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

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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 dizoic, 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 dizoic, 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

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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), 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

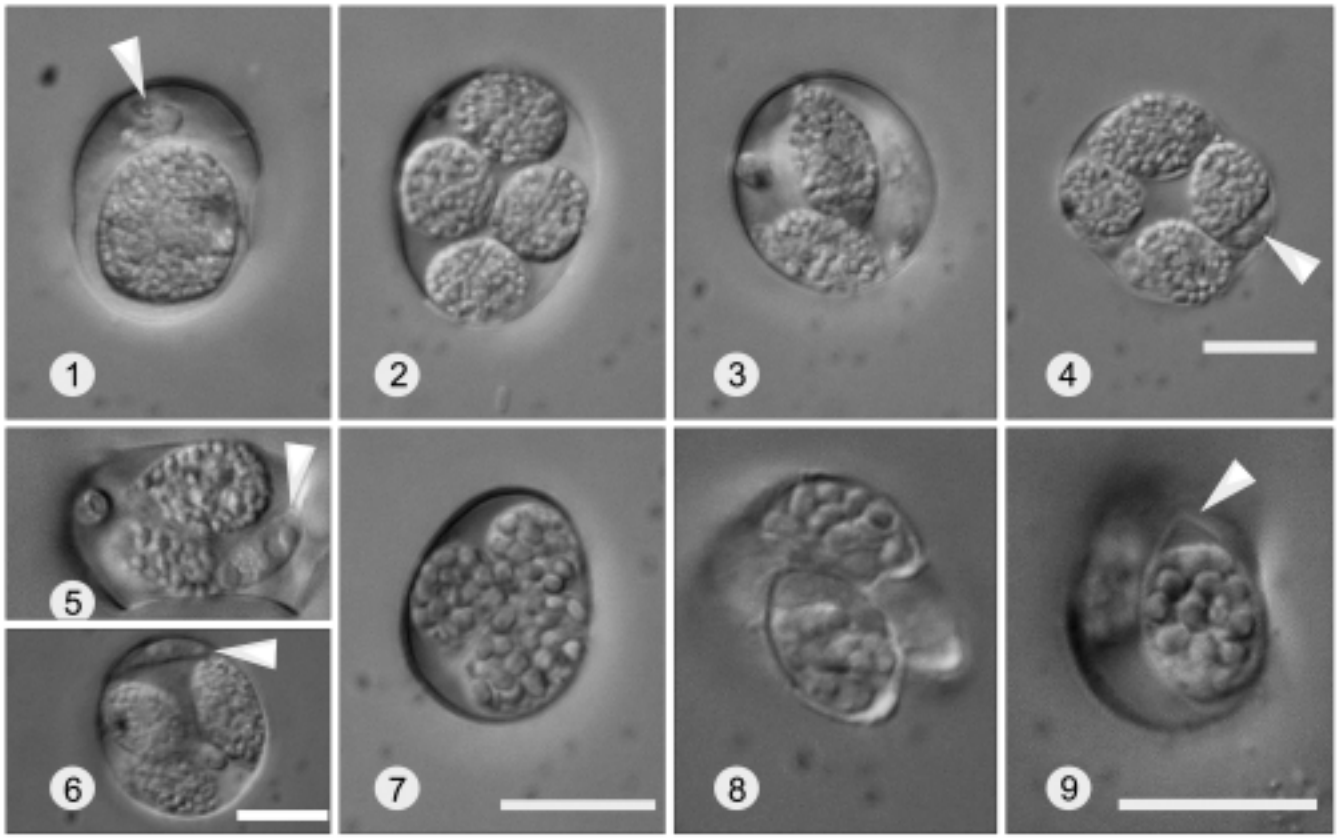
Adult *Chiromantis petersii kelleri* from Kula Mawe and a single adult *Hyperolius viridiflavus* from Wamba shed oocysts of 2 different, previously undescribed coccidia, both belonging to the genus *Eimeria*. No coccidian infection was detected in the 40 of *H. viridiflavus* from the Kakamega forest 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 zoite, 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



Figs 1-6 - Nomarski interference contrast micrographs of oocysts of *Eimeria fragilis* sp. n. in various stage of sporulation. **1** - oocyst with concentrated sporont. Note free granules near the pole (arrowhead); **2** - oocyst with four subspherical sporoblasts; **3** - sporulated oocyst. Note typical shape of the sporocyst; **4** - sporulated oocyst with collapsing oocyst wall. Arrowhead indicates the only visible part of sporozoite, almost entirely obscured by sporocyst residuum; **5, 6** - collapsed oocysts, containing free sporozoites (arrowheads). Scale bar 10 µm. Figs 1-5 are in the same scale.
Figs 7-9 - Nomarski interference contrast micrographs of oocysts of *E. wambaensis* sp. n. in various stage of sporulation. **7** - oocyst containing four (only three visible) subspherical sporoblasts; **8** - collapsed oocyst, showing typical shape of sporocysts; **9** - sporulated oocyst. Note typically pointed pole of sporocyst (arrowhead). Scale bars 10 µm. Figs 8, 9 are in the same scale.

(Fig. 13) containing approximately 5 merozoites measuring 5 × 1, are 6-7 × 4-5. Mature microgamonts (Fig. 14) of irregular shape, measured approximately 10-15 × 7.5-14. Macrogamonts (Figs 15, 16) in various stages of maturity measured 14-17 × 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.

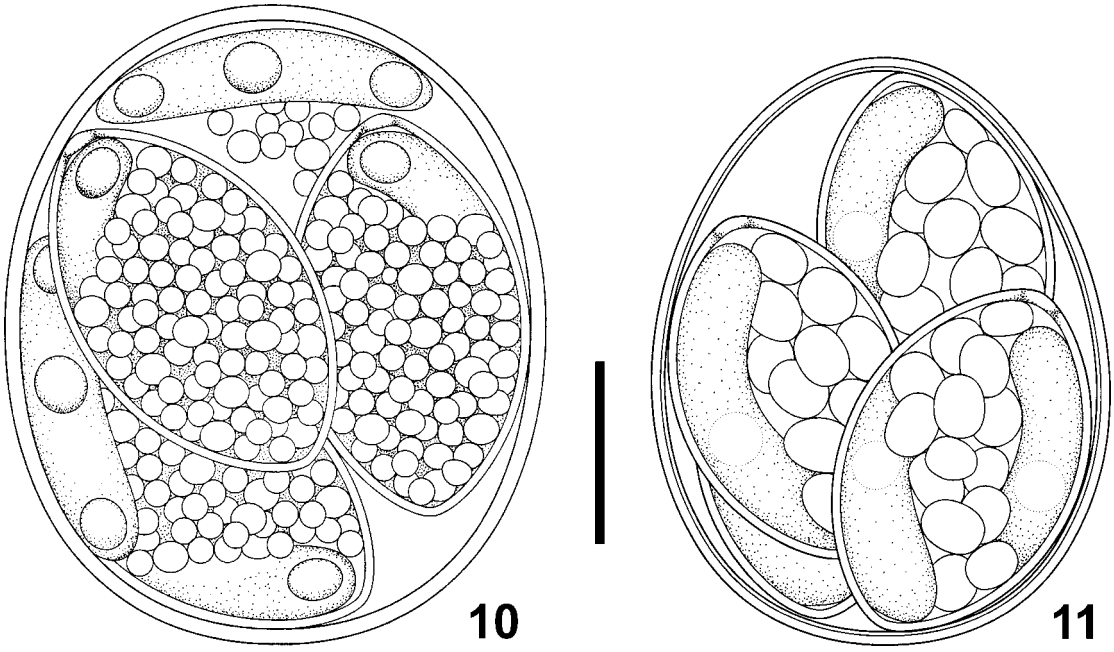
Type locality: Kula Mawe, Kenya (Eastern province, 00°34'11.1" N, 38°11'56.3" E).

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.



Figs 10, 11. Composite line drawings of sporulated oocysts, both in the same scale. **10** - *Eimeria fragilis* sp. n. containing free sporozoites and three sporocysts; **11** - *Eimeria wambaensis* sp. n. Scale bar 5 µm.

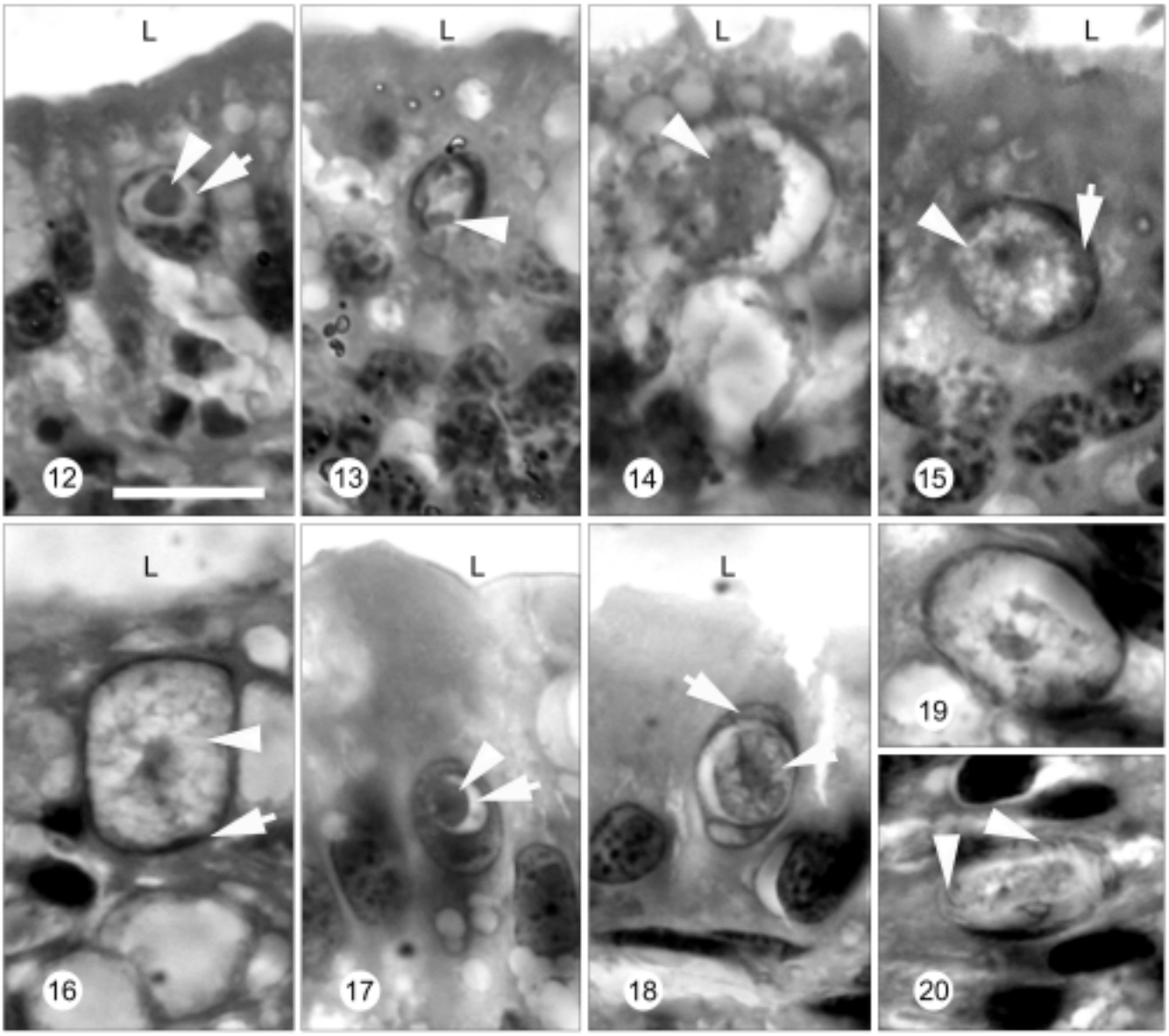
Eimeria wambaensis sp. n. (Figs 7-9, 11, 17-20)

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.



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.

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.

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 residuum composed of remarkably finer granules not exceeding 1 μm in diameter even in oocysts of different age, in contrary to large sporocyst residuum granules measuring 1.5-2 in diameter, as typical for *E. wambaensis*. 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* Linnaeus, 1758 from Europe, but this species differs in having distinct Stieda body and large oocyst residuum (Laveran and Mesnil 1902, Boulard 1975). Both new species of *Eimeria* described in this study differ from all other known anuran coccidia in oocyst or sporocyst dimensions and other morphological features. Moreover, the geographical origin and host phylogenetic distance make the conspecificity with other described species unlikely.

Recent studies on excystation structures of coccidia from poikilothermic hosts (Jirků *et al.* 2002) pointed out the importance of these structures for the classification of *Eimeria*-like coccidia. Although particular attention was given to describe the excystation structures on sporocysts of *E. fragilis* and *E. wambaensis*, we were unable to detect longitudinal sutures on the sporocysts of both *E. fragilis* and *E. wambaensis*. However, longitudinal sutures typical for sporocysts of numerous coccidia from poikilotherms usually are visible using light microscopy (pers. obs.). More importantly, both *E. fragilis* and *E. wambaensis* possessed barely distinct Stieda body on one pole of each sporocyst, and we believe these represent true Stieda bodies, clearly indicating that both of these coccidians are representatives of the genus *Eimeria*. Barely visible Stieda body also was reported in other anuran coccidia, for example in North American *E. flexuosa* Upton *et* McAllister, 1988; *E. streckeri* Upton *et* McAllister, 1988; *E. fitchi* McAllister, Upton, Trauth *et* Bursey, 1995 and South American *E. bufomarini* Paperna *et* Lainson, 1995 (Upton and McAllister 1988, McAllister *et al.* 1995, Paperna and Lainson 1995, Bolek *et al.* 2003).

Intranuclear localisation of endogenous stages observed in both *Eimeria* species described in this study was previously reported in several eimerian coccidia of the genera *Isospora*, *Cyclospora*, *Tyzzeria* and *Eimeria*, parasitizing higher vertebrates (Pellérdy 1974, Shibalova and Morozova 1979, Entzeroth and Scholtyseck 1984, Mohamed and Molyneux 1990, Paperna and Finkelman 1998, Pakandl *et al.* 2002). Among species parasitizing amphibians, intranuclear localization was reported in *E. ranarum* (Labbé, 1894), from *Rana* kl. *esculenta* and *R. temporaria* Linnaeus, 1758, and *E. salamandrae* (Steinhaus, 1889) from *Salamandra salamandra* (Linnaeus, 1758) (Steinhaus, 1889; Labbé, 1894; Pellérdy 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.

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REFERENCES

Bolek M. G., Janovy J. Jr., Irizarry-Rovira A. R. (2003) Observations on the life history and descriptions of coccidia (Apicomplexa)

from the western chorus frog. *Pseudacris triseriata triseriata*, from eastern Nebraska. *J. Parasitol.* **89:** 522-528
Boulard Y. (1975) Redescription d' *Eimeria prevoti* (Laveran et Mesnil, 1902) (Eimeriidae) parasite de la Grenouille verte en Normandiae. *Protistologica* **11:** 245-249
Chen G. J., Desser S. S. (1989) The coccidia (Apicomplexa: Eimeriidae) of frogs from Algonquin Park, with descriptions of two new species. *Can. J. Zool.* **67:** 1686-1689
Entzeroth R., Scholtyseck E. (1984) Ultrastructural study of intestinal coccidia of the European mole (*Talpa europea*). *Zbl. Bakt. Hyg.* **256:** 280-285
Frey J. K., Yates T. L., Duszynski D. W., Gannon W. L., Gardner S. L. (1992) Designation and curatorial management of type host specimens (symbiotypes) for new parasite species. *J. Parasitol.* **78:** 930-932
Frost D. R. (2004) Amphibian species of the world: an Online Reference. Version 3.0 (22 August, 2004). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.php. American Museum of Natural History, New York, USA
Jirků M., Modrý D., Šlapeta J. R., Koudela B., and Lukeš J. (2002) The phylogeny of *Goussia* and *Choleoeimeria* (Apicomplexa: Eimeriorina) and the evolution of excystation structures in coccidia. *Protist* **153:** 380-389
Laveran M. A., Mesnil F. (1902) Sur deux coccidies intestinales de la Rana esculenta. *C. r. Séanc. Soc. Biol.* **54:** 857-860
McAllister C. T., Upton S. J., Trauth S. E., and Bursey C. R. (1995) Parasites of wood frogs, *Rana sylvatica* (Ranidae), from Arkansas, with a description of a new species of *Eimeria* (Apicomplexa: Eimeriidae). *J. Helm. Soc. Wash.* **62:** 143-149
Modrý M., Šlapeta J. R., Jirků M., Oborník M., Lukeš J., Koudela B. (2001) Phylogenetic position of a renal coccidium of the European green frogs, *'Isospora' lieberkuehni* Labbé, 1894 (Apicomplexa: Sarcocystidae) and its taxonomic implications. *Int. J. Syst. Evol. Microbiol.* **51:** 767-72
Mohamed H. A., Molyneux D. H. (1990) Developmental stages of *Cyclospora talpae* in the liver and bile duct of the mole (*Talpa europea*). *Parasitology* **101:** 345-350
Molnár K. (1995) Redescription of *Goussia neglecta* n. comb. (Nöller, 1920) (Apicomplexa: Coccidia) and notes on its occurance in the gut of tadpoles. *Acta Vet. Hung*. **43:** 269-275
Pakandl M., Reynaud M. C., Chauve C. M. (2002) Electron microscopic study on the endogenous development of *Eimeria mulardi*, Chauve, Reynaud and Gounel, 1994: a coccidium from the mule duck. *Parasitol. Res.* **88:** 160-164
Paperna I., Finkelman S. (1998) The fine structure of reptilian *Isospora* species with intranuclear endogenous development. *Parassitologia* **40:** 283-296
Paperna I., Lainson R. (1995) Life history and ultrastrusture of *Eimeria bufomarini* n. sp. (Apicomplexa: Eimeriidae) of the Giant toad, *Bufo marinus* (Amphibia: Anura) from Amazonian Brazil. *Parasite* **2:** 141-148
Paperna I., Ogara W., Schein M. (1997) *Goussia hyperolisi* n. sp.: a coccidian infection in reed frog *Hyperolis viridiflavus* tadpoles which expires towards metamorphosis. *Dis. Aquat. Org.* **31:** 79-88
Pellérdy L. P. (1974) Coccidia and Coccidiosis. 2nd ed., Verlag Paul Parey, Berlin-Hamburg, Germany
Schiøtz A. (1999) Treefrogs of Africa. Chimaira Buchhandelsgesellschaft, Frankfurt am Main, Germany
Shibalova T. A., Morozova T. I. (1979) Intranuclear development of macrogametes of the coccidium *Tyzzeria parvula. Tsitologia* **21:** 669-672 (in Russian)
Upton S. J., McAllister C. T. (1988) The coccidia (Apicomplexa: Eimeriidae) of Anura, with descriptions of four new species. *Can. J. Zool.* **66:** 1822-1830

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