

A new *Eimeria* (Apicomplexa: Eimeriidae), possessing mitra-shaped oocysts, from the Neotropical chelid turtle *Batrachemys heliostemma* (Testudines: Chelidae), and its comparison with *Eimeria mitraria* (Laveran & Mesnil 1902)

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Eimeria jirkamoraveci sp. n. is described from faeces of two specimens of the toad-headed, side-necked turtle *Batrachemys heliostemma* collected at Iquitos in Peru. Oocysts are ovoid to almost spherical, 10.6 (8-12) × 8.9 (7-10) μm, without micropyle, polar granule and oocyst residuum. One conically stretched end and three blunt conical tubercles at the opposite end of oocyst give it mitra-like appearance. Sporocysts are elongated, ellipsoidal, 7.2 (6-8) × 4.1 (4-4.5) μm, with a small, knob-like Stieda body and sporocyst residuum composed of fine granules. To avoid possible conspecificity, the described new species is thoroughly compared with the most similar coccidium, *E. mitraria*, collected from its type host, *Chinemys reevesii*.

Key words: Coccidia - Apicomplexa - *Eimeria jirkamoraveci* sp. n. - turtles - *Batrachemys* - Peru

Batrachemys heliostemma McCord, Joseph-Ouni & Lamar, 2001 is a Neotropical chelid turtle described recently from the upper Amazon basin, with distribution ranging from Southern Venezuela, Western Brazil, to Peru, Ecuador, and Southeastern Colombia. It seems, that this species prefer high, non-flooded forests near permanent water bodies, and shallow and clear waters rather than the turbid, deeper waters of saturated lowlands. Little is known about other aspects of its ecology (McCord et al. 2001). Generic affiliation of this turtle has been questioned and it is even not solved definitely (Bour & Zaher 2005).

With more than sixty species, South America represents an area of rich turtle diversity. Up to recently, only eight species of *Eimeria* were described and named from turtles of this region, three of them (*E. jaboti* Carini 1942, *E. lainsoni* Hürková, Modrý, Koudela & Šlapeta 2000, and *E. motelo* Hürková, Modrý, Koudela & Šlapeta 2000) parasitize the Yellow-footed tortoise *Geochelone denticulata* (Linnaeus 1766). Three others (*E. lagunculata* Lainson, Costa & Shaw 1990, *E. mammiformis* Lainson, Costa & Shaw 1990, and *E. podocnemis* Lainson, Costa & Shaw 1990) were described from the Arrau turtle *Podocnemis expansa* (Schweigger 1812) and one species was described from each of the Galápagos tortoise *Geochelone nigra* (Quoy & Gaimard 1824) and the Big-headed turtle *Peltecephalus dumerilianus* (Schweigger 1812) (Carini 1942, Lainson et al. 1990, Couch et al. 1996, Lainson & Naiff 1998, Hürková et al. 2000).

We had an opportunity to examine the faeces of two specimens of *Batrachemys heliostemma* collected at Iquitos and found a new *Eimeria* possessing mitra-shaped oocysts, described and named in this paper. To avoid possible conspecificity with *E. mitraria* (Laveran & Mesnil 1902), a species reported from more than a dozen turtles from three continents (McAllister & Upton 1989, Lainson & Naiff 1998, Segade et al. 2004), we re-isolated this coccidium from its type host, the Chinese three-keeled pond turtle *Chinemys reevesii* (Gray 1831) and have made a thorough comparison.

MATERIALS AND METHODS

Two sub-adult females of *B. heliostemma* were collected in March 2002 at Anquilla village at Rio Nanay, 50 km west of Iquitos, Peru. They were imported alive to the Czech Republic in April 2002, placed together in one plastic box, where faecal samples were collected. Freshly obtained faeces were placed in 2.5% aqueous (w/v) potassium dichromate (K₂Cr₂O₇) solution, mixed thoroughly, and the oocysts allowed to sporulate in shallow plastic containers at 20-23°C. The material was then stored at 4°C until its examination. Samples were microscopically examined after concentration by flotation with modified Sheather's sugar solution (specific gravity 1.3). Since oocysts became quickly deformed (after circa 15 min), centrifuged sediment of further samples was used without concentration by sugar solution. Oocysts were measured and photographed using an Olympus Provis AX 70 microscope equipped with Nomarski interference contrast optics (NIC). Morphological features are described according to Duszynski and Wilber (1997). Measurements were made of 30 oocysts, using a calibrated ocular micrometer and are given in micrometers (μm) as means, followed by ranges in parentheses. For a comparison, material with oocysts of *E. mitraria* was obtained from a female *C. reevesii* originating from Japan. This turtle was

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collected in October 2003 close to Fukuoka, Kyushu island, imported into the Czech Republic by a private breeder, then housed, sampled and examined the same way.

RESULTS

Coprological examination of two *B. heliostemma* revealed presence of mitra-shaped oocysts of a previously undescribed eimerian coccidium. The new species is described and named as follows. The original description of *E. mitraria*, the species possessing most similar oocysts, contains insufficient data on its morphology necessitating a re-description of the oocyst of *E. mitraria*.

Eimeria jirkamoraveci sp. n.
(Figs 1-3, 5)

Description - Oocysts are ovoid, sub-spherical to almost spherical, $10.6 (8-12) \times 8.9 (7-10)$, oocyst shape index (SI, length/width) 1.2 (1-1.5); micropyle, polar granule and oocyst residuum absent. Oocyst wall smooth, colorless, under light microscopy single-layered, ~ 0.5 thick. One end of oocyst is conically stretched, whereas opposite end bears three blunt conical tubercles, giving the oocyst its mitra-like appearance. Sporocysts are elongated, ellipsoidal, $7.2 (6-8) \times 4.1 (4-4.5)$, with a smooth, thin and colorless sporocyst wall and a sporocyst SI of 1.75 (1.5-2). Stieda body present, small, knob-like, ~ 0.5-1 high and ~ 1-1.5 wide ($n = 8$), substieda body is not discernible. Sporocyst residuum consists of fine granules, that are either organized in a compact sphere ~ $2-4 \times 2-3$ ($n = 10$) or scattered among sporozoites. Sporozoites are elongated, arranged head to tail within the sporocyst. Each sporozoite bears a spherical to sub-spherical refractile body (~ $1.5-2 \times 2-2.5$; $n = 8$), nucleus is not discernible.

Type-host - Northern toad-headed side-necked turtle *Batrachemys heliostemma* McCord, Joseph-Ouni & Lamar, 2001 (Reptilia: Testudines: Chelidae).

Type-locality - Anquilla village, Iquitos, Departamento

Loreto, Peru ($03^{\circ}54'45''$ S, $073^{\circ}39'39''$ W).

Prevalence - Not studied, we examined a mixed faecal sample originating from both specimens.

Sporulation time - Not recorded.

Site of infection - Unknown, oocysts were acquired from faeces of living turtles.

Type-material - Photosyntypes are deposited in the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic under no. R 167/02.

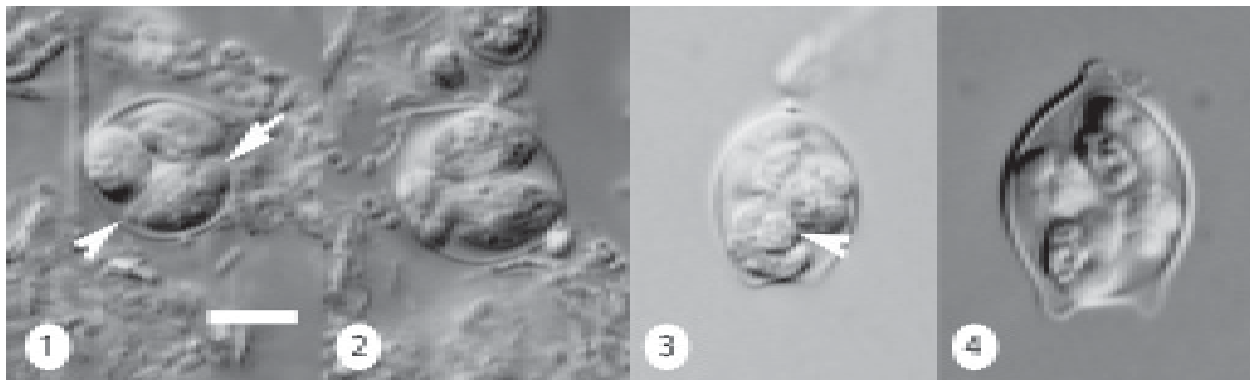
Etymology - Specific epithet "*jirkamoraveci*" is given in honour of our friend, Dr Jiř Moravec, who has contributed considerably to the knowledge of the biodiversity of the western Amazon basin.

Eimeria mitraria (Laveran & Mesnil 1902)
(Fig. 4)

Re-description - Oocysts ovoid $14.4 (13-16) \times 10.4 (9-13)$, oocyst SI 1.4 (1.23-1.58), without micropyle, polar granule and oocyst residuum. Oocyst wall smooth, colorless and, under light microscopy, single-layered, ~ 0.5 thick. One end of oocyst bears one conical projection ~ 1-1.5 long, whereas opposite end bears three similar projections, giving the oocyst mitra-like or projectile-like appearance. Sporocysts ellipsoidal, $7.1 (6-7.5) \times 4.4 (4-5)$, with smooth and colorless sporocyst wall and a sporocyst SI of 1.63 (1.33-1.88). Stieda body is present, tiny, knob-like, ~ 0.5-0.1 ($n = 7$), substieda body is not discernible. Sporozoites elongate, arranged head to tail within sporocyst. Each sporozoite bears a prominent sub-spherical refractile body $1.5-2 \times 2-3$ ($n = 9$) at one end, nucleus is not discernible. Sporocyst residuum is a compact granular mass ~ 2-4 in diameter, or is scattered among sporozoites.

DISCUSSION

Eimerian parasites are traditionally considered to be strictly host specific and geographic origin and host sys-



Nomarski interference contrast photographs of oocysts, all in the same scale. Fig. 1: sporulated oocyst of *Eimeria jirkamoraveci* sp. n.; note distinct Stieda body (arrowhead) and refractile body (arrow). Bar = 5 μ m. Figs 2-3: overall shape of sporulated oocysts of *E. jirkamoraveci* sp. n. with distinct conical tubercles; note granular sporocyst residuum (arrowhead). Fig. 4: sporulated oocyst of *E. mitraria* (Laveran & Mesnil 1902); note the different size, shape and more pronounced conical projections of the oocyst wall, compared to *E. jirkamoraveci* sp. n.

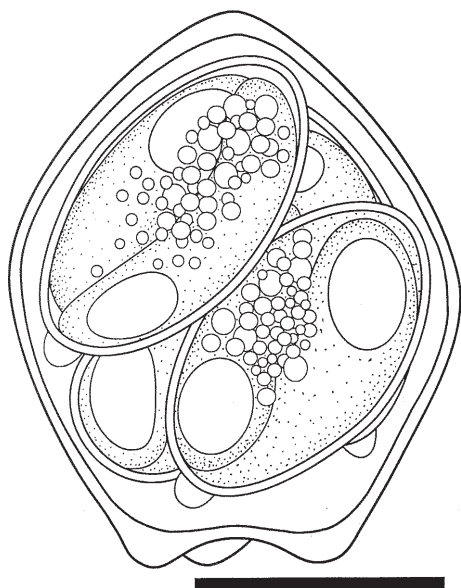


Fig. 5: composite line drawing of sporulated oocyst of *Eimeria jirkamoraveci* sp. n. Bar = 5 μ m.

tematics are common tools in their taxonomy (Duszynski 1986). Nevertheless the relevance of this trait for reptilian *Eimeria* species has been repeatedly questioned (Wacha & Christiansen 1974, 1976, McAllister & Upton 1989). This is why we are including all chelonian *Eimeria* from Neotropical zoogeographical region in our differential diagnosis. Moreover, both known *Eimeria* spp. possessing mitra-shaped oocysts are considered.

Among all *Eimeria* spp. parasitizing side-necked turtles of suborder Pleurodira (families Pelomedusidae and Chelidae *sensu* Gaffney & Meylan 1988) none is similar to *E. jirkamoraveci* sp. n., including recently discovered *Eimeria* from the African side-necked turtle *Pelomedusa subrufa* (Lacépède 1788) (Široký et al. 2006). Also none of the five remaining species of genus *Eimeria* previously described from turtles of the Neotropical region, resembles the new species described above (Carini 1942, Lainson 1968, Lainson et al. 1990, Couch et al. 1996, Lainson & Naiff 1998, Hürková et al. 2000).

Consequently, only two *Eimeria* with mitra-like oocysts resemble *E. jirkamoraveci* sp. n. - *E. mitraria* (Laveran & Mesnil 1902) described from the Chinese three-keeled pond turtle *Chinemys reevesii* (Gray 1831) and *E. stylosa* McAllister & Upton 1989, a parasite of the red-eared slider turtle *Trachemys scripta elegans* (Wied-Neuwied 1838). Oocysts of *E. stylosa* are definitely larger and have much longer and sharper projections (McAllister & Upton 1989).

E. mitraria is the most similar coccidium to *E. jirkamoraveci* sp. n., hence we give particular attention to their comparison. In the original description, Laveran and Mesnil (1902) reported oocysts of *E. mitraria* to be 15 \times 10 μ m. These data fit well the range of oocysts we obtained from *C. reevesii*. In contrast, *E. jirkamoraveci* sp.

n. has smaller, more spherical oocysts with less pronounced conical projections of oocyst wall.

McAllister et al. (1990) suggested that most species of coccidia in Testudines are host-specific at family level, although without any experimental or molecular data. *B. heliostemma* is a chelid turtle only very distantly related to geoemydid turtle *C. reevesii*. Separated evolutionary lineages of turtles of the suborders Pleurodira (families Chelidae and Pelomedusidae) and Cryptodira (all other turtle families) have been independently evolving for almost 200 millions of years (Gaffney et al. 1987). Additionally, there is also a long-term separation of Neotropical and Oriental zoogeographical regions (Smith et al. 2004). Based on the above-mentioned morphological differences, geographic isolation, and phylogenetic distance of *B. heliostemma* to any other turtle species known to be parasitized by *Eimeria* with mitra-shaped oocysts, we consider *E. jirkamoraveci* to be a new species.

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