

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 circumdata* Mertens, 1969, and *E. patta* Široký *et* Modrý, 2005, parasitizing *Melanochelys trijuga 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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(2005)], is endemic to Myanmar (formerly Burma) (Win Maung and Win Ko Ko 2002), and belongs to the rarest turtles of the world (Iverson and McCord 1997), and than being listed as critically endangered (IUCN 2004). Recently, we had a chance to examine nine specimens of *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 ($K_2Cr_2O_7$), mixed thoroughly, and then allowed to sporulate in open shallow plastic containers at 20-23°C.

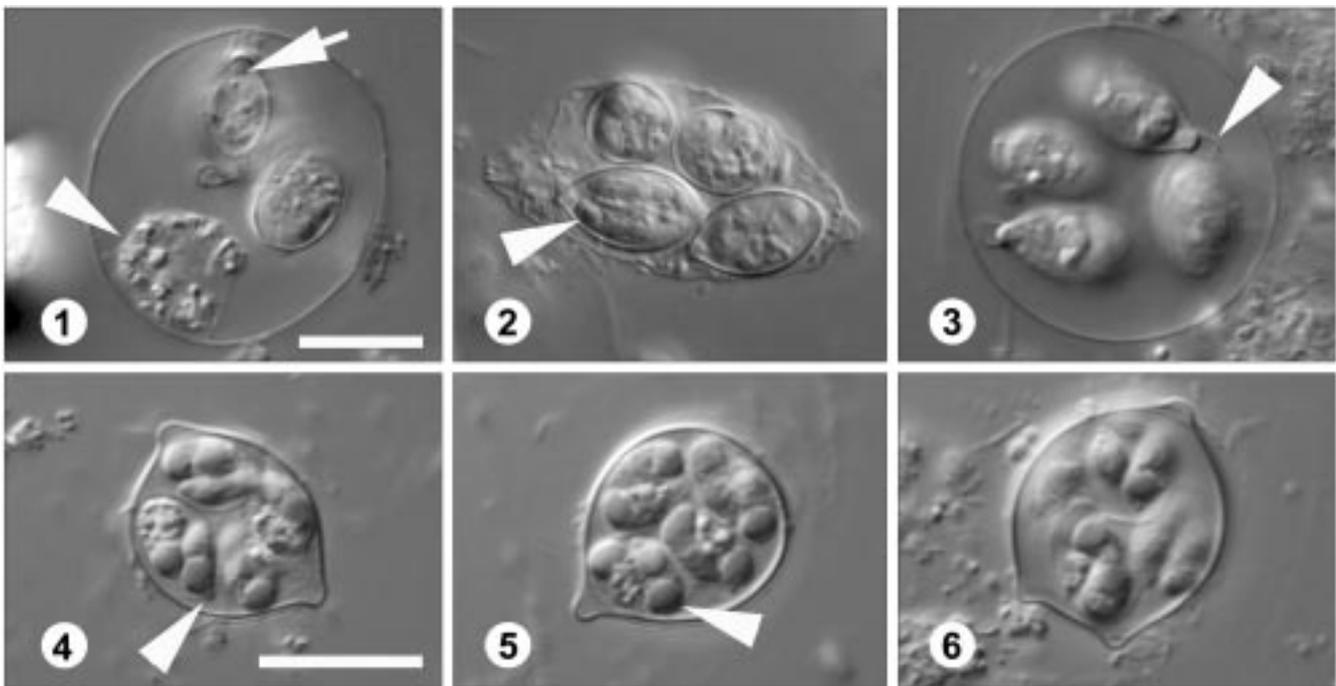
Samples were examined after concentration by flotation using a modified Sheather's sugar solution (specific gravity 1.3). Oocysts were measured and photographed using an Olympus Provis AX 70 microscope equipped with Nomarski interference contrast optics (NIC). Morphological features were described according 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) \times 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: $10\ \mu\text{m}$; **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: $10\ \mu\text{m}$; **5** - sporocysts tightly packed in sporulated oocyst, prominent refractile body is marked by arrowhead; **6** - overall shape of sporulated oocyst with distinct projections.

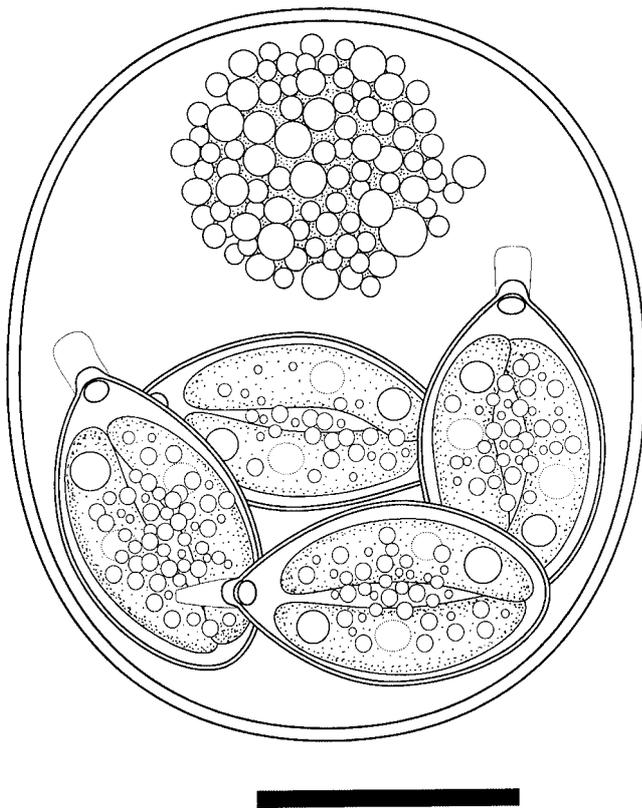


Fig. 7. Composite line drawing of sporulated oocyst of *Eimeria arakanensis* n. sp. Scale bar: 10 μ m.

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. Sporocysts ellipsoidal to oval, $12.8 (12-15) \times 7 (6-8)$, 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 ($\sim 1-2 \times 1.5-2$) at one end, smaller nucleus ($\sim 1-2$ 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.

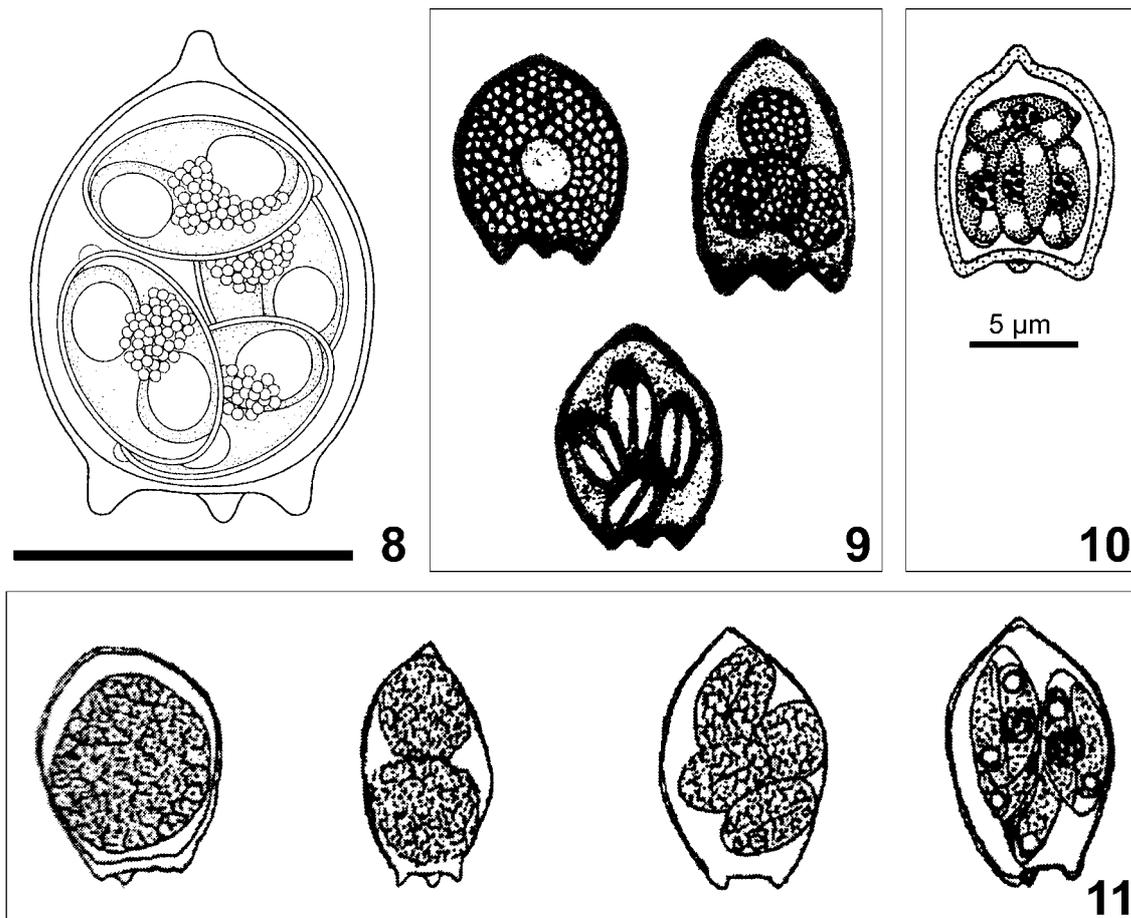
Type locality: Western Myanmar (Burma), detailed locality unknown.

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.

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

Host	Locality	Size of oocysts	References
Geoemydidae Theobald, 1868			
<i>Chinemys reevesii</i> (Gray, 1831)*	Asia	15 \times 10	Laveran and Mesnil (1902)
<i>Heosemys depressa</i> (Anderson, 1875)	Myanmar	14.9 \times 10.1	This study
Emydidae Rafinesque, 1815			
<i>Chrysemys picta bellii</i> (Gray, 1831)	Iowa (USA)	10-15 \times 5-11	Deeds and Jahn (1939)
	Iowa (USA)	10 \times 7.6	Wacha and Christiansen (1976)
<i>Emydoidea blandingii</i> (Holbrook, 1838)	Iowa (USA)	Not given	Wacha and Christiansen (1980)
<i>Emys orbicularis</i> (Linnaeus, 1758)	Galicia (Spain)	12.1 \times 9.5 (10-14 \times 7-11)	Segade <i>et al.</i> (2004)
<i>Graptemys geographica</i> (Le Seur, 1817)	Iowa (USA)	11 \times 8.3	Wacha and Christiansen (1976)
<i>Graptemys pseudogeographica</i> (Gray, 1831)	Iowa (USA)	9.2 \times 8.1	Wacha and Christiansen (1976)
<i>Graptemys versa</i> Stejneger, 1925	Texas (USA)	Not given	McAllister <i>et al.</i> (1991)
<i>Pseudemys texana</i> Baur, 1893		10 \times 8 (8-12 \times 6-9)	McAllister and Upton (1989)
<i>Trachemys scripta elegans</i> (Wied-Neuwied, 1839)	Texas (USA)	Not given	McAllister and Upton (1988)
		10 \times 8 (8-12 \times 6-9)	McAllister and Upton (1989)
Chelydridae Agassiz, 1857			
<i>Chelydra serpentina serpentina</i> (Linnaeus, 1758)	Iowa (USA)	Not given	Wacha and Christiansen (1980)
Kinosternidae Agassiz, 1857			
<i>Kinosternon flavescens flavescens</i> (Agassiz, 1857)	Texas (USA)	10 \times 8 (8-12 \times 6-9)	McAllister and Upton (1989)
<i>Kinosternon flavescens spooneri</i> Smith, 1951	Iowa (USA)	11.5 \times 8.6	Wacha and Christiansen (1976)



Figs 8-11. Comparison of *E. mitraria* from *H. depressa* with drawings from previous studies. **8** - composite line drawing of our isolates of *E. mitraria*. Scale bar: 10 µm. **9** - *E. mitraria* from original description (Laveran and Mesnil 1902). **10** - *E. mitraria* from nearctic turtle species (modified after Wacha and Christiansen 1976). **11** - process of sporulation of *E. mitraria* from *Chrysemys picta bellii* (after Deeds and Jahn 1939).

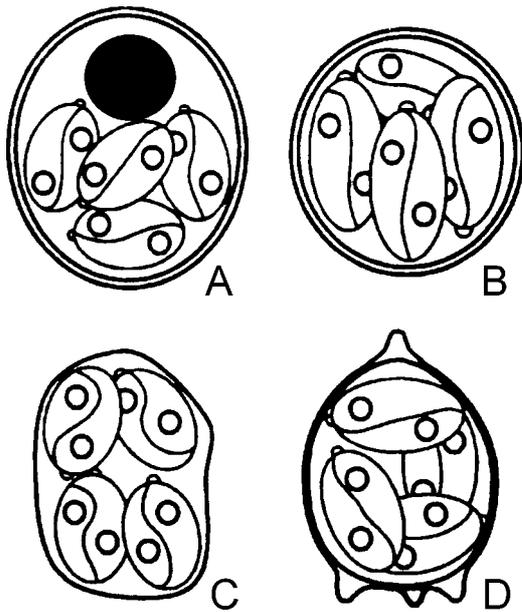
Site of infection: Unknown, collected from faeces.

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. chrysemidis* 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



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.

body not discernible. Sporozoites elongate, arranged head to tail within sporocyst. Each sporozoite bears a prominent spherical to sub-spherical refractile body ($\sim 2\text{-}3 \times 2$) at one end, nucleus not discernible. Sporocyst residuum present, usually as compact granular sphere, $\sim 2\text{-}3$ 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 phylogenies, 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 an oocyst residuum, i.e. *E. chrysemydis* 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 oocyst 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 *Chinemys reevesii* 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, synapomorphic 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.

Acknowledgements. We are indebted to Olympus C&S for generous technical support. We thank Petr Petrás and Hynek Prokop, for cooperation with sampling of their animals. Current systematic position of *H. depressa* was discussed with Uwe Fritz. Additional information about the eimerian coccidia of reptiles can be found at <http://biology.unm.edu/biology/coccidia/home.html>. This study was supported by the grant 524/03/D104 of the Grant Agency of the Czech Republic.

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Received on 20th September, 2005; revised version on 20th January, 2006; accepted on 10th February, 2006