

A New Species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the Himalayan Black Bulbul *Hypsipetes leucocephalus* (Gmelin, 1789) (Passeriformes: Pycnonotidae)

Ján JAMRIŠKA^{1,2}, F. Javier LÓPEZ⁴ and David MODRÝ^{1,3}

¹Department of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic; ²Department of Zoology, Comenius University in Bratislava, Bratislava, Slovakia; ³Institute of Parasitology, Biology Centre AS CR, České Budějovice, Czech Republic; ⁴Durrell Wildlife Conservation Trust, Les Augrés Manor, Trinity, Jersey

Summary. A new species of isosporoid coccidia (Apicomplexa: Eimeriidae) is described from captive Himalayan Black Bulbul *Hypsipetes leucocephalus* (Passeriformes: Pycnonotidae). Sporulated oocysts of *Isospora anthomassonae* n. sp. are subspherical to slightly ellipsoidal $32.0 \pm 1.5 \times 29.4 \pm 1.3$ ($30\text{--}35 \times 27\text{--}31$) μm . Micropyle, oocyst residuum and polar granule are absent. Sporocysts are broadly ellipsoidal, $24.6 \pm 1.4 \times 12.9 \pm 0.7$ ($22\text{--}28 \times 12\text{--}14$) μm , with Stieda and substiedal bodies. Sporocyst residuum is broadly ellipsoidal, $11.3 \pm 0.8 \times 9.5 \pm 0.7$ ($9\text{--}13 \times 8\text{--}11$) μm , composed of hundreds of small granules. Sporozoites are elongate and slightly curved, each with two refractile bodies.

Key words: Coccidia, *Isospora anthomassonae*, Black Bulbul, passeriformes.

INTRODUCTION

Together with haemosporidians, coccidia (Apicomplexa: Eimeriidae) are the most diversified and common endoparasites of birds (Levine 1985). Traditionally, coccidia with bisporocystic oocysts are referred to as genus *Isospora* Schneider, 1881 and their classification was, until recently, based mostly on the oocyst

morphology and presumed host specificity (Levine 1970, 1988). However, recent phylogenetic studies showed the polyphyletic character of the genus. The presence of Stieda bodies much like the merogonic development in avian isosporoid coccidia distinguishes these taxa from the isosporoid coccidia of mammals that lack Stieda bodies. The latter group of mammalian parasites has been recently transferred to the genus *Cystoisospora* Frenkel, 1977 within the family Sarcocystidae Poche, 1913 (Carreno and Barta 1999, Barta *et al.* 2005). Several species of *Isospora* from avian hosts that possess the extraintestinal development followed by the presence of developmental stages in circulating

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: jamriskaj@fns.uniba.sk

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).

Over 500 species of isosporoid coccidia have been described from passeriforms using the traditional, morphological species concept (Pelérdy 1974, Duszynski *et al.* 1999, Upton *et al.* 2001, Berto *et al.* 2011).

The Passeriformes is a large group which include a variety of avian species with worldwide distribution. Bulbuls (Pycnonotidae Gray, 1840), form a large assemblage of exotic passerines which consists of more than 130 species in 27 genera distributed in the Asian and African continents.

The genus *Hypsipetes* sp. represents a relatively homogenous group of species. The Himalayan Black Bulbul (*Hypsipetes leucocephalus* (Gmelin, 1789)) (Passeriformes: Pycnonotidae) is widely distributed around the Himalayan mountains, with presence in NE Afghanistan, NE India, S Tibet, NW Myanmar, S China, NE Thailand, N Vietnam, N Pakistan, Nepal (Fishpool and Tobias 2005). The Himalayan Black Bulbul is listed in IUCN Red List of Threatened Species as Least Concern (IUCN 2011).

An extensive investigation into atoxoplasmosis diagnosed in passerines at Durrell Wildlife Conservation Trust led to the discovery of a new species of isosporid coccidia in captive Himalayan Black Bulbuls, which is described herein.

MATERIAL AND METHODS

Thirty faecal samples were obtained from five specimens of *H. leucocephalus* kept in indoor-outdoor enclosures at Durrell Wildlife Conservation Trust (Jersey, Channel Islands). 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 for 5–7 days and stirred daily to allow the oocysts to sporulate (Long *et al.* 1976). Oocyst were concentrated by Sheather's sugar solution flotation (Sheather 1923) and examined using a Olympus AX 70 microscope equipped with Nomarski interference contrast optics. Oocysts were photographed using Olympus DP 70 digital camera. 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 five (60%) Himalayan Black Bulbuls shed unsporulated coccidian oocysts, which appeared to represent a single species of *Isospora*, description of which is presented below.

Isospora anthomassonae n. sp.

Description of the oocyst: Sporulated oocysts are subspherical to slightly ellipsoidal, $32.0 \pm 1.5 \times 29.4 \pm 1.3$ ($30\text{--}35 \times 27\text{--}31$, N = 30); shape index (length/width ratio) 1.0 (1.0–1.2). Oocyst wall is smooth and bilayered, 1.1 ± 0.1 (1.0–1.2, N = 30). The outer layer is thicker, brownish in colour and with a light stippling. Micropyle, oocyst residuum and polar granule are absent. Sporocysts are broadly ellipsoidal, $24.6 \pm 1.4 \times 12.9 \pm 0.7$ ($22\text{--}28 \times 12\text{--}14$, N = 50), with smooth, single layered wall; shape index 1.0 (1.7–2.2). Knob-like Stieda body is present, $1.1 \pm 0.1 \times 3.8 \pm 0.4$ ($0.9\text{--}1.2 \times 3.5\text{--}4$, N = 20), with a subspherical substiedal body $4.2 \pm 0.3 \times 3.4 \pm 0.4$ ($4\text{--}5 \times 3\text{--}4.5$, N = 30). A sporocyst residuum is present, which is broadly ellipsoidal, $11.3 \pm 0.8 \times 9.5 \pm 0.7$ ($9\text{--}13 \times 8\text{--}11$, N = 20) and composed of hundreds of small granules. The two sporozoites are elongated, slightly curved, each with two refractile bodies. Anterior refractile body is spherical 3.5 ($3.1\text{--}3.5$, N = 10); the posterior one is elongate $10.1 \pm 0.7 \times 4 \pm 0.3$ ($9\text{--}11.5 \times 3.5\text{--}5$, N = 20). The nuclei of the sporozoites were not well discernible.

Type-host: *Hypsipetes leucocephalus* (Gmelin, 1789) (Passeriformes: Pycnonotidae) (usually referred to as Himalayan Black Bulbul or Asian Black Bulbul). All examined birds were hatched in captivity, some originated from parents captured from the wild.

Type-locality: Durrell Wildlife Conservation Trust, Jersey, Channel Islands.

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

Sporulation: Exogenous, oocysts became sporulated within 6 days at 23°C.

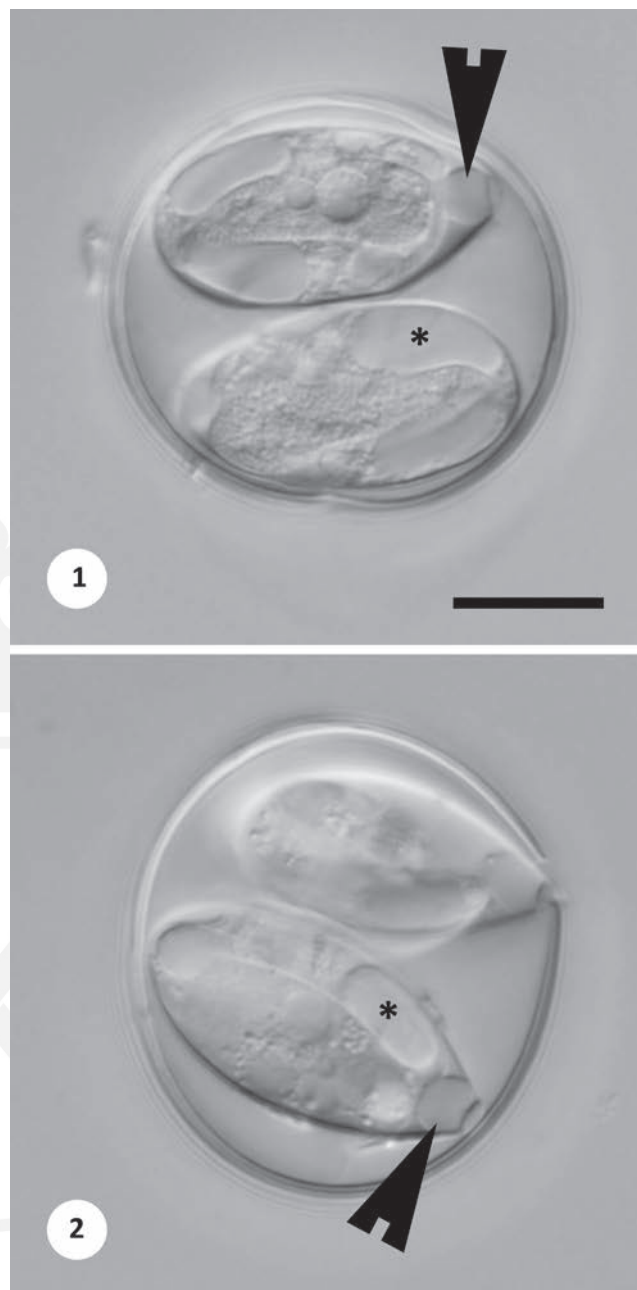
Type-specimens: Digital photomicrographs on CD deposited at the type parasitological collection of the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, No. IP ProtColl. 19.

Etymology: The species is named in honour of Ann Thomasson, a veterinary biologist from the Veterinary Department at Durrell Wildlife Conservation Trust on Jersey.

REMARKS AND DISCUSSION

The coccidia of bulbuls of the family Pycnonotidae are poorly studied. Known species of Isosporid coccidia described from hosts of this family are listed in Table 1. Comparison with available data shows that the oocysts of *Isospora anthomassonae* being similar in size to those of *I. ernsti* Todd, French, Meehan 1988, from *Pycnonotus xanthopygos* (Ehrenberg, 1833). However, unlike *I. anthomassonae*, sporulated oocysts of *I. ernsti* are described as having a single layered oocyst wall and scattered sporocyst residuum (while that of *I. anthomassonae*, is compact). Furthermore, both species differ markedly in the shape of the sporozoite's refractile bodies which are both spherical in *I. ernsti* while in *I. anthomassonae* the anterior refractile body is spherical and the posterior one is elongated. The Stieda body in *I. anthomassonae* is knob-like with a subspherical substiedal body. In *I. ernsti* the Stiedal and substiedal bodies are prominent. *I. anthomassonae* differs from all the other species of isosporid coccidia found in hosts of the family Pycnonotidae by its proportions and shape of large oocysts and sporocysts. The prolonged posterior refractile granule is a unique feature of *I. anthomassonae*. Oocysts of *I. Blackburni* Todd, French, Meehan 1988, are spherical, smaller with ovoid sporocysts. Sporocyst residuum is scattered while that in *I. anthomassonae*, is compact. *I. anthomassonae*, further differs from *I. elmahalensis* Amoudi 1987 by the presence of substiedal body and by bilayered oocyst wall. In *I. anthomassonae* there is no oocyst micropyle as it is in *I. pycnonotusi* Mandal and Chakravaty 1964. Furthermore *I. pycnonotae* Bhatia, Chauhan, Arora and Agrawai 1973, *I. gatifenensis* Amoudi 1992, *I. khobarensis* Amoudi 1992, *I. hafoufenensis* Amoudi 1992, differ from *I. anthomassonae* in the absence of polar granule, by single-layered oocyst wall and in oocyst size and shape.

Passerines are frequently parasitised with coccidia. *Isospora* spp., especially those with extraintestinal stages (former *Atoxoplasma*) cause serious problems and high mortality, mostly in young birds. The mortality rates have been observed reaching up to 80% (Partington *et al.* 1989, Upton *et al.* 2001, Schrenzel *et al.* 2001, Adkesson *et al.* 2005). Based on the analysing of occurrence of other isosporans in Durrell Wildlife Conservation Center aviary (data not shown) it seems more likely that other species (i.e. *I. rotschildi*) are re-

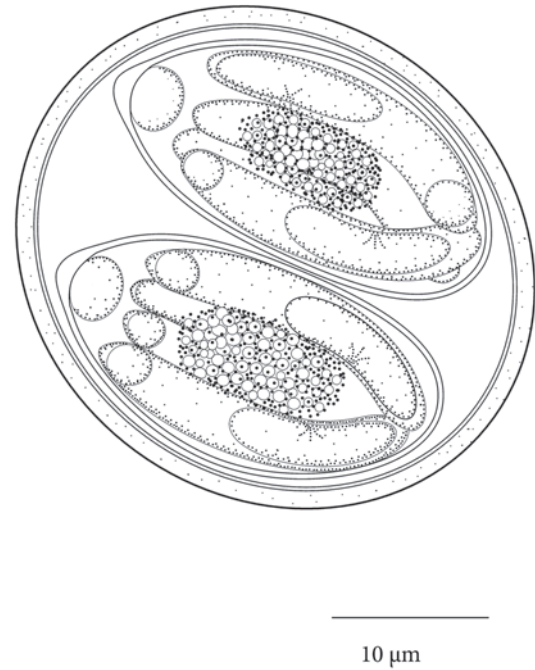


Figs 1–2. Nomarski interference micrographs of oocysts of *Isospora anthomassonae* isolated from the faeces. Note distinct elongated sporozoite refractile bodies (asterisks) and substieda body (arrowheads). Both in the same scale. Scale bar: 10 μ m.

sponsible for the avian mortality. However, additional data, especially molecular data are necessary to prove or exclude the occurrence of extraintestinal stages in the life cycle of *I. anthomassonae*.

Table 1. Isosporoid coccidia of the family Pycnonotidae listed in alphabetical order.

<i>Isospora</i>	Host	Locality	Oocyst	Sporocyst	Polar granules	Reference
<i>I. anthomassoniae</i> n. sp.	<i>Hypsipetes leucocephalus</i>	Durrell Wildlife Conservation Center	30–35 × 27–31	22–28 × 12–14	x	This paper
<i>I. blagburni</i>	<i>Pycnonotus xanthopygus</i>	Lincoln Park Zoo, Chicago	21–28 × 19–26	17–23 × 10–13	x	Todd <i>et al.</i> 1988
<i>I. elmahalensis</i>	<i>Pycnonotus leucogenys</i>	Elmahala – Saudi Arabia	19.5–22.5 × 18.5–20	14–17.5 × 7–12	x	Amoudi 1987
<i>I. ernsti</i>	<i>Pycnonotus xanthopygus</i>	Lincoln Park Zoo, Chicago	28–38 × 23–31	24–30 × 11–16	x	Todd <i>et al.</i> 1988
<i>I. gatifemensis</i>	<i>Pycnonotus leucogenys</i>	Al-Gatif – Saudi Arabia	23–26.5 × 16.5–19	17–20.5 × 8–11	1	Amoudi 1992
<i>I. hafoufenensis</i>	<i>Pycnonotus leucogenys</i>	Al-Hafouf – Saudi Arabia	22–27 × 19–24	14.5–18 × 8–13	1	Amoudi 1992
<i>I. khobarensis</i>	<i>Pycnonotus leucogenys</i>	Al-Khobar – Saudi Arabia	22–26 × 15.5–19	13–16 × 9–11.5	1	Amoudi 1992
<i>I. pycnonotae</i>	<i>Pycnonotus jocosus</i>	Calcutta – India	24.2 × 19.8	19.8 × 8.8	1	Mandal and Chakravarty 1964
<i>I. pycnonotusi</i>	<i>Pycnonotus jocosus</i>	New Delhi Zoo	17.8–22.7 × 16.2–22.7	14.2 × 8.0	1	Bhatia <i>et al.</i> 1973

**Fig. 3.** Composite line drawing of sporulated oocyst of *Isospora anthomassoniae*. Scale bar: 10 µm.

Acknowledgement. We are indebted to Ann Thomasson, veterinary biologist and to Alberto Rodríguez Barbón, veterinarian, both from the Veterinary Department at DWCT on Jersey for their help with organizing the sampling. We would like to thank the bird keepers from DWCT for their help with collecting the samples. This work was in part funded by grant IGA VFU Brno 232/2010 and by N-SPP Hlavička 72/2011.

REFERENCES

- Adkesson M. J., Zdziarski M. J., Little S. E. (2005) Atoxoplasmosis in Tanagers. *J. Zoo Wildlife Med.* **36**(2): 265–272
- Amoudi M. A. (1987) *Isospora elmahalensis* n. sp. (Apicomplexa, Eimeriidae), a Parasite of the White-Cheeked Bulbul (*Pycnonotus leucogenys*) in Saudi Arabia. *J. Protozool.* **34**(1): 26–27
- Amoudi M. A. (1992) Three new species of *Isospora* (Apicomplexa, Eimeriidae), from the White-cheeked Bulbul (*Pycnonotus leucogenys*) in Saudi Arabia. *Zool. Scr.* **21**: 1–4
- Barta J. R., Schrenzel M. D., Carreno R., Rideout B. A. (2005) The genus *Atoxoplasma* (Garnham 1950) as a junior objective synonym of the genus *Isospora* (Schneider 1881) species infecting birds and resurrection of *Cystoisospora* (Frenkel 1977) as a correct genus for *Isospora* species infecting mammals. *J. Parasitol.* **91**: 726–727
- Berto B. P., Flausino W., McIntosh D., Teixeira W. L., Lopes F. C. W. G. (2011) Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). *Syst. Parasitol.* **80**: 159–204

- 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(10)**: 944–947
- Brooks D. R. (1993) Extending the Symbiotype Concept to Host Voucher Specimens. *J. Parasitol.* **79(4)**: 631–633
- Carreno R. A., Barta J. R. (1999) An Eimeriid origin of isosporoid coccidia with stieda bodies as shown by phylogenetic analysis of small subunit ribosomal RNA gene sequences. *J. Parasitol.* **85**: 77–83
- Duszynski D. W., Upton S. J., Couch L. (1999) The Coccidia of Passeriformes (corvids, estrilids, finches, thrushes, wrens): *Isospora* spp. <http://biology.unm.edu/biology/coccidia/passer1.html>
- Fishpool L., Tobias J. (2005) Family Pycnonotidae (Bulbuls). In: Handbook of the Birds of the World. Cuckoo-Shrikes to Thrushes, (Eds. J. del Hoyo, A. Elliot and D. Christie), Vol. 10, Lynx Edicions Barcelona, 896 pp.
- IUCN (2011) IUCN Red List of Threatened Species. Version 2011.1. www.iucnredlist.org
- Levine N. D. (1970) Taxonomy of the sporozoa. *J. Parasitol.* **56**: 208–209
- Levine N. D. (1985) Veterinary Protozoology. Iowa State University Press, Ames, 414 pp.
- Levine N. D. (1988) Progress in taxonomy of the Apicomplexan protozoa. *J. Protozool.* **35**: 518–520
- Long P. L., Millard B. J., Joyner L. P., Norton C. C. (1976) A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. *Folia Vet. Latina* **6**: 201–217
- Mandal A. K., Chakravarty M. M. (1964) Studies on some aspects of avian coccidia (Protozoa: Sporozoa). Five new species of *Isospora* Schneider, 1881. *Proc. Zool. Soc. Calcutta* **17**: 35–45
- Partington C. C. J., Gardiner Ch. H., Fitz D., Phillips L. G., Montali R. J. (1989) Atoxoplasmosis in Bali Mynahs (*Leucopsar rothschildi*). *J. Zoo Wildlife Med.* **20(3)**: 328–335
- Pellérdy L. P. (1974) Coccidia and Coccidiosis. Verlag Paul Parey, Berlin und Hamburg, 959 pp.
- Sheather A. L. (1923) The detection of intestinal protozoa and mange parasites by *Flotationa flotation* technique. *J. Comp. Pathol.* **36**: 266–275
- Schrenzel M., Kenner L., McAloose D., Stalis I., Papendick R., Klieforth R., Maalouf G., Rideout B. (2001) Diagnosis and molecular characterization of *Atoxoplasma* in passerine birds. *Proc. Am. Assoc. Zoo Vet.*: 214
- Todd K. S., French R. A., Mechan T. P. (1988) Oocyst of *Isospora ernsti* n. sp. and *Isospora blagburni* n. sp. (Apicomplexa: Eimeriidae) from the black-capped bulbul, *Pycnonotus xanthopygos*. *Syst. Parasitol.* **11**: 239–241
- Todd K. S., Hammond D. M. (1971) Coccidia of anseriformes, galliformes and passeriformes. In: Infectious and Parasitic Diseases of Wild Birds, (Eds. J. D. Davis, R. C. Anderson, L. Karstad, D. O. Trainer). Iowa State University Press, Ames, 234–281
- Upton S. J., Wilson S. C., Norton T. M., Greiner E. C. (2001) A new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the Bali (Rothschild's) mynah *Leucopsar rothschildi* (Passeriformes: Sturnidae), and comments concerning the genera *Atoxoplasma* Garham, 1950 and *Isospora*. *Syst. Parasitol.* **48**: 47–53

Received on 23rd January, 2012; Revised on 6th March, 2012; Accepted on 7th March, 2012