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

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INTRODUCTION

Up to the present time, little attention was given to the eimerid 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 20th 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 (K₂Cr₂O₇) 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 edentiana*, 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 (µm), with means followed by ranges in parentheses.

### RESULTS

Coprological 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) × 13.9 (12-16), shape index (SI, length/width) 1.1 (1-1.2). Oocyst wall is optically bi-layered, colourless, ~ 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 × 1.2-1.7 (Fig. 1), in one case (3.5 %) there were two polar granules in one oocyst. Oocyst residuum is present, 5 (3.5-6) × 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) × 4.9 (4-6), sporocyst SI 1.77 (1.5-2), with a thin, smooth, colourless, and unilayered sporocyst wall. Stieda body present, knoblike, 0.5-0.8 high and 1.2-1.5 wide, covered with a fine membranous cupula-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 × 2-3) refractile body at one end, and a smaller anterior one (~ 1.5 × 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.
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 patta** 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), micropyte, 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 sporozoite 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.](image-url)
Type specimens; photosynotypes 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. *Eimeria* 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 and tortoises (i.e. McAllister & Upton, 1989b; Upton et al., 1995; Lainson & Naiff, 1998; Hrkrková 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 grapte-

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