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A new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) in Ruppell's agama *Agama rueppelli* (Vaillant) (Sauria: Agamidae) from East Africa, with a review of this genus in agamid lizards

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Abstract Coprological examinations of eight Ruppell's agamas *Agama rueppelli* (Vaillant) revealed the presence of a coccidium of the genus *Isospora* Schneider, 1881 that represents a previously undescribed species. Oöcysts of *Isospora farahi* n. sp. are spherical or subspherical, 29.1 (26–31) × 28.8 (26–31) µm, with a shape-index of 1.01 (1–1.07). An oöcyst residuum, polar granules and micropyle are absent. The oöcyst wall is bilayered, brownish and smooth, *c*. 1.5–2 µm thick. The sporocysts are oval, 16.6 (15–18) × 11.4 (11–12) µm, with a shape-index of 1.46 (1.25–1.64) and both Stieda and substieda bodies. A sporocyst residuum is present as medium-sized granules scattered irregularly among the sporozoites. The sporozoites are vermiform, with a large posterior spherical refractile body. Endogenous development is intranuclear in the epithelial cells of the small intestine. Sporulation is unknown, as oöcysts were recovered from the faeces.

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Introduction

Eimeriid coccidian parasites (Apicomplexa: Eimeriorina) of poikilotherm hosts, especially those of reptiles, represent a neglected part of our biodiversity. In contrast to eimeriids from birds and mammals, the assemblage of eimeriid coccidia from reptiles represents a phylogenetically and taxonomically complex group of protists, as reflected by repeated attempts to solve their higher-level taxonomy (Paperna & Landsberg, 1989; Jirků et al., 2002). Comparison of the diversity of lizards of the family Agamidae with the number of coccidian species described from these hosts clearly shows the under-sampling and limits of our knowledge. The Agamidae comprises over 50 genera widely distributed in Africa, Asia and Australia (Zug et al., 2001). Of these, only two genera are found in East Africa, namely *Acanthocercus* and *Agama* (see Spawls et al., 2004). Only two species of coccidia have previously been described from African agamids, *Eimeria agamae* (Laveran & Petit, 1910) Reichenow, 1921 and *E. colonorum* Prasad, 1960, both from *Agama agama* Linnaeus.

In present paper, we describe a new species of *Isospora* Schneider, 1875 from *Agama rueppelli* (Vaillant), an abundant agamid from dry, low-altitude savanna and semi-desert areas of Eastern Africa.

Materials and methods

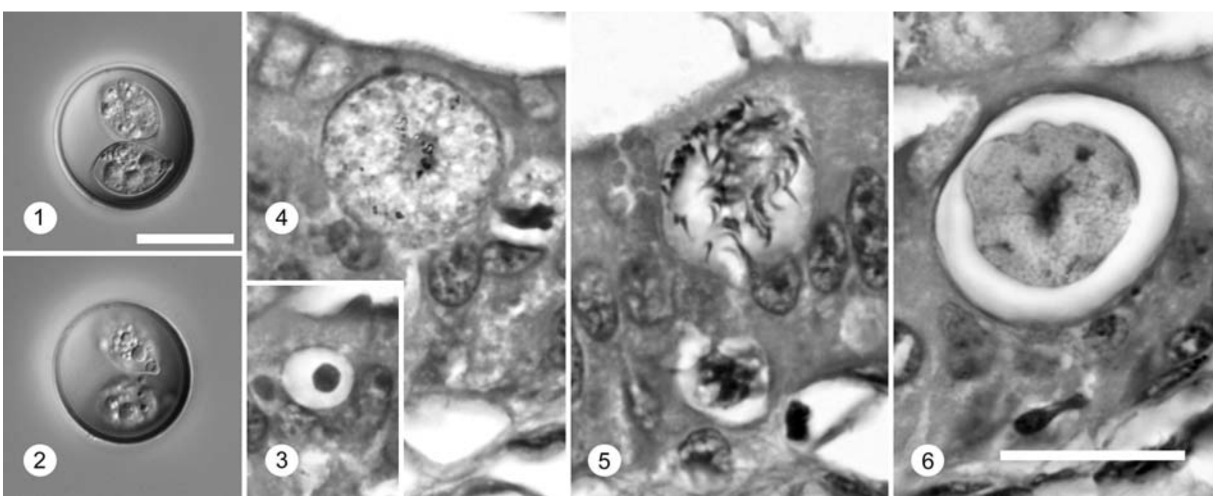
Eight specimens of *Agama rueppelli* were collected at various localities in Kenya. Animals were killed using an intra-coelomic overdose of barbiturates (Thiopental®Spofa) and dissected. Fresh contents from the terminal part of the large intestine were preserved with 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) and the gastrointestinal tract of each animal was preserved in 10% buffered formalin. Faecal samples were examined microscopically after concentration by flotation with Sheather's sugar solution (specific gravity 1.25). Oöcysts and endogenous stages were measured and photographed using differential interference contrast (DIC) optics on an Olympus AX70 microscope. Measurements were made using a calibrated ocular micrometer and are reported in micrometres, as the means, followed by the range in parentheses. After coprological examination, fixed tissues of a single infected lizard, were processed for histology using standard methods. Paraffin sections, 5–6 µm thick, were stained with haematoxylin and eosin (H&E) and examined using light microscopy.

Isospora farahi **n. sp.**

Type-host: *Agama rueppelli* (Vaillant) (Sauria: Agamidae), Ruppell's agama.
Type-locality: Kalkumpe (02°31′54″N, 36°49′20″E), Marsabit District, Kenya.
Type-material: Photosyntypes are deposited in the protozoological collection of the Institute of Parasitology of the Biology Centre of the Academy of Sciences of Czech Republic, České Budějovice, Czech Republic, under collection number IP ProtColl 6.
Symbiotype: An ethanol-preserved host specimen is deposited in the herpetological collection of the National Museum Prague, Czech Republic, under collection number NMP6V 73614.
Prevalence: One of eight examined Ruppell's agamas had oöcysts of *I. farahi* n. sp. in its intestinal contents.
Etymology: The species is named for Dr Idle Farah, the General Director of the National Museums of Kenya, in recognition of his support for collaborative research.

Description (Figs. 1–7)

Oöcysts
Fully-sporulated oöcysts (Figs. 1–2, 7) spherical or subspherical, 29.1 (26–31) × 28.8 (26–31); shape-index (SI, length/width ratio) 1.01 (1–1.07), $n = 30$.



Figs. 1–6 Micrographs of the developmental stages of *Isospora farahi* n. sp. 1–2. DIC micrographs of fully-sporulated oöcysts, both at the same scale. 3. Young trophozoite. 4. Macrogamont with peripherally localised, fine, wall-forming bodies. 5. Microgamont with microgametes. 6. Zygote with well-defined oöcyst wall. 3–6. Histological sections stained with H&E, all at the same scale. *Scale bars*: 20 µm

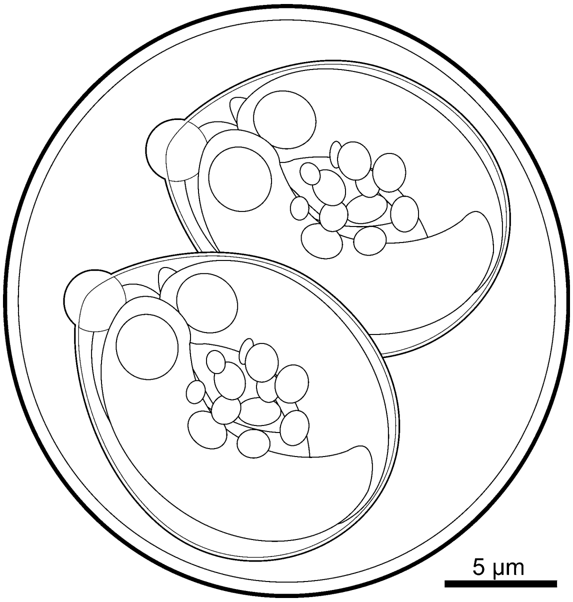


Fig. 7 Composite line drawing of sporulated oöcyst of *Isospora farahi* n. sp.

Oöcyst residuum, polar granules and micropyle absent. Oöcyst wall bilayered, *c*. 1.5–2 thick (inner layer much thinner, *c*. 0.5), brownish and smooth. Wall striations absent. Sporocysts oval, 16.6 (15–18) × 11.4 (11–12), SI = 1.46 (1.25–1.64), $n = 30$. Stieda body discoid, *c*. 1 high and 2–3 wide (Fig. 1). Substieda body globular, *c*. 2 high and 3 wide. Sporocyst residuum consists of medium-sized granules scattered irregularly among sporozoites (Fig. 1). Sporozoites vermiform, with large posterior spherical refractile body, *c*. 5 × 5 (Fig. 2), and centrally located spherical nucleus, *c*. 3 in diameter.

Site of infection and endogenous stages

Endogenous developmental stages scattered throughout mucosa of small intestine. All developmental stages intranuclear in enterocytes, localised in distinct parasitophorous vacuole. Trophozoites (Fig. 3) most prevalent. Mature macrogamonts spherical, 19–25 in diameter, containing centrally localised nucleus and fine wall-forming bodies scattered close to surface (Fig. 4). Single mature microgamont found, spherical, 20 in diameter, contains numerous curved, 2–3 long, intensively stained microgametes (Fig. 5). Few zygotes in final stages of development observed, lack wall-forming bodies and surrounded by fine oöcyst wall (Fig. 6).

Sporulation

Unknown; oöcysts recovered from faeces after few weeks of storage in potassium dichromate; but it is probably exogenous, as in other members of this genus known from saurians.

Discussion

No species of *Isospora* have previously been reported from African agamids. However, 10 species have been described from agamid lizards in Asia and Australia (Table 1). *I. farahi* n. sp. differs from all these species in oöcyst morphology. Four species (*I. amphiboluri* Cannon, 1967; *I. cannoni* Finkelman & Paperna, 1994; *I. caryophila* Rogier & Colley, 1976; and *I. gonocephali* Maupin, Diong & McQuistion, 1998) have much smaller oöcysts than *I. farahi* (see Cannon, 1967; Rogier & Colley, 1976; Finkelman & Paperna, 1994; Maupin et al., 1998). Moreover, *I. caryophila* also differs by having a rather ellipsoidal oöcyst shape. Sporocyst shape differentiates *I. farahi* from other three species (*I. phrynocephali* Ovezmukhammedov, 1971; *I. rayi* Mandal, 1966; and *I. rustamovi* Ovezmukhammedov, 1977) (see Mandal, 1966; Ovezmukhammedov, 1971, 1977). *I. choochotei* Finkelman & Paperna, 1994 lacks a substieda body (Finkelman & Paperna, 1994), whereas in *I. farahi* the substieda body is clearly visible. *I. lacertae* Saum, Diong & McQuistion, 1997 has light perpendicular striations in the outer oöcyst wall (Saum et al., 1997), which are evidently absent in *I. farahi*. *I. deserti* Finkelman & Paperna, 1994 is the most similar species in terms of oöcyst morphology, but differs by possessing slightly smaller oöcysts, relatively smaller sporocysts and less prominent Stieda bodies (Finkelman & Paperna, 1994).

However, further distinguishing features can be drawn from the localisation and appearance of the stages of endogenous development. Basically, isosporan coccidia from reptilian hosts exhibit two modes of endogenous development: intracytoplasmic or intranuclear. Although it is exceptional among coccidia from homeotherms, intranuclear localisation, which is the case in *I. farahi* n. sp., is a rather common feature of *Isospora* spp. from saurian hosts (Finkelman & Paperna, 1994; Paperna & Finkelman, 1998). Also, among species parasitising agamid lizards, intranuclear localisation is known in at least in four other species (Table 1). Among them, *I. deserti,* the

Table 1 Revised checklist with key taxonomic characters of *Isospora* species from lizards of the family Agamidae

Species	Host	Oöcyst shape and size	Sporocyst shape and size	Endogenous development	Geographical origin
I. farahi n. sp.	*Agama rueppelli*	Subspherical 29.1 (26–31) × 28.8 (26–31)	Ovoid 16.6 (15–18) × 11.4 (11–12)	Intranuclear	Kenya
I. amphiboluri Cannon, 1967	*Pogona barbata*, *P. vitticeps*	(Sub)spherical 24.9 (22.1–26.8) × 24.2 (22.1–26.8)	Oval 14.6 (13.8–15.7) × 10.3 (9.1–11.0)	Intracytoplasmic	Australia
I. cannoni Finkelman & Paperna, 1994	*Diporiphora australis*	Subspherical 22.8 (20–25) × 24.8 (22.5–27.5)	Ovoid 14.7 (14–15.5) × 10.2 (10–11.5)	Intranuclear	Australia
I. caryophila Rogier & Colley, 1976	*Gonocephalus grandis*	Ellipsoidal–subspherical 23.5 (21–30) × 21.9 (18–29)	Ovoid 13.2 (9–15) × 8.2 (7–10)	Intranuclear	Malaysia
I. choochotei Finkelman & Paperna, 1994	*Calotes mystaceus*	(Sub)spherical 29.3 (24–32) × 29.5 (28–32.5)	Ovoid 16.5 (15.5–18) × 11.2 (11)	Intranuclear	Thailand
I. deserti Finkelman & Paperna, 1994	*Trapelus pallidus*, *T. mutabilis*	Spherical 27.7 (25–28) × 27.7 (25–28)	Ovoid 16.1 (14–17.5) × 10.7 (10–11)	Intranuclear	Israel
I. gonocephali Maupin, Diong & McQuistion, 1998	*Gonocephalus grandis*	Subspherical–ovoid 22.3 (19–25) × 18.7 (17–23)	Almond-shaped 13.5 (12–15) × 9.2 (8.5–10.0)	No data	Malaysia
I. lacerate Saum, Diong & McQuistion, 1997	*Calotes versicolor*	Subspherical–ovoid 28.1 (23.0–31.0) × 26.5 (23.0–28.0)	Ovoid 14.6 (13.0–15.0) × 10.3 (7.0–11.0)	No data	Singapore
I. phrynocephali Ovezmukhammedov, 1971	*Phrynocephalus helioscopus*	Spherical 26.2 (24.3–27.0) × 26.2 (24.3–27.0)	Subspherical 14.7 (13.5–18.9) × 9.2 (8.1–13.5)	No data	Turkmenia
I. rayi Mandal, 1966	*Ptyctolaemus gularis*	Spherical 26.3 (25.5–27.4) × 26.3 (25.5–27.4)	Naviculoid 15.4 (14.5–16.3) × 8.6 (9.5–10.5)	No data	India
I. rustamovi Ovezmukhammedov, 1977	*Phrynocephalus reticulatus*	Spherical 26.2 (18.9–32.4) × 26.2 (18.9–32.4)	Pyriform 16.5 (13.5–18.9) × 11.7 (10.8–13.5)	No data	Turkmenia

All sizes are in micrometres

only species with a similar oöcyst morphology, differs in the appearance of wall-forming bodies which are evidently larger than those of *I. farahi* (cf. our Fig. 4 with figure 8 of Finkelman & Paperna, 1994).

Only limited information exists on the pathogenicity of reptilian species of *Isospora*. In the case of *I. amphiboluri* from an Australian agamid in captivity, the destruction of the intestinal epithelium and resulting deaths have been reported (McAllister et al., 1995). However, under natural condition, these coccidia appear to have little effect on parasitised hosts, which is also evident in the absence of histopathological changes in our material.

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