Eimeria fragilis and E. wambaensis, Two New Species of Eimeria Schneider (Apicomplexa: Eimeriidae) from African Anurans

Miloslav JIRKŮ¹ and David MODRÝ¹,²

¹Department of Parasitology, University of Veterinary & Pharmaceutical Sciences, Brno; ²Institute of Parasitology of Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

Summary. Two new species of coccidia (Apicomplexa: Eimeriidae) are described from Kenyan frogs. Eimeria fragilis sp. n., described from Chiromantis petersii, has ellipsoidal oocysts, 18.5 (17-19.5) × 15.2 (14.5-16) µm; lacking micropyle, oocyst residuum and polar granule. Sporocysts are dizzoic, navicular, 10.6 (9.5-12) × 6.8 (6-7) µm. Oocysts of Eimeria wambaensis sp. n. found in Hyperolius viridiflavus are ellipsoidal to ovoidal, 17.0 (15.0-18.5) × 13.0 (11.0-14.0) µm; without micropyle, oocyst residuum and polar granule. Sporocysts are dizzoic, navicular, 8.7 (8.0-10.5) × 6.0 (5.5-7.0) µm. Described species are the first Eimeria reported from African anurans.

Key words: Africa, anura, Apicomplexa, Chiromantis petersii, coccidia, Eimeria fragilis sp. n., E. wambaensis sp. n., Hyperolius viridiflavus, intranuclear development, Kenya.

INTRODUCTION

To date, there are 30 valid species of anuran coccidia, described within four genera: Eimeria Schneider, 1875 (16 species), Goussia Labbé, 1896 (2 species), Hyaloklossia Labbé, 1896 (1 species) and Isospora Schneider, 1881 (11 species) (Upton and McAllister 1988, Chen and Desser 1989, McAllister et al. 1995, Molnár 1995, Paperna and Lainson 1995, Paperna et al. 1997, Modrý et al. 2001, Bolek et al. 2003). Most named species originate from the holarctic region (Upton and McAllister 1988), with only a single species being described from anurans from the African continent. Goussia hyperolisi Paperna, Ogara et Schein, 1997 has been reported from central Kenya, parasitizes the digestive tract of tadpoles of the Common reed frog Hyperolius viridiflavus (Duméril et Bibron, 1841) (Paperna et al. 1997).

The coccidians described in this report represent first members of the genus Eimeria described from African anurans. Peters’ foam-nest treefrog, Chiromantis petersii kelleri Boettger, 1893, and Common reed frog, Hyperolius viridiflavus, are African anurans of the families Rhacophoridae and Hyperoliidae, respectively. Chiromantis p. kelleri is restricted in its distribution to...
xeric areas covered by dry savannah from northern Kenya through Ethiopia to northern Somalia. In contrary, *H. viridiflavus* is widely distributed throughout the tropical savannah from Senegal to the Indian Ocean coast (Schiøtz 1999).

**MATERIALS AND METHODS**

Animals were collected during two field trips to Kenya in 2003 and 2004. Forty adult *Hyperolius viridiflavus* were collected in September 2003 by hand at night from a pond, approximately 0.5 km from Udo’s camp site in Kakamega forest reserve in western Kenya (Western province, 00°20'55.7” N, 34°51'56.2” E). Two more adults of *H. viridiflavus* were captured in February 2004, by hand at night in a creek in the vicinity of Wamba (Rift Valley province, 00°56'58.4” N, 37°20'56.9” E). A single adult *Chiromantis petersii kelleri* was collected in February 2004, by local people in Kula Mawe (Eastern province, 00°34'11.1” N, 38°11'56.3” E). Frogs were identified according to Schiøtz (1999). Animals were housed for several hours in individual plastic boxes until they defecated, afterwards they were euthanized by overdosing with barbiturates (Thiopental® Spofa), according to Schiøtz (1999). Animals were housed for several hours in individual plastic boxes until they defecated, afterwards they were euthanized by overdosing with barbiturates (Thiopental® Spofa), dissected, and processed for following standard parasitological protocol.

Equidistantly spaced portions of the gastrointestinal tract, liver, kidney and muscle samples were fixed in 10% buffered formalin, processed routinely for histology, stained with haematoxylin and eosin, and examined for the presence of endogenous stages of coccidia. Measurements of endogenous stages were made on 10-15 individuals of each stage.

Faecal samples were placed immediately in 2.5% potassium dichromate, stored at room temperature for 4 weeks, transported to the laboratory in the Czech Republic, stored for next 3 months in 6-7°C, and examined for the presence of coccidian oocysts. Oocysts were concentrated by flotation method, using modified Sheather’s sugar solution (specific gravity 1.30), and examined using Nomarski interference contrast optics (NIC).

Measurements were made on 20 oocysts, using an Olympus AX 70 microscope equipped with a calibrated ocular micrometer and are reported in micrometers (µm), usually as the mean, followed by range in parentheses.

**RESULTS**

Adult *Chiromantis petersii kelleri* from Kula Mawe and a single adult *Hyperolius viridiflavus* from Wamba shed oocysts of 2 different, previously undescribed coccidia, both belonging to the genus *Eimeria*. No coccidian infection was detected in the 40 of *H. viridiflavus* from the Kakamega forest reserve. All *H. viridiflavus* from both Wamba and Kakamega were breeding individuals. Breeding status of *C. p. kelleri* was not recognized.

*Eimeria fragilis* sp. n. (Figs 1-6, 10, 12-16)

Description of oocysts: Unsporulated oocysts (Fig. 1) contain a subspherical sporont, approximately 13 × 11, composed of fine granules, 0.5-1 in diameter, containing vacuolised area (probably nucleus) ~2.5 in diameter. Outside this granular mass, usually a few larger granules, 1.5-3.5 in diameter, are present near one end of the oocyst. In sporulating oocysts (Fig. 2), 4 subspherical sporoblasts are present, measuring approximately 8.5 × 7. Fully sporulated oocysts (Figs 3-6, 10) are broadly ellipsoidal, 18.5 (17-19.5) × 15.2 (14.5-16); shape index (length: width ratio, SI) 1.2 (1.1-1.3). Micropyle, oocyst residuum and polar granule are absent. Oocyst wall is smooth, colorless, appearing as single-layered in light microscopy, ~0.5 thick. Sporocysts are dizoic, navicular (Fig. 3), 10.6 (9.5-12) × 6.8 (6-7), SI 1.57 (1.46-1.71), with smooth, colourless and single-layered sporocyst wall. One pole of each sporocyst is slightly thickened, with a barely distinct Stieda body. Sporocyst residuum present, usually as a mass composed of fine granules ~1 in diameter. Sporocyst residuum (even in completely sporulated oocysts) almost completely fills the sporocyst, leaving only small parts of the sporozoites visible (Fig. 4). Sporozoites are elongated, 10 × 2, arranged head to tail within sporocyst. Each sporozoite possesses two spherical refractile bodies 1-1.5 in diameter, located on the opposite ends of the sporozoite, and a centrally located nucleus, 1.5 in diameter. Sporozoite cytoplasm is finely granulated (Fig. 5).

One of the most distinctive features of *E. fragilis* sp. n. is its fragility. There is a tendency of sporocysts in sporulated oocysts to disintegrate and release free sporozoites into the oocyst’s content (Figs 5, 6). After four months of storage, 80% of sporulated oocysts contained free zoites. Additionally, oocysts start to break down immediately in hypertonic conditions of Sheather’s solution, and after ~15-20 min, oocyst walls are completely broken, leaving only collapsed sporocysts.

Morphology of endogenous stages: All endogenous stages putatively identified as *E. fragilis* are surrounded by a parasitophorous vacuoles and develop within nuclei of epithelial cells, in the small intestine. Occasionally, we observed more than one trophozoite in some nuclei of host cells. Additionally, when multiple developmental stages of *E. fragilis* were present within an individual host cell nucleus, each stage evidently possessed its own parasitophorous vacuole. Early trophozoites (Fig. 12), 3 × 2, were located within their vacuole (5 × 3.5-4) inside a host cell nucleus. Meronts
New *Eimeria* spp. from African anurans

(Fig. 13) containing approximately 5 merozoites measuring $5 \times 1$, are $6.7 \times 4.5$. Mature microgamonts (Fig. 14) of irregular shape, measured approximately $10-15 \times 7.5-14$. Macrogamonts (Figs 15, 16) in various stages of maturity measured $14-17 \times 11-17$ and contained a large nucleus and numerous eosinophilic granules, 0.5-1 in diameter.

**Type host:** *Chiromantis petersii kelleri* Boettger, 1893, (Anura: Rhacophoridae), Peters’ foam-nest treefrog or Central foam-nest tree frog (common names according to Frost (2004)).

**Type material:** Photosyntypes of sporulated oocysts and histological sections with endogenous stages are deposited under collection number R 60/04 in the collection of Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic. Voucher specimen of *C. p. kelleri* [a symbiotype sensu Frey *et al.* (1992)] is deposited in the herpetological collection of the National Museums of Kenya, Nairobi under collection number A/4138.

**Type locality:** Kula Mawe, Kenya (Eastern province, 00°34’11.1” N, 38°11’56.3” E).

**Prevalence:** Only a single animal was examined.

**Sporulation and sporulation time:** Unknown, oocysts obtained from faeces preserved in 2.5% potassium dichromate.

**Site of infection:** Intranuclear in epithelial cells of small intestine.

**Etymology:** Specific epithet refers to very fragile oocyst and sporocyst walls and tendency of sporocysts to disintegrate and release free sporozoites in to the lumen of oocyst.
**Eimeria wambaensis** sp. n. (Figs 7-9, 11, 17-20)

**Description of oocysts:** Oocysts (Figs 7-9, 11) are ellipsoidal to ovoidal, 17.0 (15.0-18.5) × 13.0 (11.0-14.0); SI 1.3 (1.15-1.55). Micropyle, oocyst residuum and polar granule absent. Oocyst wall is smooth, appearing bilayered in light microscopy, outer layer approximately 0.5-0.7 thick, inner layer ~ 0.1-0.2 thin. Sporocysts are dizoic, navicular, 8.7 (8.0-10.5) × 6.0 (5.5-7.0), SI 1.4 (1.2-1.6), with smooth, colourless and single-layered sporocyst wall. One pole of sporocyst is slightly thickened, with a barely distinct Stieda body. Sporocyst pole opposite to Stieda body pointed (Fig. 9). Oocyst wall encloses sporocysts quite tightly and sporocysts sometimes seem a little bit deformed. Sporocyst residuum almost completely fills the sporocyst, composed of granules of irregular shape, 1.5-2 in diameter, leaving only small parts of the sporozoites visible. Sporozoites are arranged head to tail within the sporocyst. Each sporozoite possesses 1 spherical body (probably nucleus), 1.5 in diameter, located in the central part of the zoite. Sporozoite cytoplasm is finely granulated.

**Morphology of endogenous stages:** All endogenous stages putatively identified as *E. wambaensis* are surrounded by a parasitophorous vacuoles and develop within nuclei of epithelial cells, of the small and large intestine, with most of the infection occurring in the anterior and central part of small intestine. Early trophozoites (Fig. 17) are 3.5 × 2-3, located within a vacuole (5 × 4) inside the host cell nucleus. Mature microgamonts (Fig. 20) are spherical to elongately ellipsoidal, approximately 10-15 × 8-10. Macrogamonts, in various stages of maturity, (Figs 18, 19) were 11-16 × 7-12 and contained large nucleus and numerous eosinophilic granules resembling wall forming bodies, up to 1 in diameter. No meronts were observed.

**Type host:** *Hyperolius viridiflavus* (Duméril et Bibron, 1841), (Anura: Hyperoliidae), Common reed frog or Kenyan reed frog (common names according to Frost (2004)).

**Type material:** Photosyntypes of sporulated oocysts and histological sections with endogenous stages are deposited in the collection of the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, R 65/04. Voucher specimen of *Hyperolius viridiflavus* (a symbiotype) is deposited in the herpetological collection of National Museums of Kenya, Nairobi, A/4130.
New *Eimeria* spp. from African anurans 171

**Type locality:** Kenya, Wamba, (Rift Valley province, 00°56'58.4" N, 37°20'56.9" E).

**Prevalence:** 1/2 (50%) frogs from Wamba was infected; all 40 specimens from Kakamega forest were coccidia free.

**Sporulation and sporulation time:** Unknown, oocysts obtained from faeces preserved in 2.5% potassium dichromate.

**Site of infection:** Intranuclear in epithelial cells of both small and large intestine.

**Figs 12-20.** Micrographs of endogenous stages. 12 - early trophozoite of *Eimeria fragilis* sp. n. (arrowhead), surrounded by distinct parasitophorous vacuole (arrow) located within host cell nucleus; 13 - meront containing merozoites (arrowhead); 14 - nearly mature microgamont of *E. fragilis* (arrowhead); 15 - immature macrogamont of *E. fragilis* (arrowhead) surrounded by residuum of host cell nucleus (arrow); 16 - mature macrogamont of *E. fragilis* sp. n. (arrowhead). Note that host cell nucleus surrounds macrogamont tightly, forming thin layer on its surface (arrow); 17 - early trophozoite of *E. wambaensis* (arrowhead), surrounded by parasitophorous vacuole (arrow), located within the host cell nucleus; 18 - immature macrogamont of *E. wambaensis* (arrowhead). Note deformed and enlarged host cell nucleus (arrow) containing distinct parasitophorous vacuole; 19 - mature intranuclear macrogamont of *E. wambaensis*; 20 - mature microgamont of *E. wambaensis* containing fully developed microgametes (arrowheads). L - intestinal lumen. Scale bar 10 µm.
Etymology: Specific name wambaensis is derived from the Wamba, a village in northern Kenya, which is the type locality.

DISCUSSION

Both species of coccidia described in this study differ clearly from each other in oocyst and sporocyst shape and size. Oocysts of E. wambaensis are smaller and often have an ovoidal shape, with one end thickened, compared to ellipsoidal oocysts of E. fragilis. Sporocysts of the two described species differ both in their dimensions, and also in shape. Sporocysts of E. fragilis are longer, with SI exceeding 1.5, containing sporocyst dimensions, and also in shape. Sporocysts of E. wambaensis are measured 1.5-2 in diameter, as typical for age, in contrary to large sporocyst residuum granules exceeding 1 µm in diameter even in oocysts of different species. Additionally, the tendency of sporocysts of E. fragilis to disintegrate and release free sporozoites into the oocyst content is another distinguishing feature. However, oocysts of E. fragilis were first examined after four months of storage, when 80% of sporulated oocysts contained free sporozoites. Thus, it is possible, that free sporozoites do not occur in freshly sporulated oocysts. Among all anuran coccidia, presence of free sporozoites in oocysts is reported only in Eimeria prevoti (Laveran et Mesnil, 1902) from Rana kl. esculenta and R. temporaria Linnaeus, 1758, and E. salamandraceae (Steinhäus, 1889) from Salamandra salamandra (Linnaeus, 1758) (Steinhäus, 1889; Labbé, 1894; Pelléry 1974). However, no comparable micrographs and adequate description of endogenous stages morphology were provided by authors, and intranuclear development of these two taxa must be confirmed by further studies.

The diversity of African Anura sharply contrasts with low number of described coccidian parasites. It is evident, that apicomplexan parasitic organisms represent a neglected part of African biodiversity and as such require further studies.

Acknowledgements. The research on diversity of parasites of East African vertebrates was facilitated by Biota East Africa; we are deeply indebted to Jörn Köhler and Stefan Lüters for generous help and useful discussion. We thank Richard Bagine (Kenyan Wildlife Service) for help and issuing necessary permits; Damaris Rotich (National Museums of Kenya) helped with organisation of the trip and kindly provided necessary laboratory space in Nairobi and organised all the logistics of the expedition to northern Kenya. The comments of two anonymous referees helped in improving an earlier draft of this manuscript. Finally, we thank members of 2003 and 2004 expeditions to Kenya, namely Jana Kopečná, Martin Kamler, Pavel Široký, Tomáš Mazuch, Luděk Kofrený and Petr Neckas for help with field work. We are indebted to Olympus C&S for generous technical support. This study was supported by the grants No. 206/03/1544 and 524/03/H133 from the Grant Agency of the Czech Republic.

REFERENCES

from the western chorus frog. Pseudacris triseriata triseriata, from eastern Nebraska. J. Parasitol. 89: 522-528
Schiøtz A. (1999) Treefrogs of Africa. Chimaira Buchhandelsgellschaft, Frankfurt am Main, Germany

Received on 9th November, 2004; revised version on 13th January, 2005; accepted on 18th January, 2005