

## TWO NEW SPECIES OF *EIMERIA* (APICOMPLEXA: EIMERIIDAE) FROM ASIAN GEOEMYDID TURTLES *KACHUGA TENTORIA* AND *MELANOCHELYS TRIJUGA* (TESTUDINES: GEOEMYDIDAE)

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### Summary:

Coprological examination of ten Pink-ringed tent turtles *Kachuga tentoria circumdata*, recently imported from India, and three Burmese black turtles *Melanochelys trijuga edeniana*, imported from Myanmar, revealed the presence of two new species of *Eimeria*. Oocysts of *Eimeria kachua* n. sp. from *K. t. circumdata* are broadly oval to subspherical, 15.3 (13-18) × 13.9 (12-16) µm, with polar granule and subspherical oocyst residuum. Sporocyst elongately oval to spindle-shaped, 8.7 (7.5-10) × 4.9 (4-6) µm, with a knoblike Stieda body, covered with fine membranous cupola-like structures. Thin walled oocysts of *Eimeria patta* n. sp. from *M. t. edeniana*, have an irregular shape, influenced by the position of sporocysts, frequently with lobular irregularities, 12.6 (11-16) × 9.1 (7.5-12) µm. Sporocysts are oval to ellipsoidal, 5.8 (5-7) × 4.2 (3.5-5) µm.

**KEY WORDS :** Coccidia, Apicomplexa, *Eimeria*, taxonomy, Geoemydidae, *Kachuga*, *Melanochelys*.

**Résumé :** DEUX ESPÈCES NOUVELLES D'*EIMERIA* (APICOMPLEXA : EIMERIIDAE) DE TORTUES D'ASIE *KACHUGA TENTORIA* ET *MELANOCHELYS TRIJUGA* (TESTUDINES : GEOEMYDIDAE)

L'examen coprologique de dix tortues *Kachuga tentoria circumdata*, récemment importées d'Inde, et de trois tortues *Melanochelys trijuga edeniana*, importées du Myanmar (Birmanie) révèle la présence de deux espèces nouvelles d'*Eimeria*. Les oocystes d'*Eimeria kachua* n. sp. provenant de *K. t. circumdata* sont ovales ou subsphériques – 15,3 (13-18) × 13,9 (12-16) µm – avec un granule polaire et un résidu oocystaire subsphérique. Les sporocystes ont une forme ovale allongée à fusiforme – 8,7 (7,5-10) × 4,9 (4-6) µm – avec un corps de Stieda protubérant et sont couverts d'une fine structure membranaire. Les rares oocystes d'*Eimeria patta* n. sp. provenant de *M. t. edeniana* ont une forme irrégulière, influencée par la position des sporocystes, avec des irrégularités lobulaires fréquentes – 12,6 (11-16) × 9,1 (7,5-12) µm –. Les sporocystes sont de forme ovale à ellipsoïdale – 5,8 (5-7) × 4,2 (3,5-5) µm –.

**MOTS CLÉS :** Coccidia, Apicomplexa, *Eimeria*, taxonomie, Geoemydidae, *Kachuga*, *Melanochelys*.

## INTRODUCTION

Up to the present time, little attention was given to the eimeriid parasites from turtles of South and Southeast Asia, the area of the turtles' highest diversity (Ernst & Barbour, 1989). A few studies are from first half of the 20<sup>th</sup> century (Simond, 1901; Laveran & Mesnil, 1902; Das Gupta, 1938; Chakravarty & Kar, 1943; Kar, 1944). These papers, all but one, were focused on protozoan parasites from soft-shelled turtles of the family Trionychidae. The only exception – *Eimeria mitraria* (Laveran & Mesnil, 1902) – was described from the Chinese three-keeled pond turtle *Chinemys reevesii* (Gray, 1831), member of the most plentiful turtle family Geoemydidae (formerly

Bataguridae). In a frame of wide-scale study of turtles' coccidia, we have been allowed to examine freshly imported groups of two turtle species: Pink-ringed tent turtle *Kachuga tentoria circumdata* Mertens, 1969, and Burmese black turtle *Melanochelys trijuga edeniana* Theobald, 1876. We found two species of coccidia, which are described in this paper as new.

## MATERIALS AND METHODS

A group of ten specimens of *K. tentoria circumdata* were imported into the Czech Republic from an animal market in Bombay, India, in April 2004. Three *M. trijuga edeniana* were imported from Myanmar (formerly Burma) by a pet animal dealer to China in August 2003, and then were immediately transported into the Czech Republic. Based on the dealer's information, all specimens were freshly caught by local people in central Myanmar, although the exact locality was not given. All animals of both species were in a state of good health. All *K. tentoria circumdata* were placed together in one tank with shallow water, whereas three specimens of the second species were housed separately in plastic boxes, where fecal samples were col-

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lected for parasitological examination. Fresh faeces of all turtles were placed in 2.5 % aqueous (w/v) potassium dichromate ( $K_2Cr_2O_7$ ) solution, mixed thoroughly, then were allowed to sporulate in Petri dishes at 20-23° C, and repeatedly examined over a period of one month. All samples from *K. tentoria circumdata* were microscopically examined after concentration by flotation with modified Sheather's sugar solution (specific gravity 1.3). Because of the extraordinarily thin and fragile oocyst wall of *Eimeria* species from *M. trijuga edeniana*, it was impossible to obtain their undamaged oocysts in the same way. That's why, in a case of this species, sediment of centrifuged samples was used without concentration with sugar solution. Coccidian oocysts were measured and photographed using Nomarski interference contrast optic microscopy (NIC) at Olympus Provis AX-70. Measurements were made on 30 oocysts, using a calibrated ocular micrometer and throughout are reported in micrometers ( $\mu m$ ), with means followed by ranges in parentheses.

## RESULTS

Coprolological examination revealed the presence of two previously undescribed coccidian species of the genus *Eimeria*. Both new coccidia are described as follows.

### *EIMERIA KACHUA* N. SP. (Figs 1-2, 5)

Oocysts broadly oval to subspherical, exceptionally spherical, 15.3 (13-18)  $\times$  13.9 (12-16), shape index (SI, length/width) 1.1 (1-1.2). Oocyst wall is optically bi-

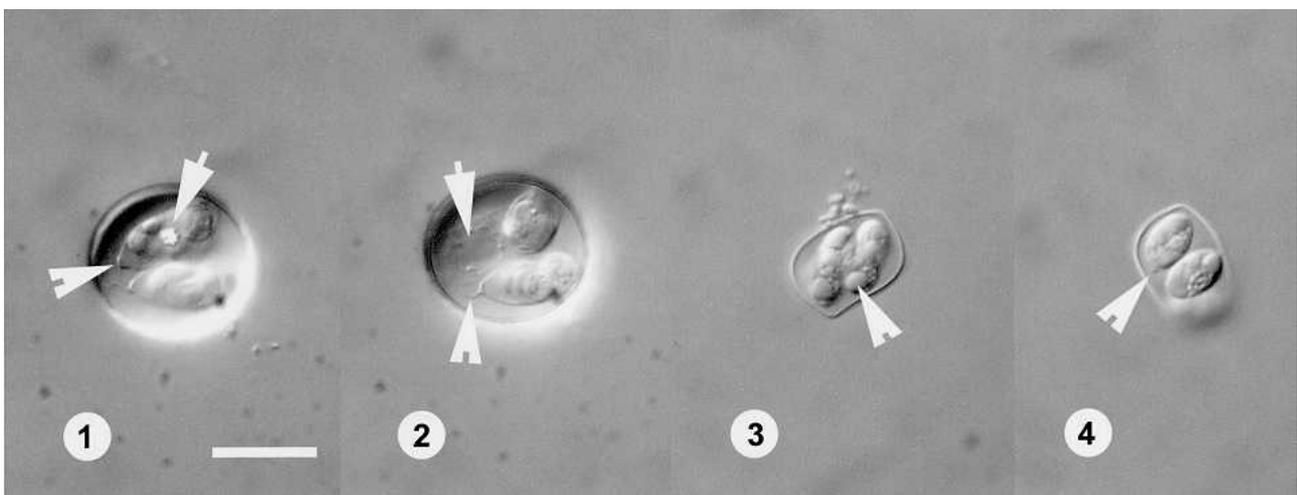
layered, colourless,  $\sim 0.8$  (0.7-0.9) thick, having both layers of approximately the same thickness. Micropyle is absent, polar granule is present, 1-1.5  $\times$  1.2-1.7 (Fig. 1), in one case (3.3 %) there were two polar granules in one oocyst. Oocyst residuum is present, 5 (3.5-6)  $\times$  5.5 (4-7), composed of fine granules, and covered with a very thin membranous layer (Fig. 2). During the sporogony, the quantity of granular matter in oocyst residuum decreases, making an impression of a nearly empty membranous vacuole. Sporocysts are elongately oval to spindle-shaped, 8.7 (7.5-10)  $\times$  4.9 (4-6), sporocyst SI 1.77 (1.5-2), with a thin, smooth, colourless, and unlayered sporocyst wall. Stieda body present, knoblike, 0.5-0.8 high and 1.2-1.5 wide, covered with a fine membranous cupola-like structure, 1.2-1.8 wide and 1.8-2.0 high (n = 12). Substieda body was not detected. Sporocyst residuum present as a little amount of small granules scattered among sporozoites. Sporozoites elongate, laying head to tail within the sporocyst. Each sporozoite has a prominent posterior oval to subspherical (2-2.5  $\times$  2-3) refractile body at one end, and a smaller anterior one ( $\sim 1.5 \times 2$ ) at the opposite end. The nucleus of the sporozoite is not discernible.

### Taxonomic summary

Type host: Pink-ringed tent turtle *Kachuga tentoria circumdata* Mertens, 1969 (Testudines: Geoemydidae).

Type locality: India, detailed locality unknown. All turtles bought at a market in Bombay.

Type specimens: photosyntypes are deposited in Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, under No. R 79/04.



Figs 1-4. – Nomarski interference contrast photographs of oocysts of described coccidia, all in the same scale. Fig. 1. Sporulated oocyst of *E. kachua* n. sp., note distinct Stieda body (arrowhead) and polar granule (arrow). Scale bar = 10  $\mu m$ . Fig. 2. Sporulated oocyst of *E. kachua* n. sp., note membranous cupola-like structure covering the Stieda body (arrowhead) and oocyst residuum (arrow). Fig. 3. – Sporulated oocyst of *E. patta* n. sp., note distinct sporozoite refractile body (arrowhead). Fig. 4. Sporulated oocyst of *E. patta* n. sp., note fine Stieda body (arrowhead).

Site of infection: unknown, samples were obtained from live turtles.

Sporulation: exogenous. Some oocysts became fully sporulated within 3-5 days at 20-23°C, but not all oocysts became sporulated up to the end of observation period (one month).

Prevalence: not studied, examined sample originated from a group of ten animals.

Etymology: specific epithet "*kachua*" is adopted from Hindi language, meaning a turtle; it is given, in accordance with International Code of Zoological Nomenclature (Article 31.1) as a noun in apposition (ICZN, 1999).

*EIMERIA PATA* N. SP. (Figs 3-4, 6, 7)

Oocysts have an irregular shape, they range from oval, ellipsoidal to almost subspherical, 12.6 (11-16) × 9.1 (7.5-12); oocyst SI 1.4 (1.08-1.63), micropyle, polar granule and oocyst residuum absent. Oocyst wall is smooth, colourless, very thin (~ 0.2-0.3), under light microscopy appears to be single-layered. Because of a thin and fragile oocyst wall, the overall shape of the oocysts is remarkably variable, lobularly irregular, influenced by the position of sporocysts inside (Fig. 7). Sporocysts are oval to ellipsoidal, 5.8 (5-7) × 4.2 (3.5-

5), with a smooth, colourless, single-layered very thin sporocyst wall; sporocyst SI 1.4 (1.2-1.75). Stieda body is present as a low flat projection, substieda body is not discernible. Sporozoites are elongated, encircled one another, and arranged head-to-tail within the sporocyst. Each sporozoit bears spherical to subspherical refractile bodies (~ 2-3 × 2-2.5) at both ends. Sporocyst residuum present, usually in a form of granular matter scattered among sporozoites. The nucleus is not discernible.

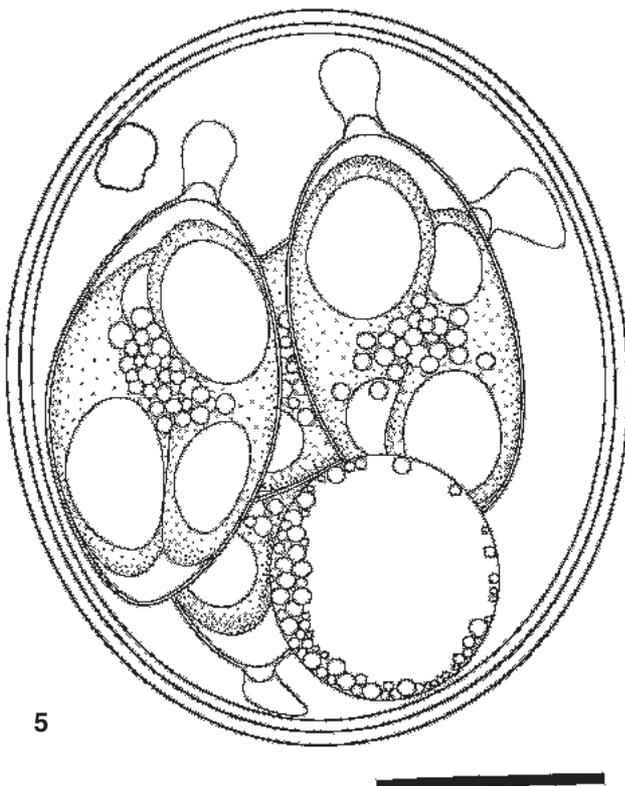
Taxonomic summary

Type host: Burmese black turtle *Melanochelys trijuga edeniana* Theobald, 1876 (Testudines: Geoemydidae).

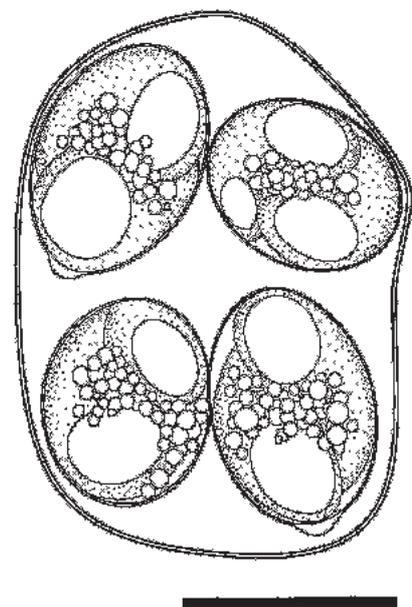
Type locality: Central Myanmar (Burma), detailed locality unknown.



Fig. 7. – Schematic line drawings showing variability in shape of sporulated oocysts of *Eimeria patta* n. sp. Bar = 10 µm.



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Figs 5-6. – Composite line drawings of sporulated oocysts, scale bar = 5 µm. Fig. 5. *Eimeria kachua* n. sp. Fig. 6. *Eimeria patta* n. sp. Parasite, 2005, 12, 9-13

Type specimens: photosyntypes are deposited in the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, under No. R 78/04.

Site of infection: unknown, samples were obtained from live turtles.

Sporulation: probably exogenous, two day old faeces contained only fully sporulated oocysts.

Prevalence: all three examined specimens of *M. trijuga edeniana* were infected. This fact can be skewed by the shared transport box during the importation.

Etymology: “Leik patta” is one of the vernacular names of the type host used in Myanmar (Win Maung & Win Ko Ko, 2002), the specific epithet “*patta*” is given, in accordance with International Code of Zoological Nomenclature (Article 31.1) as a noun in apposition (ICZN, 1999).

## DISCUSSION

Increasing numbers of described coccidian species lead to the overlap of morphological characters, used as principle diagnostic features. Eimerian parasites are traditionally considered to be highly host specific, affecting only a narrow spectrum of closely related hosts (Duszynski, 1986; Duszynski & Wilber, 1997). Then, host systematics and geographic origin are commonly used criteria in taxonomy of the genus *Eimeria*. Thus, in the following differential diagnosis, only *Eimeria* spp. described and named from turtles of families Emydidae, Geoemydidae, and Testudinidae (superfamily Testudinoidea, sensu Gaffney & Meylan, 1988) are included, as conspecificity of described species with coccidia from other hosts is unlikely.

Some of the previous authors reviewed coccidian parasites isolated from turtles and tortoises (i.e. McAllister & Upton, 1989b; Upton *et al.*, 1995; Lainson & Naiff, 1998; Hůrková *et al.*, 2000). Thus, altogether 26 *Eimeria* spp. are affecting testudinoid hosts, all but three (*E. brodeni* Cerruti, 1930; *E. delagei* [Labbé, 1893]; *E. mitraria* [Laveran & Mesnil, 1902]) are described from chelonians of the New World. In spite of different geographic origin, we include them into the differential diagnosis, to avoid possible conspecificity, potentially caused by the recent extensive international pet trade. *Eimeria brodeni* and *E. delagei* are significantly bigger than both our isolates (Labbé, 1893; Cerruti, 1930), and *E. mitraria* is a distinctive coccidium with a unique mitre-shaped oocyst (Laveran & Mesnil, 1902). *Eimeria kachua* sp. n. is a medium sized to smaller coccidium. Its shape and size are similar to six other *Eimeria* from testudinoids. *E. carri* Ernst & Forrester, 1973 differs in having longer sporocysts, oocysts without a polar granule, and a tiny Stieda body. *Eimeria grape-*

*mydos* Wacha & Christiansen, 1976 is a bit smaller, differing in its single-layered and thinner oocyst wall. *Eimeria jaboti* Carini, 1942 is bigger, with a different sporocyst shape, without a Stieda body, and oocyst residuum. *Eimeria juniataensis* Pluto & Rothenbacher, 1976 has smaller and more spherical oocysts, with a thinner oocyst wall composed of only a single layer. Moreover, its oocysts lack a polar granule. *Eimeria megalostiedai* Wacha & Christiansen, 1974 differs by its thinner oocyst wall, large Stieda body and absence of polar granules. *Eimeria ornata* McAllister & Upton, 1989 has larger oocysts and sporocysts and a thick single-layered oocyst wall, Stieda body is indistinct. Further, proposed endogenous sporulation of *E. ornata* contrasts with the observed large portion of unsporulated oocysts in our isolate (Carini, 1942; Ernst & Forrester, 1973; Wacha & Christiansen, 1974, 1976; Pluto & Rothenbacher, 1976; McAllister & Upton, 1989a). Definitely, *E. kachua* can be easily distinguished from all above mentioned similar species by its fine membranous structure covering its Stieda body.

*Eimeria patta* sp. n. is a small coccidium, with a uniquely thin oocyst wall, when compared to other *Eimeria* spp. from chelonian hosts. Moreover, markedly irregular shape of oocysts of *E. patta* distinguishes this species from all but one *Eimeria* described from chelonians. *E. motelo* Hůrková, Modrý, Koudela & Šlapeta, 2000, a species from the neotropical Yellow-footed tortoise *Geochelone denticulata* (Linnaeus, 1766) has also oocysts of irregular shape, but contrary to *E. patta*, oocysts and sporocysts of *E. motelo* are remarkably longer (Hůrková *et al.*, 2000).

Based on the above-mentioned morphological differences, and, further, on geographic isolation of hosts, we consider the found *Eimeria* to be a new species.

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