

Eimeria terraepokotorum n. sp. (Apicomplexa: Eimeriidae) from *Hoplobatrachus occipitalis* (Anura: Ranidae) from Kenya

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Summary. *Eimeria terraepokotorum* n. sp. (Apicomplexa: Eimeriidae) is described from *Hoplobatrachus occipitalis* from Kenya. Endogenous stages develop extranuclearly, in the cytoplasm of epithelial cells of the small intestine. Oocysts ovoidal to broadly elliptical, 20.2 (18.0-24.5) × 16.0 (13.5-18.5) μm; lacking micropyle and polar granule. Oocyst residuum present. Sporocysts dizoic, 9.8 (8.5-11.5) × 7.2 (6.0-8.0) μm, possessing a prominent Stieda body.

Key words: Africa, Anura, Apicomplexa, Coccidia, *Eimeria terraepokotorum* n. sp., *Hoplobatrachus occipitalis*, Kenya.

INTRODUCTION

During a long-term study of parasites of African vertebrates we examined fecal samples from numerous anurans from Kenya, and recently described 2 new *Eimeria* species (Jirků and Modrý 2005).

Here, we provide a summary of all anurans, examined during 3 field trips to Kenya in 2003 and 2004, together with the description of a new species of *Eimeria*. The type host of this new *Eimeria*, *Hoplobatrachus occipitalis* (Günther, 1858), the Afri-

can Tigrine Frog (Anura: Ranidae), is one of the most widely distributed anuran species of Afro-tropical zoogeographic realm. It inhabits a wide range of habitats from wells and seasonal water courses in arid areas to permanent water bodies, swamps and rivers in tropical savanna (Schleich *et al.* 1996, Rödel 2000, Channing and Howell 2006).

MATERIALS AND METHODS

Animals were collected during 3 field trips to Kenya in 2003 and 2004. Fecal samples of all animals (Table 1) were examined coprologically using a flotation method (see below). Most dissected animals were preserved in 70% alcohol and voucher specimens are deposited in the Herpetological Collection of the National Museums of Kenya, Nairobi.

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Five specimens (1 juvenile, 2 subadults, 2 adults) of *H. occipitalis* were collected at the beginning of the rainy season on the end of September 2004. The frogs were caught in temporary pools and seasonal water course near Nginyang village in west-central Kenya. Frogs were identified according to Channing and Howell (2006). Animals were placed for several hours into plastic boxes until they defecated, than euthanized by overdosing them with barbiturates (Thiopental® Spofa), dissected and processed for the following protocol.

Pieces of liver, kidney, muscle and equidistantly spaced portions of the gastrointestinal tract 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.

Fecal samples were placed (and suspended) immediately in 2.5% aqueous solution of potassium dichromate ($K_2Cr_2O_7$) in vials containing enough air, stored at room temperature for 2 weeks, transported to the laboratory in the Czech Republic, and examined for the presence of coccidian oocysts. Oocysts were concentrated by flotation method, using modified Sheather's sugar solution (specific gravity 1.3), and examined using Nomarski interference contrast optics (NIC).

Measurements were made on 10-15 individuals of each endogenous developmental stage, and on 30 oocysts, using an Olympus AX 70 microscope equipped with a calibrated ocular micrometer and

are reported in micrometers (μm), usually as the means, followed by ranges in parentheses.

RESULTS

In total, 209 specimens of 16 anuran taxa were examined coprologically (Table 1). Of all the frogs examined, 5 (2.4%) possessed coccidian oocysts in their feces. Samples from 3 host species from 3 localities provided material suitable for further taxonomic work. As a result, 3 new species *Eimeria* were characterized morphologically, based both on endogenous and exogenous developmental stages, and described as a new species. Two of these species were described previously (Jirků and Modrý 2005). Additionally, unsporulated oocysts of undetermined coccidium, were found in feces of a single specimen of *Ptychadena* sp. 1 from Cheptongei village (Southern Cherangani Hills).

One juvenile and one subadult of *Hoplobatrachus occipitalis* from Nginyang shed oocysts of a previously

Table 1. Summary of anurans examined coprologically during the 3 field trips to Kenya in 2003 - 2004. Footnotes: a - samples collected in August 2003; b - samples collected in January 2004; c - samples collected in September 2004. Abbreviations: R - results of coprological examination (n examined / n infected with *Eimeria* sp.); UC - unsporulated coccidian oocysts observed in feces; RV - Rift Valley province; C - Coast province; E - East province; W - West province.

Taxon	Locality	R	coccidia found
<i>Afrana</i> sp. 1	Morijo ^a RV	4/0	–
<i>Afrana</i> sp. 2	E slope of Mt. Warges ^c RV	1/0	–
<i>Afrana</i> sp. 2	Wamba ^c RV	3/0	–
<i>Bufo</i> cf. <i>garmani</i>	Wamba ^c RV	2/0	–
<i>Bufo</i> cf. <i>garmani</i>	South Horr ^c RV	4/0	–
<i>Bufo gutturalis</i>	Witu ^c C	2/0	–
<i>Bufo</i> sp. 1	Loyangalani ^c E	2/0	–
<i>Bufo</i> sp. 2	Nginyang ^c RV	4/0	–
<i>Chiromantis petersii kelleri</i>	Kula Mawe ^b E, Mt. Forole ^c E	1/1 ^b , 1/0 ^c	<i>Eimeria fragilis</i>
<i>Hoplobatrachus occipitalis</i>	Nginyang ^c RV	5/2	<i>Eimeria terraepokotorum</i>
<i>Hyperolius viridiflavus</i>	Kakamega ^a W, Wamba ^c RV	40/0 ^a , 6/1 ^c	<i>Eimeria wambaensis</i>
<i>Hyperolius kivuensis</i>	Kakamega ^a W	20/0	–
<i>Kassina senegalensis</i>	South Horr ^c RV	20/0	–
<i>Tomopterna</i> sp.	Nginyang ^c RV	15/0	–
<i>Tomopterna</i> sp.	South Horr ^c RV	10/0	–
<i>Ptychadena</i> sp. 1	Cheptongei ^b RV	2/1	UC
<i>Ptychadena</i> sp. 2	E slope of Mt. Warges ^c RV	4/0	–
<i>Ptychadena</i> sp. 3	Kakamega ^b W	3/0	–
<i>Ptychadena</i> sp. 2	Wamba ^c RV	2/0	–
<i>Xenopus</i> sp.	Cheptongei ^b RV	15/0	–
<i>Xenopus</i> sp.	Kakamega ^b W	15/0	–
<i>Xenopus</i> sp.	Loita Hills ^a and Nguruman Esc. ^c RV	25/0	–
<i>Xenopus</i> sp.	Marsabit ^a E	10/0	–
Total of 16 taxa	14 localities	209/5 (2.4%)	3 spp. in total

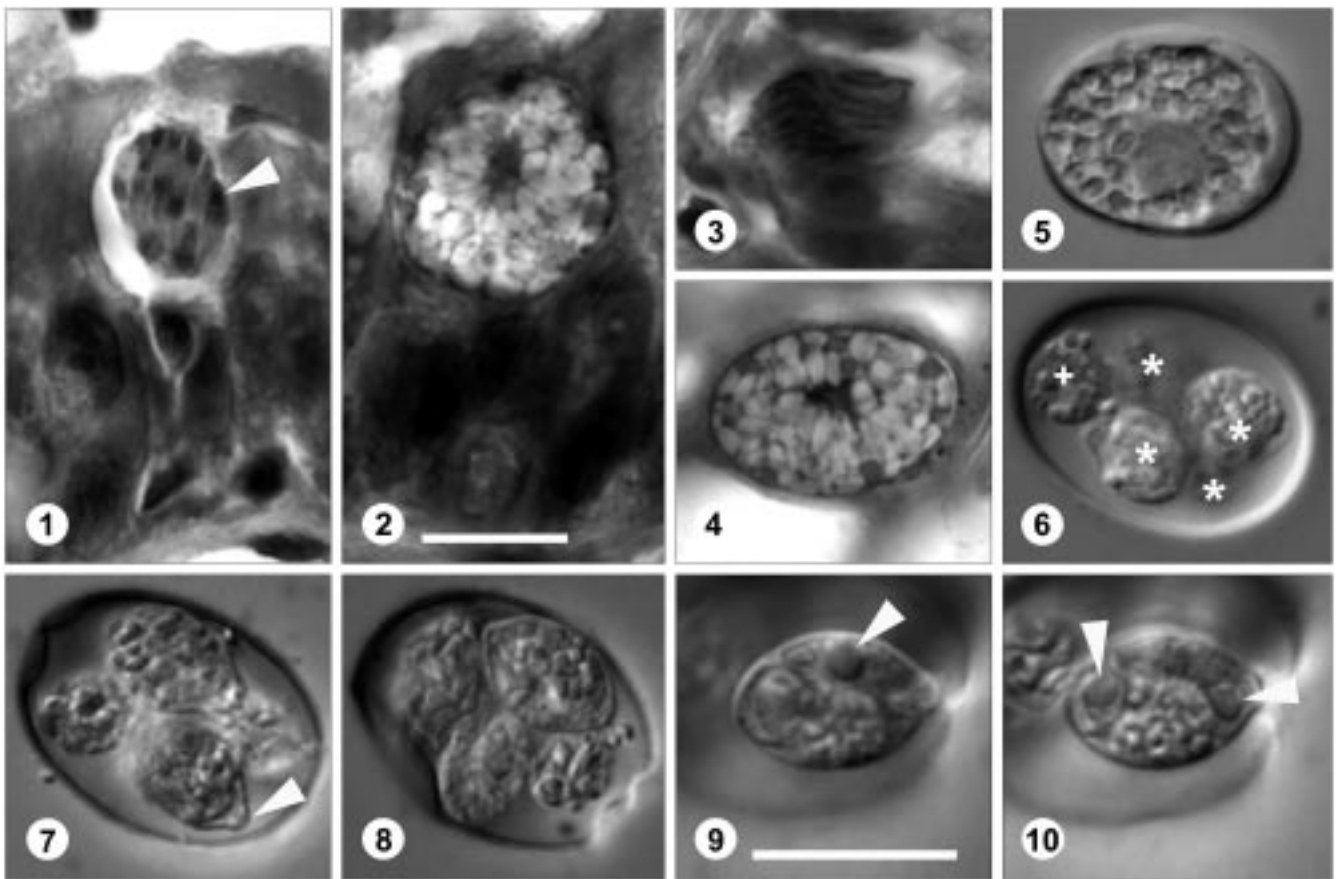
undescribed coccidium, evidently belonging to the genus *Eimeria* s. str. Neither coccidian oocysts nor endogenous stages were detected in another juvenile and two adult *H. occipitalis* from the same locality.

***Eimeria terraepokotorum* n. sp.**

Endogenous stages: All endogenous stages, putatively identified as those belonging to this species develop in the cytoplasm of epithelial cells of the small intestine. Mature meronts (Fig. 1) are spherical to broadly elliptical, $8-12 \times 5-8$, containing approximately 20, somewhat spirally arranged merozoites. In haematoxylin & eosin stained sections, one end of each merozoite (probably apical complex) is usually more

intensively stained (Fig. 1, arrowhead). Mature macrogamonts (Fig. 2), $16-19 \times 13-17$, are spherical or elliptical, containing a few distinct wall forming bodies (1.5-2 in diameter). Mature microgamonts elliptical, $10-12 \times 6-9$, containing numerous relatively thick-bodied microgametes (Fig. 3). In histological sections, unsporulated oocysts (Fig. 4), measuring $18-20 \times 11.5-15$, were found in the posterior part of the small intestine, either still attached to the intestinal mucosa, or freely in the intestine lumen. In most cases, oocysts were easily distinguishable from macrogamonts by the presence of thin, but clearly visible oocyst wall.

Exogenous stages, oocysts: Unsporulated oocysts isolated from preserved feces (Fig. 5) contain sporont,



Figs 1-4. Micrographs of endogenous stages of *Eimeria terraepokotorum* in histological sections stained with haematoxylin and eosin. **1** - mature meront possessing numerous merozoites with one end intensely stained (arrowhead); **2** - mature macrogamont with a few wall forming bodies on its periphery; **3** - mature microgamont showing relatively rough bodied microgametes; **4** - unsporulated oocyst within intestinal lumen. Note that fine oocyst wall is already clearly visible on its surface.

Figs 5-10. Nomarski interference micrographs of oocysts isolated from feces. **5** - unsporulated oocyst showing vacuolated area within sporont; **6** - oocyst in process of sporulation, showing 4 sporocysts (stars) and oocyst residuum (cross). Note that granules of the oocyst residuum resemble those forming sporocyst residua. **7** - fully sporulated oocyst with a sporocyst showing typical shape of Stieda body (arrowhead); **8** - fully sporulated oocyst; **9** - sporocyst possessing fully developed sporozoite with distinct nucleus (arrowhead); **10** - same sporocyst as on Fig. 9., focused to show 2 refractile bodies (one on each end) of the sporozoite (arrowheads). Scale bars: 5 μ m. Magnification of Figs 1-8, refer to scale bar of Fig. 2, and Figs 9, 10 to scale bar of Fig. 9.

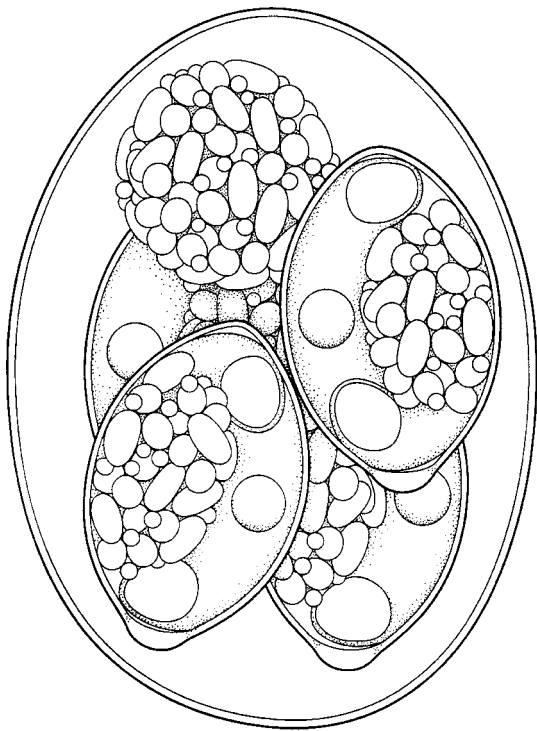


Fig. 11. Composite line drawing of sporulated oocyst of *Eimeria terraepokotorum* n. sp. Scale bar: 5 μ m.

composed of rough, elongate granules (2×1.5). Spherical halo (nucleus), up to 8 in diameter, is visible within sporont. Fully sporulated oocysts (Figs 7-11), relatively variable both in size and shape (compare Figs 5-8), are ovoidal to broadly elliptical, 20.2 (18.0 - 24.5) \times 16.0 (13.5 - 18.5), shape index (length: width ratio, SI) 1.3 (1.1 - 1.4). Oocyst residuum (7-11 in diameter) present, composed of spherical to subspherical mass of granules resembling those forming sporocyst residua (see below). Micropyle and polar granule absent. Oocyst wall colorless, smooth, approximately 0.6 thick, unilayered in light microscopy. Sporocysts dizoic, 9.8 (8.5 - 11.5) \times 7.2 (6.0 - 8.0), possessing prominent Stieda body (1.5 - 2×0.5 - 0.7) (Fig. 7). Sporocyst pole, opposite to Stieda body is usually slightly pointed. Finely granulated sporozoites without visible striation, contain probably 2 (one on each end) refractile bodies (2 - 3×1.5 - 2), and centrally located nucleus (2 in diameter). Sporocyst residuum composed by the mass of granules completely filling space between sporozoites (Figs 7, 8) or forming subspherical mass (Figs 9, 10). Granules of the sporocyst residuum are of two types: elongated ones (2 - 2.5×1 - 1.5) accompanied by distinctly finer spherical granules (0.5 - 1 in diameter).

Generally, the size and shape of granules, forming both sporocyst and oocyst residua is quite variable. Such variability may be associated with the process of sporulation, since granules of sporont are considerably coarser, than those forming residua of mature oocysts and sporocysts (compare Fig. 5 with Figs 6-10).

Type host: *Hoplobatrachus occipitalis* (Günther, 1858), (Anura: Ranidae), African Tigrine Frog.

Type material: Photosyntypes of oocysts in various stages of sporulation and hematoxylin and eosin stained paraffin sections with endogenous stages, together with piece of type-hosts' liver (for eventual DNA isolation) are deposited in the collection of the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic under the collection number R 113/05. Voucher specimen of *Hoplobatrachus occipitalis* [a symbiotype sensu Frey *et al.* (1992)] is deposited in herpetological collection of National Museums of Kenya, Nairobi, under collection number: NMK A/4246.

Type locality: Kenya, Nginyang (Rift Valley province, $01^{\circ}09'33''$ N, $37^{\circ}15'47''$ E). All examined *H. occipitalis* were collected in temporary pools in riverside sand sediments along the river flowing through the Nginyang village.

Prevalence: $2/5$ (40 %) frogs from Nginyang were infected.

Sporulation and sporulation time: Exogenous, only unsporulated oocysts were observed in intestine in histological sections.

Site of infection: Epithelial cells of the entire small intestine, extranuclear.

Etymology: Specific name *terraepokotorum* is a genitive case of Latin term Terra Pokotorum (= Land of Pokots); it reflects the location of the type locality, situated in the hearth of territory of Pokot tribe.

DISCUSSION

Of all 18 *Eimeria* spp. described from anuran hosts to date, only 3 species are similar enough to be compared to *E. terraepokotorum* n. sp. *Eimeria cyanophlyctis* Chakravarty *et al.*, 1944 from India differs in having distinctly narrower (11.0×4.4 - 6.6 . vs. 8.5 - 11.5×7.2 6.0 - 8.0 in *E. terraepokotorum* n. sp.), spindle-shaped sporocysts lacking sporocyst residuum (Chakravarty and Kar 1952). *Eimeria leptodactyli* Carini, 1931 from S America, most closely resembling *E. terraepokotorum* n. sp. by oocysts and sporocyst size, differs in general

appearance of oocyst residuum (granules arranged in rosettes) and presence of only scanty sporocyst residuum (Carini 1931). *Eimeria streckeri* Upton et McAllister, 1988 from North America differs in oocyst shape (spherical), presence of distinct vacuole within oocyst residuum (absent in *E. terraepokotorum* n. sp.), and presence of indistinct Stieda body (distinct in *E. terraepokotorum* n. sp.) (Upton and McAllister 1988). Additionally, *E. terraepokotorum* n. sp. differs clearly from both congeners (*Eimeria fragilis* and *Eimeria wambaensis*) recently described from African amphibians (Jirků and Modrý 2005) by oocyst and sporocyst size and shape, by the presence of oocyst residuum, and by the presence of distinct Stieda body. Moreover, the localization of endogenous stages of *E. terraepokotorum* n. sp. is different, as both *E. fragilis* and *E. wambaensis* are strictly intranuclear.

Although Table 1 is self explanatory two aspects of coccidian infections in anurans obtained during our study are worth to note. Both infected *H. occipitalis* were immature. This result is in contradiction to suggestions of other authors (Upton and McAllister 1988, Bolek et al. 2003) that coccidian infections in frogs may be restricted mainly to breeding animals. This might be true in case of terrestrial species, but our data show that, at least in semiaquatic species, infections can be common also in immature, non-breeding individuals. On the other hand, we examined 65 individuals of African claved frogs (*Xenopus* spp.) of various age from 4 localities, without any positive record of coccidian infection. All *Xenopus* spp. are strictly aquatic anurans with extraordinarily diverse parasitofauna (Tinsley 1996). Despite the fact that high numbers of *Xenopus* spp. were imported to Europe and USA as laboratory animals, no coccidia have ever been reported from these hosts. The apparent absence of coccidia in *Xenopus* spp. is surprising, since aquatic life-style in dense populations might normally favor infection by coccidian parasites with direct life-cycle.

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REFERENCES

- Bolek M. G., Janovy J., 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
- Carini A. (1931) *Eimeria leptodactyli* n. sp., rencontrée dans l'intestin du *Leptodactylus ocellatus*. *C. R. Seances Soc. Biol. Ses Fil.* **106**: 1019
- Chakravarty M., Kar A. B. (1952) The life history and affinities of two salientian Coccidia, *Isospora stomaticae* and *Eimeria cyanophlyctis*, with a note on *Isospora wenyoni*. *Proc. Zool. Soc. Bengal.* **5**: 11-18
- Channing A., Howell K. M. (2006) Amphibians of East Africa. Cornell University and Edition Chimaira, Ithaca, London and Frankfurt, NY, UK and Germany
- Frey J. K., Yates T. L., Duszynski D. W., Gannon W. L., Gardner S. L. (1992) Designation and curatorial management of type host specimens (syntypes) for new parasite species. *J. Parasitol.* **78**: 930-932
- Jirků M., Modrý D. (2005) *Eimeria fragilis* and *E. wambaensis*, two new species of *Eimeria* Schneider (Apicomplexa: Eimeriidae) from African anurans. *Acta Protozool.* **44**: 167-173
- Rödel M. (2000) Herpetofauna of West Africa, Vol. I Amphibians of the West African Savanna. Edition Chimaira, Frankfurt am Main, Germany
- Schleich H. H., Kästle W., Kabisch K. (1996) Amphibians and Reptiles of North Africa. Koeltz Scientific Publishers, Koenigstein, Germany
- Tinsley R. C. (1996) Parasites of *Xenopus*. In: The Biology of *Xenopus*, (Eds. R. C. Tinsley, H. R. Kobel). The Zoological Society of London, 233-261
- 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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