Two Eimerian Coccidia (Apicomplexa: Eimeriidae) from the Critically Endangered Arakan Forest Turtle *Heosemys depressa* (Testudines: Geoemydidae), with Description of *Eimeria arakanensis* n. sp.

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Summary. Coprological examination of nine Arakan forest turtles *Heosemys depressa* freshly imported from Myanmar revealed the presence of two species of *Eimeria*, one of which is described as a new species. Oocysts of *Eimeria arakanensis* n. sp. are broadly oval to subspherical, 28.0 (24-30) × 23.6 (22-25) µm, with a smooth, colourless, uni-layered wall ~ 0.6 µm thick, possessing a large globular oocyst residuum. Sporocysts are ellipsoidal to oval, 12.8 (12-15) × 7 (6-8) µm, with a smooth and colourless sporocyst wall; Stieda body present, knob-like, ~ 1 × 1-2 µm, substieda body homogenous, sub-globular, 1-1.5 × 1.5-2 µm. Additionally, oocysts of *Eimeria cf. mitraria* (Laveran et Mesnil, 1902) were found in all nine examined turtles. *H. depressa* represents a new host for this coccidium. Host specificity and taxonomy of *E. mitraria*, yet recorded by previous authors from turtles of four families, is discussed.

Key words: Apicomplexa, Coccidia, *Eimeria arakanensis* n. sp., *Eimeria cf. mitraria*, Geoemydidae, *Heosemys depressa*, Myanmar.

INTRODUCTION

Although the South and Southeast Asia represent an area of the highest diversity of turtle species (Van Dijk et al. 2000), only nine species of *Eimeria* are described from turtles of this region. Six of them parasitize softshell turtles (family Trionychidae) of India (Simond 1901, Das Gupta 1938, Chakravarty and Kar 1943, Kar 1944) and three are eimeriids from turtles of the speciose family Geoemydidae (= Bataguridae). Those in the latter family are *E. mitraria* (Laveran et Mesnil,1902) from *Chinemys reevesii* (Gray,1831), *E. kachua* Široký et Modrý, 2005 from *Kachuga tentoria circundata* Mertens, 1969, and *E. patta* Široký et Modrý, 2005, parasitizing *Melanochelys triglua edeniana* Theobald, 1876 (Laveran and Mesnil 1902, Široký and Modrý 2005). Among these species, *E. mitraria* is exceptional in its wide host spectrum and geographical range of distribution, as previous authors have reported it from 12 turtle species of four families.

The Arakan forest turtle *Heosemys depressa* (Anderson, 1875) [for the latest taxonomy see Diesmos et al.]
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(H. depressa, freshly imported from western Myanmar. Two species of *Eimeria* were found to parasitize this turtle, one of which is described in this paper as new.

**MATERIALS AND METHODS**

Nine Arakan forest turtles, *H. depressa*, were imported into the Czech Republic from China and are currently kept alive in a private collection. Originally, these animals were exported by a Chinese pet animal dealer from western Myanmar to China in August 2003. Based on the dealer’s information all specimens were freshly caught in the wild, which corresponds with observed ticks’ infestation and perfect state of health of the animals. Despite this fact, exact locality of their origin was not given.

During quarantine, turtles were housed separately in plastic boxes. Individually collected fresh faeces were placed in 2.5% aqueous (w/v) potassium dichromate (K₂Cr₂O₇), mixed thoroughly, and then allowed to sporulate in open shallow plastic containers at 20-23°C. Samples were examined after concentration by flotation using a modified Sheather’s sugar solution (specific gravity 1.3). Oocysts were measured and photographed using an Olympus Provis AX 70 microscope equipped with Nomarski interference contrast optics (NIC). Morphological features were described according to Duszynski and Wilber (1997), and measurements were made on 30 oocysts using a calibrated ocular micrometer and are reported in micrometers (µm) as means, followed by ranges in parentheses.

**RESULTS**

Coprological examination revealed eggs of undetermined nematodes and the presence of oocysts of two eimerians. Three *H. depressa* were infected with a thus far undescribed *Eimeria* species; all nine harboured the oocysts of *Eimeria cf. mitraria* (Laveran et Mesnil, 1902). Moreover, two ticks of the genus *Amblyomma* were found on a single turtle during the initial inspection.

**Eimeria arakanensis n. sp. (Figs 1-3, 7)**

Description of oocysts: Oocysts are broadly oval to sub-spherical, 28.0 (24-30) × 23.6 (22-25), oocyst shape index (SI, length/width) 1.19 (1.08-1.36), oocyst wall

**Figs 1-3.** Nomarski interference contrast photographs of oocysts of *Eimeria arakanensis* n. sp., all in the same scale. 1 - sporulated oocyst, note large oocyst residuum (arrowhead) and subglobular substieda body (arrow). Scale bar: 10 µm; 2 - sporocysts in collapsed oocyst, note refractile body (arrowhead); 3 - sporulated oocyst from a polar view, note membranous cupola-like structure covering the Stieda body (arrowhead).

**Figs 4-6.** Nomarski interference contrast photographs of oocysts of *Eimeria cf. mitraria* (Laveran et Mesnil, 1902), all in the same scale. 4 - sporulated oocyst, note the Stieda body (arrowhead). Scale bar: 10 µm; 5 - sporocysts tightly packed in sporulated oocyst, prominent refractile body is marked by arrowhead; 6 - overall shape of sporulated oocyst with distinct projections.
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Single-layered, colourless, smooth and ~ 0.6 thick. Micropyle and polar granule absent. Globular oocyst residuum composed of fine granules present, measuring approximately 10 - 15 in diameter. Sporocyst ellipsoidal to oval, 12.8 (12-15) × 7 (6-8), sporocyst SI 1.84 (1.63-2.17), with smooth, colourless and less than 0.5 thick sporocyst wall. Stieda body present, knob-like, ~ 1 high and 1-2 wide (n = 12). Substieda body homogenous, sub-globular, 1-1.5 high and 1.5-2 wide (n = 12). Very thin, membranous, cap-like structure over-layering Stieda body. Sporocyst residuum present as small granules of irregular sizes usually scattered in high number among sporozoites. Sporozoites elongate, cucumber-shaped, lying head to tail within sporocyst. Each sporozoite bears one sub-spherical to spherical refractile body (~1-2 × 1.5-2) at one end, smaller nucleus (~1-2 in diameter) located sub-centrally.

**Type host:** Arakan forest turtle, *Heosemys depressa* (Anderson, 1875) (Reptilia: Testudines: Geoemydidae).

**Type material:** Photosyntypes are deposited at the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, under collection number R 100/05.

**Type locality:** Western Myanmar (Burma), detailed locality unknown.

**Prevalence:** Three of nine examined specimens of *H. depressa* were infected.

**Sporulation:** Exogenous. Oocyst became fully sporulated within 3-5 days at 20-23°C.

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**Table 1.** Review of turtle species reported to date as hosts of *Eimeria mitraria* (Laveran *et al.*, 1902), asterisk marks the type host.

<table>
<thead>
<tr>
<th>Host</th>
<th>Locality</th>
<th>Size of oocysts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geoemydidae</strong> Theobald, 1868</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chinemys reevesii</em> (Gray, 1831)*</td>
<td>Asia</td>
<td>15 × 10</td>
<td>Laveran and Mesnil (1902)</td>
</tr>
<tr>
<td><em>Heosemys depressa</em> (Anderson, 1875)</td>
<td>Myanmar</td>
<td>14.9 × 10.1</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Emydidae</strong> Rafinesque, 1815</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chrysemys picta belli</em> (Gray, 1831)</td>
<td>Iowa (USA)</td>
<td>10-15 × 5-11</td>
<td>Deeds and Jahn (1939)</td>
</tr>
<tr>
<td><em>Emys orbicularis</em> (Linnaeus, 1758)</td>
<td>Iowa (USA)</td>
<td>10 × 7.6</td>
<td>Wacha and Christiansen (1976)</td>
</tr>
<tr>
<td><em>Graptemys pseudogeographica</em> (Gray, 1831)</td>
<td>Iowa (USA)</td>
<td>11 × 8.3</td>
<td>Wacha and Christiansen (1976)</td>
</tr>
<tr>
<td><em>Graptemys versa</em> Stejneger, 1925</td>
<td>Iowa (USA)</td>
<td>9.2 × 8.1</td>
<td>Wacha and Christiansen (1976)</td>
</tr>
<tr>
<td><em>Pseudemys tenax</em> Bair, 1893</td>
<td>Texas (USA)</td>
<td>Not given</td>
<td>McAllister <em>et al.</em> (1991)</td>
</tr>
<tr>
<td><em>Trachemys scripta elegans</em> (Wied-Neuwied, 1839)</td>
<td>Texas (USA)</td>
<td>10 × 8 (8-12 × 6-9)</td>
<td>McAllister and Upton (1989)</td>
</tr>
<tr>
<td><strong>Chelydridae</strong> Agassiz, 1857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chelydra serpentina serpentina</em> (Linnaeus, 1758)</td>
<td>Iowa (USA)</td>
<td>Not given</td>
<td>Wacha and Christiansen (1980)</td>
</tr>
<tr>
<td><strong>Kinosternidae</strong> Agassiz, 1857</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Kinosternon flavescens flavescens</em> (Agassiz, 1857)</td>
<td>Texas (USA)</td>
<td>10 × 8 (8-12 × 6-9)</td>
<td>McAllister and Upton (1989)</td>
</tr>
<tr>
<td><em>Kinosternon flavescens spooneri</em> Smith, 1951</td>
<td>Iowa (USA)</td>
<td>11.5 × 8.6</td>
<td>Wacha and Christiansen (1976)</td>
</tr>
</tbody>
</table>
Site of infection: Unknown, collected from faeces.

Etymology: The specific epithet arakanensis is derived from the name of Arakan hills, the “terra typica” of the type host.

Remarks: Eimeria brodeni Cerruti, 1930 described from Testudo graeca Linnaeus, 1758 from Cagliari, Sardinia, Italy is the only Eimeria from turtles of the Testudinoidea that resembles Eimeria arakanensis n. sp. morphologically. However, oocysts of E. brodeni are narrower and lack an oocyst residuum (Cerruti 1930). E. chrysemysid Deeds et Jahn, 1939 from Chrysemys picta bellii (Gray, 1831) from Iowa, USA possesses oocysts of similar size, but are pear-shaped (Deeds and Jahn 1939) compared to broadly oval of E. arakanensis n. sp. All other coccidian species from testudinoid hosts are significantly smaller.

Eimeria cf. mitraria (Laveran et Mesnil, 1902) (Figs 4-6, 8-11)

Description of oocysts: Oocysts ovoid, 14.9 (13-16) × 10.1 (9-12); SI is 1.48 (1.27-1.78); micropyle, polar granule and oocyst residuum absent. Oocyst wall smooth, colourless, under light microscopy single-layered, ~0.5 thick. Each pole of oocyst bears blunt conical projections, ~1.2 (1-1.5) long. Most oocysts possess three projections on one (“flat”) pole, and one on the opposite (“conical”) end, giving the oocyst projectile-like appearance. Nevertheless, specimens with two and three projections were rarely seen. Sporocysts oval to ellipsoidal, 6.8 (6-8) × 4.2 (3.5-5), with smooth and colourless sporocyst wall and sporocyst SI 1.64 (1.3-2). Stieda body present as tiny knob-like projection. Substieda
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body not discernible. Sporozoites elongate, arranged head to tail within sporocyst. Each sporozoite bears a prominent spherical to sub-spherical refractile body (~ 2-3 × 2) at one end, nucleus not discernible. Sporocyst residuum present, usually as compact granular sphere, ~ 2-3 in diameter.

DISCUSSION

Eimerian parasites are traditionally considered to be highly host specific (Duszynski 1986) and host systematics and geographic origin are common tools in their taxonomy. However, strict host specificity of Eimeria spp. parasitizing reptiles was repeatedly questioned (i.e. Wacha and Christiansen 1974, 1976; McAllister and Upton 1989). Thus, Eimeria spp. described and named from turtles of families Emydidae, Geoemydidae, and Testudinidae [superfamily Testudinoidea sensu Gaffney and Meylan (1988)] are considered in the differential diagnosis of E. arakanensis n. sp. given above.

Genus Eimeria represents the most specious genus within both protozoan and metazoan organisms. Until recently, more than 1700 named Eimeria species have been described on the base of the qualitative and quantitative traits of their sporulated oocyst and their host specificity (Duszynski and Upton 2001). Despite the great oocyst variability, there is an evident trend of repetition of typical morphological features in Eimeria species, originating from different hosts from different zoogeographic regions. Thorough evaluation of oocyst morphology reveals many such “morphotypes”. Eimeria leukarti Reichenow, 1940 from horses and E. cameli Reichenow, 1952 from dromedaries can be used as a most prominent example of such similarity. Recent studies on rodent and bat Eimeria species revealed that some morphological features (namely the presence of the oocysts residuum) show a clear correlation to the phylogenetic relationships as determined by the molecular genetic methods (Zhao and Duszynski 2001a,b; Zhao et al. 2001). It is probable, that further studies reveal phylogeneys, showing even more such “subgroups” within evidently polyphyletic genus Eimeria.

Also among chelonian Eimeria, the oocyst shape and absence vs. presence of the oocyst residuum can be used to group species together (Fig. 12). Howsoever arbitrary such a grouping looks like, above-mentioned case of rodent coccidia suggests the evolutionarily significance of some aspects of the oocyst morphology.

Then, the following morphological types can be delimited within chelonian Eimeria: (a) species with oocysts of various shape possessing distinct oocyst residuum, B - elliptical, sub-spherical to spherical oocysts lacking oocyst residuum; C - thin-walled oocysts with shape influenced by position of sporocysts inside, and lacking oocyst residuum; D - mitra-shaped (Eimeria mitraria - like) oocysts without oocyst residuum.

Figs 12A-D. Schematic drawings of morphological types of oocysts of Eimeria spp. described from turtles. A - oocysts of various shape possessing distinct oocyst residuum, B - elliptical, sub-spherical to spherical oocysts lacking oocyst residuum; C - thin-walled oocysts with shape influenced by position of sporocysts inside, and lacking oocyst residuum; D - mitra-shaped (Eimeria mitraria - like) oocysts without oocyst residuum.

Then, the following morphological types can be delimited within chelonian Eimeria: (a) species with oocysts of various shape possessing distinct oocyst residuum, i.e. E. chrysemidis Deeds et Jahn, 1939; E. cooteri McAllister et Upton, 1989; E. delagei (Labbé, 1893); E. kachua Široký et Modrý, 2005; E. marginata Deeds et Jahn, 1939; (b) species with elliptical, sub-spherical to spherical oocysts without the oocyst residuum (E. geochelona Couch, Stone, Duszynski, Snell et Snell, 1996; E. koormae Das Gupta, 1938; E. mascoutini Wacha et Christiansen, 1976; E. paynei Ernst, Fincher et Stewart, 1971; E. serpentina McAllister, Upton et Trauth, 1990); (c) Eimeria spp. with peculiar thin walled oocysts of polymorphic shape without oocyst residuum (E. motelo Hůrková, Modrý, Koudela et Šlapeta, 2000; E. patta Široký et Modrý, 2005); and, (d) species with Eimeria mitraria-like oocysts, which also lack oocyst residuum and typically possess peculiar projections of the oocyst wall [E. mitraria (Laveran et Mesnil, 1902); E. stylosa, McAllister et Upton, 1989]. At least the latter case is probably a case of synapomorphy rather than convergence and it is possible, that actually each of these morphologically defined groups represent a separate evolutionary lineage, emergence of which predates the separation of continents.
Chelonians of Eurasian and American continents have been geographically separated since the middle Miocene (Danilov 2005). Originally, E. mitraria was described from Chinemens reevesi from SE Asia. Later authors considered further eimerian isolates from 10 Nearctic turtle taxa of other three families (including phylogenetically distant families Chelydridae and Kinosternidae) to be conspecific with E. mitraria (Table 1, Figs 8-11). Moreover, we currently found morphologically very similar oocysts in Neotropical chelid turtle Batrachemys heliostemma McCord, Joseph-Ouni et Lamar, 2001 from Peru (data not shown).

It is hard to imagine that protozoan with a short generation intervals can survive for 15 millions years without further speciation. Thus, wide occurrence of E. mitraria in hosts of two suborders in two distant continents makes the conspecificity of isolates rather improbable. Moreover, all E. mitraria-like coccidia reported to date from Nearctic turtles have smaller oocysts (Table 1) comparing to the size given for E. mitraria by Laveran and Mesnil (1902). We believe that mitra-shaped oocysts reported to date from 13 turtle hosts (McAllister and Upton 1989, Lainson and Naiff 1998, Segade et al. 2004) is a common, synapomorph trait of Eimeria from aquatic turtles. Thus, E. mitraria, as presently used, possibly represents a “morphotype” rather than a species. Similar cases can be found in coccidia of other hosts; i.e. Eimeria callospermophyli Henry, 1932 is reported from both Palaeartic and Nearctic ground squirrels (Wilber et al. 1998).

So far, a universal species concept is not even discussed in the area of coccidian taxonomy and variable combination of morphological, biological and evolutionary species concepts is used throughout the taxonomical studies by various authors. Such a discouraging situation calls for intense research and broad, interdisciplinary discussion aimed to provide a species concept adequate to the current state of knowledge.

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