CARYOSPORA MATATU N. SP., A NEW COCCIDIAN PARASITE (APICOMPLEXA: EIMERIIDAE) FROM THE HORNED BUSH-VIPER, *ATHERIS CERATOPHORUS* WERNER, 1895 FROM TANZANIA

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Summary:

Caryospora matatu n. sp. is the first species of coccidia reported from the a horned bush viper, *Atheris ceratophorus* endemic to Tanzania. Oocysts are spherical or slightly subspherical, 19.8 (16–23) µm, a micropyle and an oocyst residuum are absent, a single polar granule is present. An oocyst wall is bilayered, ~ 1.5 µm thick, brownish and distinctly pitted. Sporocysts are ellipsoidal, 15.6 (12.5–17.0) × 10.1 (8–12) µm, tightly fitting an internal oocyst diameter, Stieda and substieda bodies are present. An experimental inoculation of SCID mice did not confirm a facultatively heteroxenous life cycle in the studied coccidium. Morphology and evolutionary history of *Caryospora* species from viperids is is reviewed and discussed.

KEY WORDS : Coccidia, Apicomplexa, *Caryospora matatu* n. sp., taxonomy, Serpentes, *Atheris ceratophorus*, Tanzania.

Résumé : *CARYOSPORA MATATU* N. SP., UNE NOUVELLE COCCIDIE PARASITE (APICOMPLEXA: EIMERIIDAE) DE LA VIPÈRE À CORNE, *ATHERIS CERATOPHORUS* WERNER, 1895 DE TANZANIE

Caryospora matatu n. sp. est la première coccidie rapportée chez la vipère à corne Atheris ceratophorus, *endémique en Tanzanie. Les oocystes sont sphériques ou légèrement sub-sphériques, de 19,8 µm (16-23), sans mycropyle et sans résidu d'oocyste, un granule polaire unique est présent. La paroi de l'oocyste est double, d'environ 1,5 µm d'épaisseur, brunâtre et distinctement excavée. Les sporocystes sont ellipsoïdes, de 15,6 (12,5-17,0) × 10,1 (8-12) µm, remplissant l'ooyste ; le corps et le sous-corps de Stieda sont présents. Une inoculation expérimentale de souris SCID ne confirme pas la possibilité d'un cycle hétéroxène chez la coccidie étudiée. La morphologie et l'évolution des* Caryospora *des vipères sont revues et discutées.*

MOTS CLÉS : *coccidie, Apicomplexa,* Caryospora matatu *n. sp., taxonomy, serpent,* Atheris ceratophorus, *Tanzanie.*

INTRODUCTION

Coccidian parasites of the genus *Caryospora* Léger, 1904 are widespread among ophidian hosts. So far, about 43 species are described from snakes, including four named species parasitising members of the snake family Viperidae (Modrý, 1999; Upton *et al.*, 1986). The majority of known *Caryospora* species are probably monoxenous. However, experimentally studied *Caryospora* spp. from vipers and rattlesnakes possess a distinct heteroxenous mode of the life cycle (Koudela, 1993; Wacha & Christiansen, 1982). An oocyst infection leads to a dermal caryosporosis and a development of subcutaneous caryocysts in a secondary hosts, i.e. rodents (Koudela, 1993; Modrý *et al.*, 1997; Upton & Barnard, 1988; Wacha & Christiansen, 1982). Recently, a severe combined immunodeficient (SCID) mice were used and suggested as a model for testing the heteroxenity of *Caryospora* spp. (Koudela *et al.*, 2000).

In this study, a new species of *Caryospora* is described from the horned bush viper, *Atheris ceratophorus,* a species endemic to northern part of the Eastern Arc Mts. in Tanzania. Furthermore, we used the SCID mice to test the heteroxenity of the species.

MATERIALS AND METHODS

PARASITE ISOLATION AND EXAMINATION

In 2000, faecal samples from four newly imported adult specimens of *Atheris ceratophorus* Werner, 1895 were collected in quarantine facilities. The snakes imported by a pet trader from Tanzania were kept individually in glass terraria and fed on suckling mice, the faecal samples were collected repeatedly from cages during the period of four weeks. The fresh faecal samples were then placed into the plastic vials with 2.5 % (w/v) potassium dichromate ($K_2Cr_2O_7$),

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mixed thoroughly and transported to the laboratory for immediate examination. The samples containing unsporulated coccidian oocysts were allowed to sporulate in Petri dishes at room temperature (19-21° C) and examined daily to determine the stage of sporulation. All faecal samples were examined microscopically after concentration by flotation with modified Sheather's sugar solution (s. g. 1.30). and stored at 4-5° C. The oocysts were measured and photographed using Nomarski differential contrast optics (NIC) with Olympus PROVIS microscope. Measurements were made using a calibrated ocular micrometer and are reported in micrometers, as means, followed by the range in parentheses.

Experimental infection

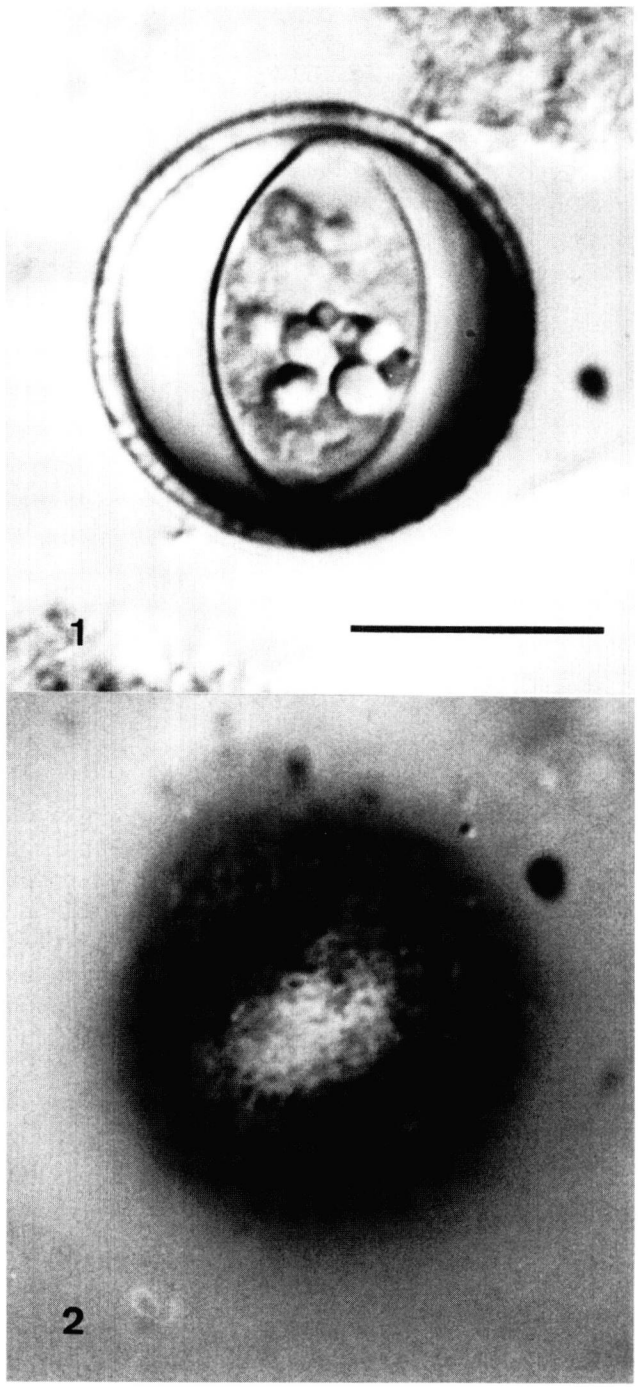
Experimental SCID mice were used as described in Koudela *et al.* (2000). A total of five SCID mice 11 weeks old were used in experimental trial. The sporulated oocysts (~ 2×10^3) were administered orally to each mouse; the mice were monitored daily for clinical signs of a disease. On day 15, 22, 30 post inoculation (DPI) mice were euthanised and examined as described previously (Koudela *et al.*, 2000).

RESULTS

A coprological examination revealed the presence of oocysts of an undescribed species of *Caryospora* Léger, 1904 (Apicomplexa: Eimeriidae) in one of four vipers examined. The type host was shedding the oocysts during the entire period of four weeks in the quarantine. Neither clinical signs of dermal coccidiosis nor caryosporan developmental stages were observed in any of the five experimental SCID mice.

Caryospora matatu n. sp. (Figs 1-3)

Description: oocysts spherical or, rarely, slightly subspherical, 19.8 (16-23) in diameter, n = 30; shape index (SI, length: width ratio) 1.0-1.05. A micropyle and an oocyst residuum absent, however, in some oocyst 2-5 very small granules occur in the oocyst content. A single distinct polar granule present in all oocysts examined, usually ~ 1 in diameter. An oocyst wall bilayered, ~ 1.5 thick (inner layer much thinner, < 0.5), brownish, distinctly pitted; various debris and contaminant bacteria tend to adhere to the oocyst surface, giving the oocyst wall distinctly rough appearance. Sporocysts ellipsoidal, 15.6 (12.5-17.0) × 10.1 (8-12), n = 30; sporocyst SI = 1.55 (1.42-1.67). Typically, the sporocyst tightly fits the internal diameter of the oocyst. A Stieda body is dome-like, ~ 1 high and 2-3 wide. The substieda body barely distinguishable, in some sporocysts ellipsoidal, two high and four wide. In the majority of the sporocysts, substieda body appears as slightly irregular mass forming a distinct halo between the Stieda body and the sporozoites. A sporocyst residuum present, consisting of numerous small granules scattered among the sporozoites and concentrated in the central part of the sporocyst. The eight sporozoites are vermiform, possessing slightly striated surface and



Figs 1-2. – Nomarski interference contrast photograph of oocyst of *Caryospora matatu* n. sp., both in the same scale. Fig. 1. Sporulated oocyst. Scale bar = 10 µm. Fig. 2. Surface of the oocyst wall showing distinct pitting.

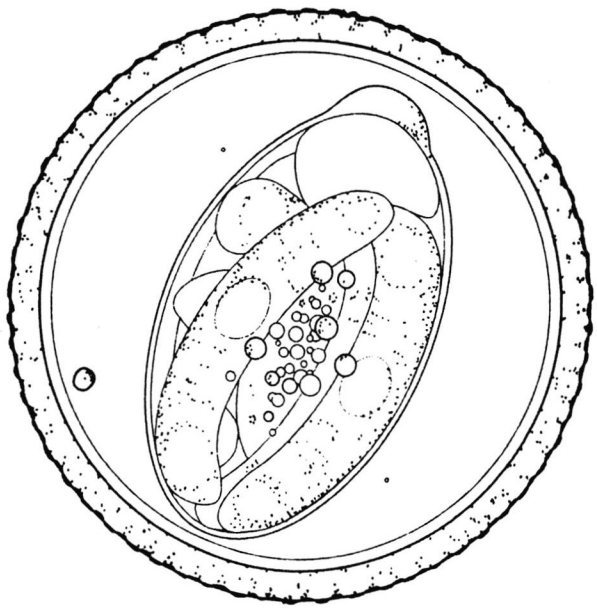


Fig. 3. – Composite line drawing of sporulated oocyst of *Caryospora matatu* n. sp. Scale bar = 10 µm.

two barely distinguishable refractile bodies and centrally located nucleus.

Type host: *Atheris ceratophorus* Werner, 1895 (Serpentes: Viperidae), horned bush-viper.

Type locality: detailed locality unknown, examined snakes originated from Usambara Mts. *Atheris ceratophorus* is endemic to Usambara, Udzungwa and possibly also Uluguru Mts. in eastern Tanzania.

Prevalence: one of four examined snakes shed oocysts in faeces.

Site of infection: unknown, oocysts recovered from faeces.

Sporulation: exogenous, fresh oocysts recovered from faeces were unsporulated, complete sporulation occurs within five days (at 19-21° C).

Type material: photo-syntypes deposited under the collection No. R 03/2002 at Dept. of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno.

Etymology: the specific epithet *matatu*, given as a noun in apposition, is adopted from the a local term describing the most common mean of public transportation in East Africa.

DISCUSSION

So far, there are eight species of *Caryospora* described from snakes from a sub-Saharan Africa (Bray, 1960; Daszak & Ball 2001a, b; Hoare, 1933; Matuschka, 1982; Upton *et al.*, 1992). Only a single named species, *C. maculatus* from *Causus maculatus* Upton, Freed & Freed, 1992, originates from a member of the family Viperidae. All *Caryospora* spp. from African snakes can be easily distinguished from *C. matatu* n. sp. by the general oocyst morphology. *C. maculatus* differs greatly by smaller oocyst dimensions, smooth oocyst wall;, the absence of polar granule and the different appearance of Stieda and substieda bodies. None of three species of *Caryospora* named from members of the family Viperidae from other zoogeographical regions is similar enough to be confused with *C. matatu* sp. n. All of them are typical in having smaller (10-16 µm) spherical oocysts with thin and smooth oocyst wall and different Stieda and

Species of *Caryospora*	Type host	Shape (SI)	PG	Size	Shape	Size	Sb ssb	Distribution	Heteroxenity	Reference
bigenetica	*Crotalus horridus*	spherical	+	13.2 (10.9-15.4)	ellipsoid ovoid	10.1 (8.3–11.5) × 7.7 (6.4-9.0)	+/+	USA - Iova, Georgia	+	Wacha & Christiansen, 1982
simplex	*V. x. xanthina*	spherical	+	14.9 (13.5-16.2)	ovoid	11.6 (10.4-12.6) × 8.9 (8.1-9.5)	+/+	Palaearctic	+	Upton *et al.*, 1983; Modrý *et al.*, 1997
jararacae	*Bothrops jararaca*	spherical	–	13.0–14.0	ovoid	9.0-10.0 × 7.0-8.0		Brazil	n. t.	Carini, 1939
maculatus	*Causus maculatus*	spherical	+/–	12.1 (11-13)	ovoid	10.1 (9.5-11) × 7.9 (7.5-8.5)	+/+	Cameroon	n. t.	Upton *et al.*, 1992
sp.	*Atheris nitschei*	spherical	–	11.9 (11-13)	ovoid	9.4 (9-10) × 7.4 (7-7.5)	+/+	Uganda	+	Koudela *et al.*, 2000; Modrý, unpubl.
sp.	*Calloselasma rhodostoma*	spherical	+	12.1 (11-13)	ovoid	9.5 (8.5–10) × 7.8 (7-8.5)	+/+	SE Asia	+	Koudela *et al.*, 2000; Modrý, unpubl.
matatu n. sp.	*Atheris ceratophorus*	spherical	+	9.8 (16–23)	ellipsoid	15.6 (12.5–17.0) × 10.1 (8-12)	+/+	Tanzania	–	This study

Abbreviations: Sb: Stieda body, ssb: substieda body, n. t.: not tested.

Table I. – Species of *Caryospora* Léger, 1904 reported from members of the family Viperidae.

substieda bodies and can be, therefore, easily distinguished (Table I).

In general, the oocyst morphology, the oocyst wall texture and the shape of the Stieda and the substieda body distinguish *C. matatu* n. sp. well from the most of the ophidian species of *Caryospora* . Only *C. carajasensis, C. constanciae, C. duszynskii, C. epicratesi, C. heterodermus* and *C. peruensis* possess moderately to distinctly pitted or striated oocyst wall rough enough to be compared with *C. matatu* n. sp. (Lainson *et al.*, 1991; Upton *et al.*, 1984; Upton *et al.*, 1989; Upton *et al.*, 1992). However, all these species differ in the fine oocyst morphology, dimensions, host systematics and geographical origin and as such could not be confused with species described herein as a new.

ACKNOWLEDGEMENTS

The research on parasites of East African reptiles was kindly supported by Masokombinát Písek a.s. and by the Nadání Josefa a Marie Hlávkových Endowment. We are indebted to Olympus C & S for generous technical support. This study was supported by the grant No. 524/00/P015 of the Grant Agency of the Czech Republic.

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Reçu le 8 juin 2002
Accepté le 25 septembre 2002