The Blue-crowned Laughingthrush, *Dryonastes courtoisi* (Ménégaux, 1923), is endemic to China. It occupies an extremely small fragmented breeding range in Jiangxi Province. The entire known wild population was estimated over 250 individuals in 2011 and current trends in the wild have not been estimated. In addition, there were 170 individuals known in captivity in 2012; this species is classified as Critically Endangered (Wilkinson and Gardner 2011; IUCN 2012).

Isosporoid infections with extraintestinal stages are usually in adult birds asymptomatic but the mortality in juvenile birds can approach 80% between two and nine months of age (Dorrestein *et al.* 1985, Ritchie *et al.* 1994). This represents serious problem in conservation of endangered bird species.

Presented study is one of the outputs of a detailed investigation of atoxoplasmosis among the passerines kept at Durrell Wildlife Conservation Trust, describing a new species of isosporoid coccidia in captive Blue-crowned Laughingthrushes.

## MATERIAL AND METHODS

Faecal samples were obtained from 9 specimens of *D. courtoisi* kept in indoor-outdoor enclosures at Durrell Wildlife Conservation Trust (Jersey, Channel Islands). Fresh faecal samples were collected individually from the enclosure's floor and placed into vials containing 2.5% aqueous (w/v) potassium dichromate ( $K_2Cr_2O_7$ ) solution. The vials were maintained at room temperature ( $\sim 23^\circ C$ ) for 5–7 days to allow the oocysts to sporulate (Long *et al.* 1976). Oocyst were concentrated by modified Sheather's sugar solution (SG = 1.30) flotation (Sheather 1923) and examined using Olympus AX 70 microscope equipped with Nomarski interference contrast op-

tics. Oocysts were photographed using a Olympus DP 70 digital camera. CorelDRAW 11 was used for preparing the composite line drawing. Measurements were taken using a calibrated ocular micrometer and are presented in micrometers as means followed by the ranges in parentheses.

## RESULTS

Three out of nine (33%) Blue-crowned Laughingthrushes shed unsporulated coccidian oocysts, which appeared to represent a single species of *Isospora*, description of which is presented below.

### *Isospora courtoisi* n. sp.

#### Description of the oocyst

Sporulated oocysts are elongately ellipsoidal,  $14.5 \times 24.5$  ( $23\text{--}24.5 \times 13\text{--}15$ , N = 25); shape index (length/width ratio) 1.7 (1.6–1.75). Oocyst wall bi-layered, 1 (0.8–1.5, N = 25). Micropyle and oocyst residuum are absent. One irregularly-shaped polar granule is present, about  $1 \times 2.0$ . Sporocysts are elongate ellipsoidal, slightly asymmetrical, cramped on one pole,  $8.5 \times 15.9$  ( $14.5\text{--}16.5 \times 7\text{--}9$ , N = 25), with smooth, single layered wall; shape index 2.1 (1.7–2.2). Stieda body is present, dome-like, 1.3 high  $\times$  1.5 wide ( $1.0\text{--}1.2 \times 1.5\text{--}1.6$ , N = 15) (Fig. 2), substieda body is spherical  $1.8 \times 2.4$  ( $1.7\text{--}1.9 \times 2.3\text{--}2.6$ , N = 15). Sporocyst residuum is scattered, composed of numerous small granules, smaller than  $1 \mu m$  in diameter (Fig. 1). Sporozoites are elongated, slightly curved, each with two refractile bodies. Anterior refractile body is spherical  $1.8 \times 2.4$  ( $1.8 \times 2.4\text{--}2.5$ , N = 10); the posterior one is elongate  $2.7 \times 4.8$  ( $4.5\text{--}4.9 \times 2.6\text{--}2.8$ , N = 10) (Fig. 4). The nuclei of the sporozoites were not well discernible.

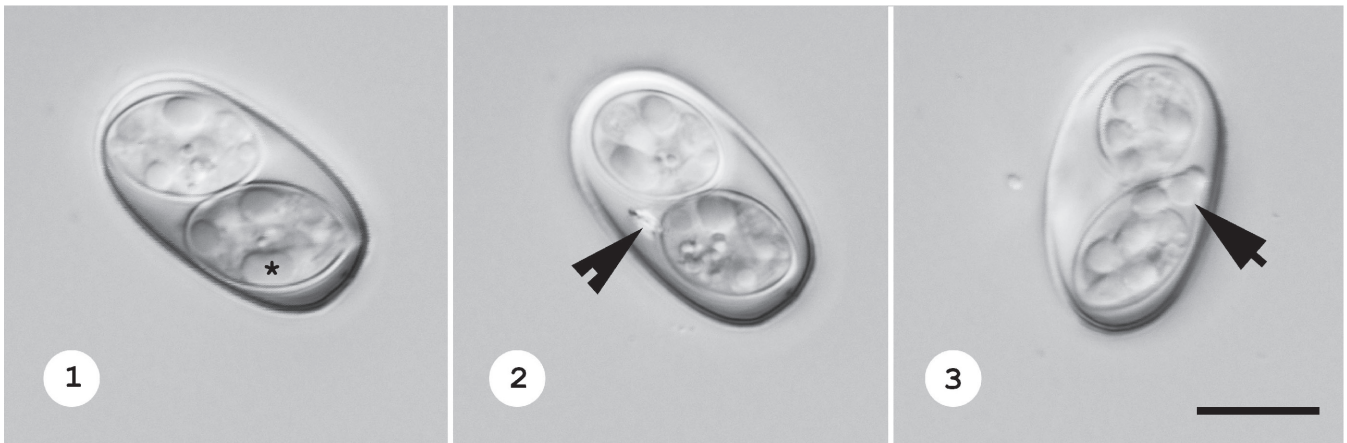
**Type-host:** *Dryonastes courtoisi* (Ménégaux, 1923) (Passeriformes: Timaliidae) (usually referred to as Blue-crowned Laughingthrush or Courtoisi's Laughingbird). Previously placed into paraphyletic genus *Garrulax*. Treated as critically endangered species. All examined birds were hatched in captivity (but parents reared).

**Type-locality:** Durrell Wildlife Conservation Trust, Jersey, Channel Islands, British Crown Dependency, GPS coordinates of the Park:  $49.22949^\circ N$ ,  $2.07338^\circ W$ .

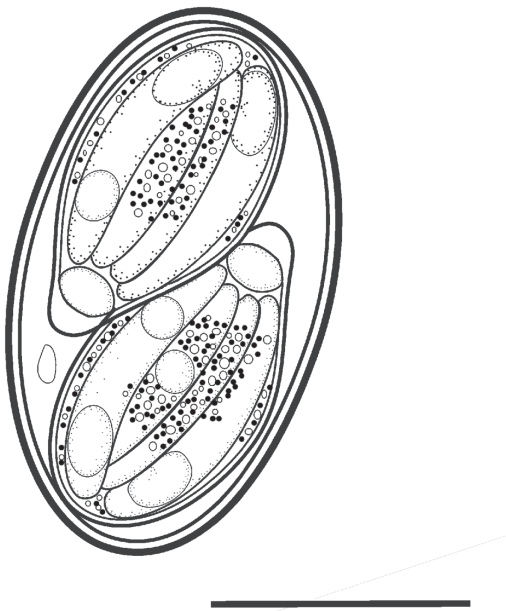
**Site of infection:** Unknown, oocysts were recovered from faeces.

**Sporulation:** Exogenous, oocysts became fully sporulated within 6 days at  $23^\circ C$ .

**Type-specimens:** Digital photomicrographs on CD deposited at the type parasitological collection of the



**Figs 1–3.** Nomarski interference contrast micrographs of sporulated oocysts of *I. courtoisi* isolated from the faeces of *Dryonastes courtoisi*. Note distinct elongated sporozoite refractile bodies (asterisk), polar granule (arrowhead) and globular substieda body (arrow); all in the same scale. Scale bar: 10  $\mu$ m.



**Fig. 4.** Composite line drawing of sporulated oocyst of *I. courtoisi*. Scale bar: 10  $\mu$ m.

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**Etymology:** The specific epithet of the new isosporan reflects the specific epithet of the scientific name of the type host.

## REMARKS AND DISCUSSION

Overall, the coccidia of the Thrushes of the family Timaliidae are still poorly studied. Known species of isosporid coccidia described from hosts of this family are listed in Table 1. Among these species, *I. courtoisi* is unique by extensive ellipsoidal oocyst shape. The oocysts of *I. courtoisi* have no oocyst residuum, which distinguishes them from *I. garrulae* Ray *et al.*, 1952 recorded from *Garrulax lineatus* (Vigors, 1831). Unfortunately, descriptions of *I. garrulae* and *I. pycnonotae* Mandal and Chakravarty, 1964 lack the information about substieda body. In *I. garrulae* information about polar granule and sporocyst residuum is also missing. *I. capistrata* Sinha and Sinha, 1981, differs from *I. courtoisi* by the shape of the sporulated oocyst and by the absence of polar granule. Comparing *I. courtoisi* with previously described isosporid species from the family Timaliidae, we consider it as a new species.

Evaluation of diversity and host specificity of isosporid coccidia can help not only to understand the taxonomy of these parasites, but can shed some light on the epidemiology of coccidiosis/atoxoplasmosis in captive populations of endangered passeriform birds. Passerines are frequently parasitized by coccidia (Pellérdy 1974, Duszynski *et al.* 1999), and those with extraintestinal stages are under captive conditions highly pathogenic (Barbón *et al.* 2013) and cause serious health alteration in young birds; the mortality rates reaching up to 80% have been observed (Partington *et al.* 1989,

**Table 1.** Isosporoid coccidia of the family Timaliidae listed in alphabetical order. All measurements are given in  $\mu\text{m}$ .

Species	Host	Oocyst	Oocyst shape index	Oocyst residuum	Micropyle	Polar granule	Sporocyst	Sporocyst shape index	Sporocyst residuum	Stieda body	Substieda body	Reference
<i>I. capistrata</i>	<i>Leioptila capistrata</i>	18.2 × 24.6	—	no	no	no	11–16.9 × 8.8–10.4	—	present	yes	—	Sinha and Sinha 1981
<i>I. courtoisi</i> n. sp.	<i>Dryonastes courtoisi</i>	23–24.5 × 13–15	1.7	no	no	yes (1)	14.5–16.5 × 7–9	1.09	scattered	yes	yes	Present study
<i>I. garrulae</i>	<i>Garrulax lineatus</i>	20–22.5 × 17.5–21.25	—	yes (1)	no	—	10–15.5 × 7.5–12.5	—	—	yes	—	Ray <i>et al.</i> , 1952
<i>I. pycnonotae</i>	<i>Turdoides striatus</i>	19.8 × 24.2	—	no	no	yes (1)	8.8 × 19.8	—	scattered	yes	—	Mandal and Chakravarty 1964

Upton *et al.* 2001, Adkesson *et al.* 2005). Intestinal coccidiosis pathogenicity is primarily caused by the depth of the intestinal lesions and those lesions are specific to the species of coccidia infecting the host (Saif *et al.* 2008; Répérant *et al.* 2012).

We have no direct evidence for the extraintestinal development in case of *I. courtoisi*. Based on our further research of the isosporoid coccidia in Durrell Wildlife Conservation Trust aviary (data not shown), it seems more likely that other isosporan species (i.e. *I. rotschildi*) are responsible for the avian mortality. However, further data are necessary to prove or exclude the occurrence of extraintestinal stages in the life cycle of *I. courtoisi*.

Case of *I. courtoisi* demonstrates sad controversy of veterinary care and parasite control in captive populations of endangered animals. Considering the critically endangered status of *Dryonastes courtoisi* (Wilkinson and Gardner 2011; IUCN 2012), also *I. courtoisi* can be therefore classified as critically endangered, as there is high risk that host specific parasites (as isosporan coccidian are), will become extinct, either intentionally or accidentally (Dunn *et al.* 2009).

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