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Eimeriid coccidia (Apicomplexa: Eimeriidae) from Geoemydid Turtles (Testudines: Geoemydidae) with a Description of Six New Species

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Summary. The genus *Eimeria* Schneider, 1875, comprises of > 1,000 described species, making it the most species-rich among coccidians. Within chelonian hosts, only 61 *Eimeria* spp. have been described to date, with the majority known from the New World. Yet, the Southeast Asia region has the highest turtle diversity in the world, from which only a few species have been discovered. Furthermore, the largest turtle family, Geoemydidae Theobald, 1868, has received little attention. In the past decade we examined samples from wild-caught geoemydid turtles from Southeast Asia and adjacent areas. Our work revealed considerable diversity of turtles' coccidia and we describe six new species of *Eimeria* in this report. *Eimeria surinensis* sp. n. from *Malayemys subtrijuga* caught in Thailand possesses almost spherical oocysts (22.6 × 21.4), with spindle-shaped to ellipsoidal sporocysts pointed at both poles (13.4 × 6.9). *Eimeria pangshurae* sp. n. from *Pangshura sylhetensis* residing in India has ellipsoidal to spherical oocysts (16.5 × 13.2) and broadly-ellipsoidal to flask-shaped sporocysts (11.1 × 5.7), with a Stieda body frequently connected with two to four filaments. *Eimeria hynekprokopi* sp. n. from Vietnamese *Cuora galbinifrons* has extremely thin-walled and fragile, elongated oocysts, and is a bit pointed at one pole (15.6 × 8.7), carrying ellipsoidal to broadly oval sporocysts (6.5 × 4.3). *Eimeria zbatagura* sp. n. from *Batagur baska* sampled in Singapore has tiny sub-spherical to broadly ellipsoidal oocysts (7.4 × 6.3 µm) with almost oval but relatively small sporocysts (5.5 × 3 µm). The broadly ellipsoidal, fragile oocysts of *Eimeria petrasi* sp. n. (22.9 × 18.6 µm) collected from *Cyclemys dentata* in the Philippines possess a wavy outer surface of the oocyst wall, and oval to ellipsoidal sporocysts (12.3 × 6.5 µm). *Eimeria palawanensis* sp. n. from *Cyclemys dentata* found in the Philippines has thin-walled and fragile spherical oocysts (14–17 µm wide), plus elongated, ellipsoidal sporocysts (12.6 × 5.8 µm). We also recorded *Eimeria arakanensis* Široký *et* Modrý, 2006 from the new host species – *Cuora flavomarginata* collected in China. Since many chelonians are critically endangered species, their parasite biodiversity will be lost following the collapse of their populations.

Key words: Chelonians, oocysts, parasite diversity, Southeast Asia, Systematics.

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INTRODUCTION

The diversity of parasites surpasses the diversity of their hosts (e.g. Windsor 1998, Poulin and Morand 2000). Among protistan parasites, the majority of taxa still await discovery and formal description, and furthermore will most likely be lost forever as their hosts are driven to extinction. Monoxenous coccidia (e.g. Eimeriidae *sensu lato*) are excellent organisms for studies of protistan diversity and taxonomy because they are easily obtained from the feces of their hosts, their exogenous oocysts possess relatively simple morphology and they sometimes elicit clinical disease, especially in domestic animals under crowded conditions.

Undoubtedly, the genus *Eimeria* Schneider, 1875 is the most species-rich coccidian genus, with more than a thousand described species (Duszynski and Upton 2001, Power *et al.* 2009). Traditionally, insectivores, rodents and ruminants are the most often studied hosts of *Eimeria* spp. (Duszynski and Upton 2001) and data about the diversity of coccidia in these hosts are rather complex. In contrast, only 61 eimerian taxa are named from chelonian hosts (turtles, tortoises, and terrapins) up to now. Most of them originate from the New World (McAllister and Upton 1989a, 1992; McAllister *et al.* 1990a, b; Upton *et al.* 1990, 1992, 1995; Lainson *et al.* 1990, 2008; Lainson and Naiff 1998; Široký *et al.* 2006a). Only four *Eimeria* spp. were discovered in chelonians of the western Palaearctic realm (Labbé 1893, Cerruti 1930, Segade *et al.* 2006), and only a single *Eimeria* was described from a chelonian host inhabiting Sub-Saharan Africa (Široký *et al.* 2006b).

The Oriental region is the area of highest turtle diversity (Ernst and Barbour 1989, Fritz and Havaš 2007). However, there have been few surveys of turtles from this region for eimerians. In particular, little attention has been given to turtles in the Geoemydidae Theobald, 1868. *Eimeria mitraria* (Laveran *et* Mesnil, 1902) from *Mauremys reevesii* (Gray, 1831) is the only coccidian species described from this family in over a century (Laveran and Mesnil 1902). Recently, three species were described from geoemydid hosts, including *E. kachua* Široký *et* Modrý, 2005, from *Pangshura tentoria circumdata* (Mertens, 1969); *E. patta* Široký *et* Modrý, 2005, from *Melanochelys trijuga edeniana* Theobald, 1876; and *E. arakanensis* Široký *et* Modrý, 2006, from *Heosemys depressa* (Anderson, 1875) (Široký and Modrý 2005, 2006).

Further complicating surveys for eimerians in these hosts is that many of these chelonians are near extinction while others are rare and protected by law; thus, fecal samples are difficult to obtain. In past decade, we had the opportunity of examine a set of fecal samples from wild-caught turtles of the family Geoemydidae from Southeast Asia and adjacent areas. Here we report six new and one previously described *Eimeria* species from these hosts.

MATERIALS AND METHODS

Six yellow-margined box turtles *Cuora flavomarginata* (Gray, 1863), were sampled from Qing Ping Market, Kanton City, Guangzhou Province, China in 2003; 34 Indochinese box turtles *Cuora galbinifrons* Bourret, 1939, were collected from Central Vietnam and imported through China into the Czech Republic in 2003–2004; five river terrapins *Batagur baska* (Gray, 1830), were sampled from a turtle farm in Singapore in 2004; six Asian leaf turtles *Cyclemys dentata* (Gray, 1831), were collected from the Malinao River, Palawan Island, Philippines in 2006; four Malayan snail-eating turtles *Malayemys subtrijuga* (Schlegel et Müller, 1845), were sampled from Surin, Isaan Province, Thailand in 2007; three Assam roofed turtles *Pangshura sylhetensis* Jerdon, 1870, were sampled from Assam, India in 2007.

The turtles were housed separately, or in groups in the cases of *B. baska* and *P. sylhetensis*, in plastic boxes, to facilitate the proper isolation of individual turtle species. Collected fresh feces were placed in 2.5% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$), mixed thoroughly, allowed to sporulate in open shallow plastic vials at room temperature and then transported to the laboratory to be examined. The fecal samples were examined after concentration by flotation using a modified Sheather's sugar solution (specific gravity 1.3). Because oocysts from *C. galbinifrons* and *C. dentata* were thin and fragile and could not withstand flotation by this method, oocysts for these samples were obtained by directly sampling the sediment in centrifuged samples. Oocysts were measured and photographed using an Olympus Provis AX 70 microscope equipped with Nomarski differential interference contrast (DIC) optics. Morphological features were described according to Duszynski and Wilber (1997). Measurements were made on 30 oocysts using a calibrated ocular micrometer and are reported in micrometers (μm) as the mean, followed by ranges in parentheses. The chelonian taxonomy proposed by Fritz and Havaš (2007) is used for classifying host species throughout this paper.

RESULTS

Coprological examination revealed the presence of sporulated oocysts of seven coccidian species; six of them are described in this paper as new. Furthermore, one of the isolated coccidia is considered to be conspecific with a species already described.

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***Eimeria surinensis* sp. n. (Figs 1–3, 19)**

Description of oocysts: Sporulated oocysts spherical to slightly sub-spherical, 22.6 (21–25) × 21.4 (20–25) (n = 30); shape index (SI; length/width ratio) 1.06 (1–1.15). Oocyst wall smooth, colorless, optically bi-layered, 1–1.5 thick. Micropyle and polar granule absent. Oocyst residuum distinct, globular, 5–7 wide, composed of small globules and fine granular matter. Dizoic sporocysts elongate, ellipsoidal to spindle shaped, pointed to both ends, 13.4 (13–15) × 6.9 (6–8), SI = 1.94 (1.75–2.33). Stieda body present, robust, cup-like, 2 × 1.5–2. Substieda body present, homogenous, sub-globular, 1.5–2 × 1–1.5. Sporozoites cucumber-shaped, laying head to tail within sporocyst; each bears spherical to sub-spherical refractile bodies (2–3 × 2–3) at both ends. Sporocyst residuum present, consisting of granular matter scattered among sporozoites. Nucleus not discernible.

Type-host: Malayan snail-eating turtle *Malayemys subtrijuga* (Schlegel *et* Müller, 1845) (Testudines: Geoemydidae).

Type-locality: Surin, Isaan province, Thailand.

Type-specimens: Photosyntypes are deposited in collection of Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. IP ProtColl P9.

Symbiotype: An alcohol preserved voucher specimen of the host is deposited in Zoological collection of the National Museum Prague under collection number NMP6V 73559.

Site of infection: Unknown; oocysts were collected from the feces of live animals.

Sporulation: Probably exogenous in spite of examined samples containing only fully sporulated oocysts.

Prevalence: All four examined *Malayemys subtrijuga* were infected (100%).

Etymology: The specific epithet *surinensis* is derived from Surin, the "terra typica" of this species.

Remarks: *Eimeria surinensis* could distantly resemble *E. tetradacrutata* Wacha *et* Christiansen, 1976 and *E. carbonaria* Lainson, da Silva, Franco *et* de Souza, 2008. Contrary to *E. surinensis*, *E. tetradacrutata* has smaller oocyst (19.5 × 19.2 vs. 22.6 × 21.4) with thicker oocyst wall (1.3–1.9 vs. 1–1.5) having remarkably different structure (single-layered and striated vs. bi-layered and colorless), and membrane bounded oocyst residuum. Also shape of sporocyst and Stieda body is much different in *E. tetradacrutata* comparing to *E. surinensis* (see Wacha and Christiansen 1976). Oocysts of *E. carbonaria* are smaller (18.7 × 18.1 vs. 22.6 × 21.4) and have no oocyst residuum (Lainson *et al.* 2008).

***Eimeria pangshurae* sp. n. (Figs 4–6, 20)**

Description of oocysts: Sporulated oocyst spherical to ellipsoidal, 16.5 (15–17) × 13.2 (10–17) (n = 20); SI = 1.28 (1–1.6). Oocyst wall smooth, colorless, single-layered, up to 1 thick, micropyle absent. Polar granule present, single, spherical to sub-spherical, 1.5–2 wide. Oocyst residuum absent. Dizoic sporocysts elongated, broadly ellipsoidal to flask-shaped, single-layered, 11.1 (10–12) × 5.7 (5–7) (n = 20); sporocyst SI 1.97 (1.57–2.2). Stieda body present as flat projection, frequently connected with 2–4 filamentous structures. Substieda body not discernible. Sporocyst residuum present, small, composed of tiny granules, sometimes scattered among sporozoites. Sporozoites laying head to tail (sometimes curved together) possess two refractile bodies, usually one bigger and one smaller, at both ends. Small nucleus (1–1.5 wide), when visible, located sub-centrally. Sporozoites are sometimes striated towards one end.

Type-host: Assam roofed turtle *Pangshura sylhetensis* Jerdon, 1870 (Testudines: Geoemydidae).

Type-locality: Assam, India.

Type-specimens: Photosyntypes are deposited in collection of Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. IP ProtColl P10.

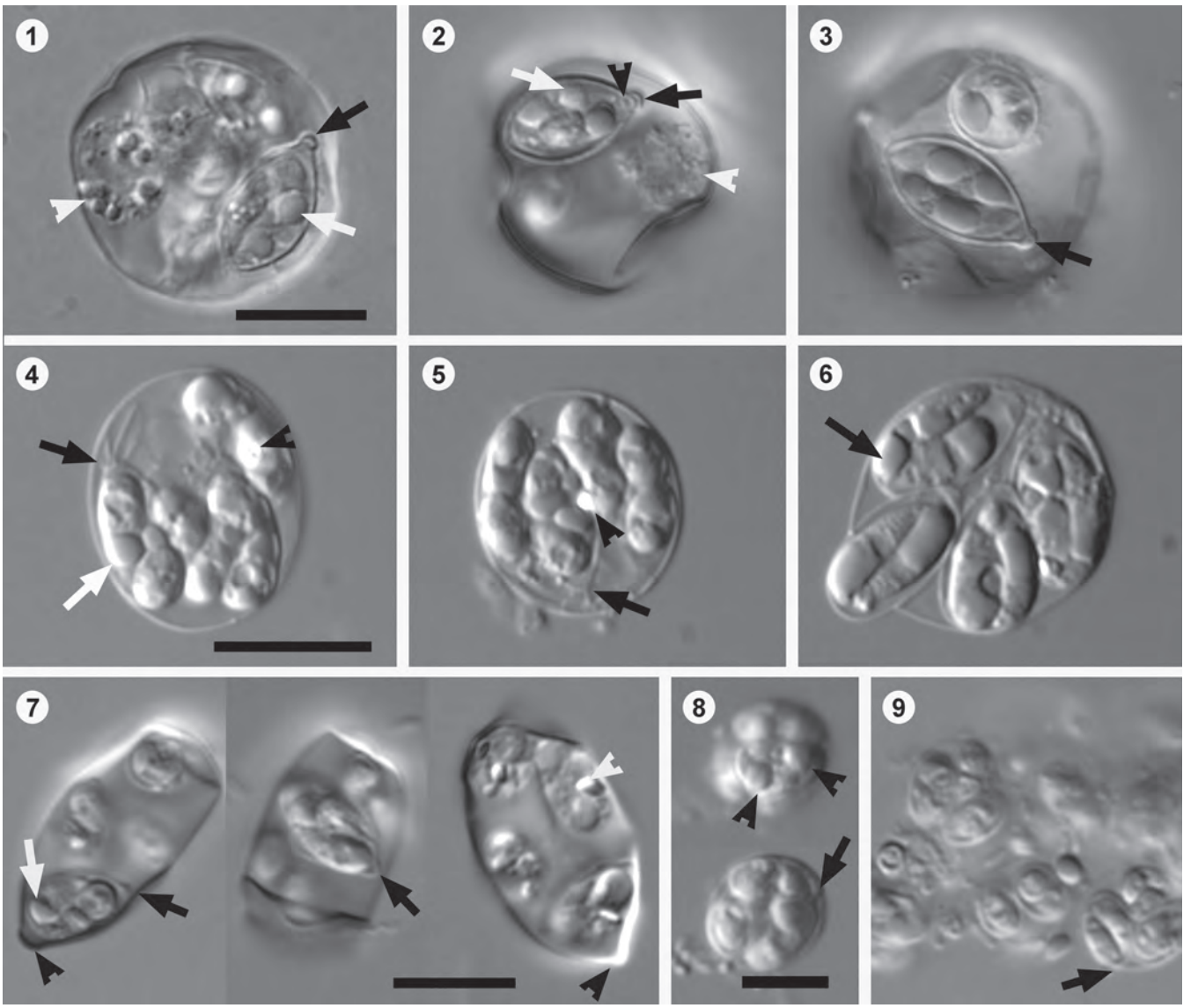
Site of infection: Unknown; oocysts were collected from living animals.

Sporulation: Despite the fact that examined samples contained only fully sporulated oocysts probably exogenous.

Prevalence: Not studied; examined samples originated from a group of three turtles.

Etymology: The specific epithet *pangshurae* is derived as a genitive of the host generic name *Pangshura*, which is grammatically a feminine.

Remarks: Oocysts of five already described *Eimeria* spp. from chelonians distantly resemble those of *E. pangshurae*. *Eimeria carri* Ernst *et* Forrester, 1973 and *E. juniataensis* Pluto *et* Rothenbacher, 1976 both have contrary to *E. pangshurae* the oocyst residuum (Ernst and Forrester 1973, Pluto and Rothenbacher 1976). *Eimeria galaeciaensis* Segade, Crespo, Ayres, Cordero, Arias, García-Estévez *et* Iglesias Blanco, 2006 also possess oocyst residuum, its oocysts are generally bigger than those of *E. pangshurae* (19.3 × 16 vs. 16.5 × 13.2), and its Stieda body possess different shape (see



Figs 1–3. Nomarski differential interference contrast (DIC) photographs of oocysts of *Eimeria surinensis* sp. n., at the same scale. **1 –** sporulated oocyst, note the prominent Stieda body (black arrow), refractile body (white arrow) and large oocyst residuum (white arrowhead). Scale bar: 10 µm; **2 –** collapsing sporulated oocyst, the Stieda body is marked by black arrow, substieda body by black arrowhead, refractile body by white arrow, and oocyst residuum by white arrowhead; **3 –** sporulated oocyst with well outlined sporocyst, the black arrow marks the Stieda body. **Figs 4–6.** DIC photographs of oocysts of *Eimeria pangshurae* sp. n., at the same scale. **4 –** sporulated oocyst with four visible sporocysts, note the Stieda body with visible filaments marked by a black arrow, polar granule (black arrowhead), and refractile body (white arrow). Scale bar represents 10 µm; **5 –** sporulated oocyst, the Stieda body equipped with filaments is marked by a black arrow, polar granule is marked by a black arrowhead; **6 –** slightly compressed oocyst with exiting sporocyst, the refractile body is marked by a black arrow. **Fig. 7.** DIC photographs of three sporulated fragile oocysts of *Eimeria hynekprokopi* sp. n., all shown at the same scale. Stieda bodies are marked by black arrows, pointed poles of oocysts are marked by black arrowheads, refractile body is marked by a white arrow, and sporocyst residuum is marked by a white arrowhead. Scale bar: 5 µm. **Figs 8–9.** DIC photographs of sporulated oocysts of *Eimeria zbatagura* sp. n., both shown at the same scale. **8 –** two oocysts, note the relatively small Stieda body (black arrow), and refractile bodies (black arrowheads). Scale bar: 5 µm; **9 –** oocysts during massive infection, the black arrow marks the Stieda body.

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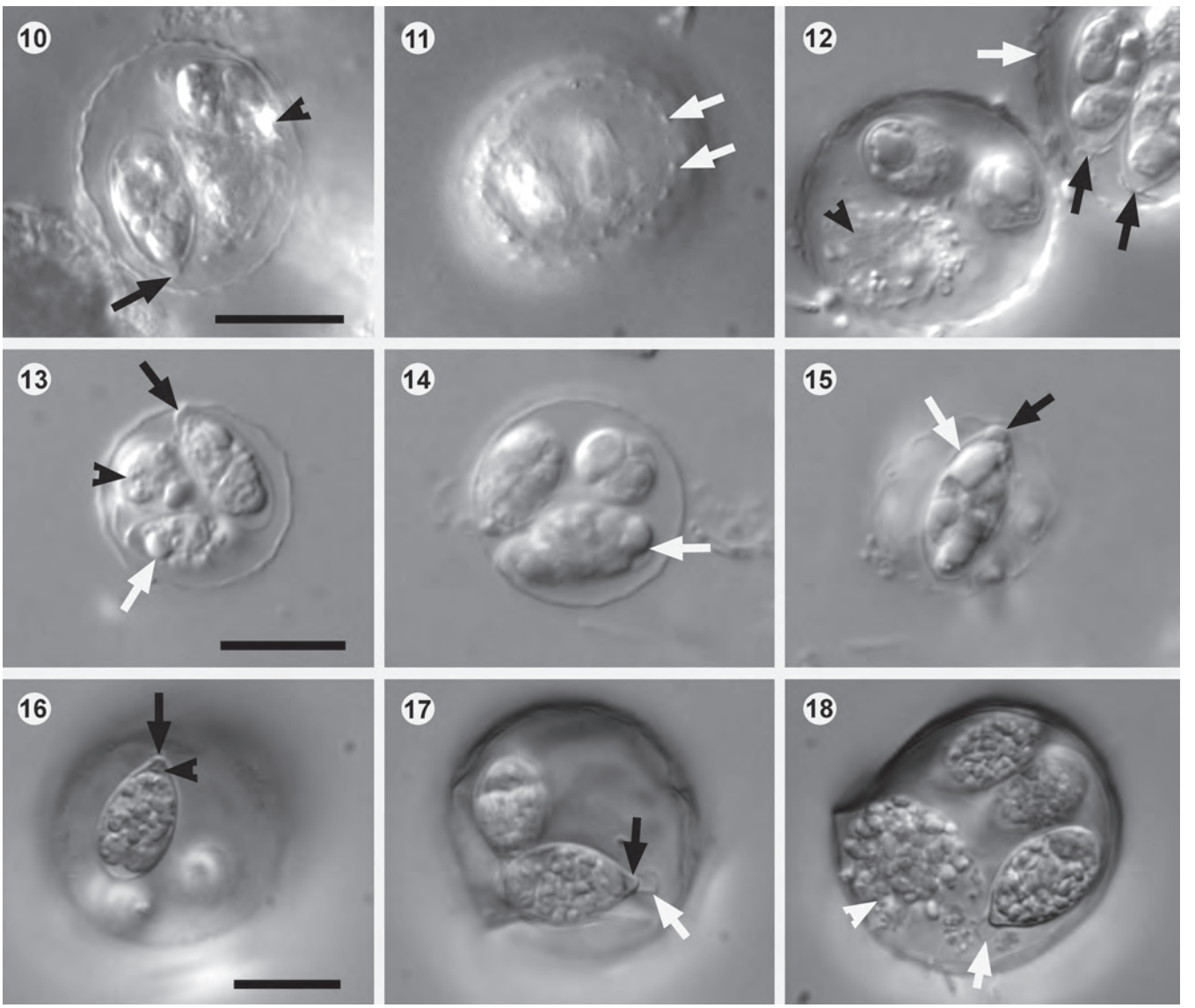
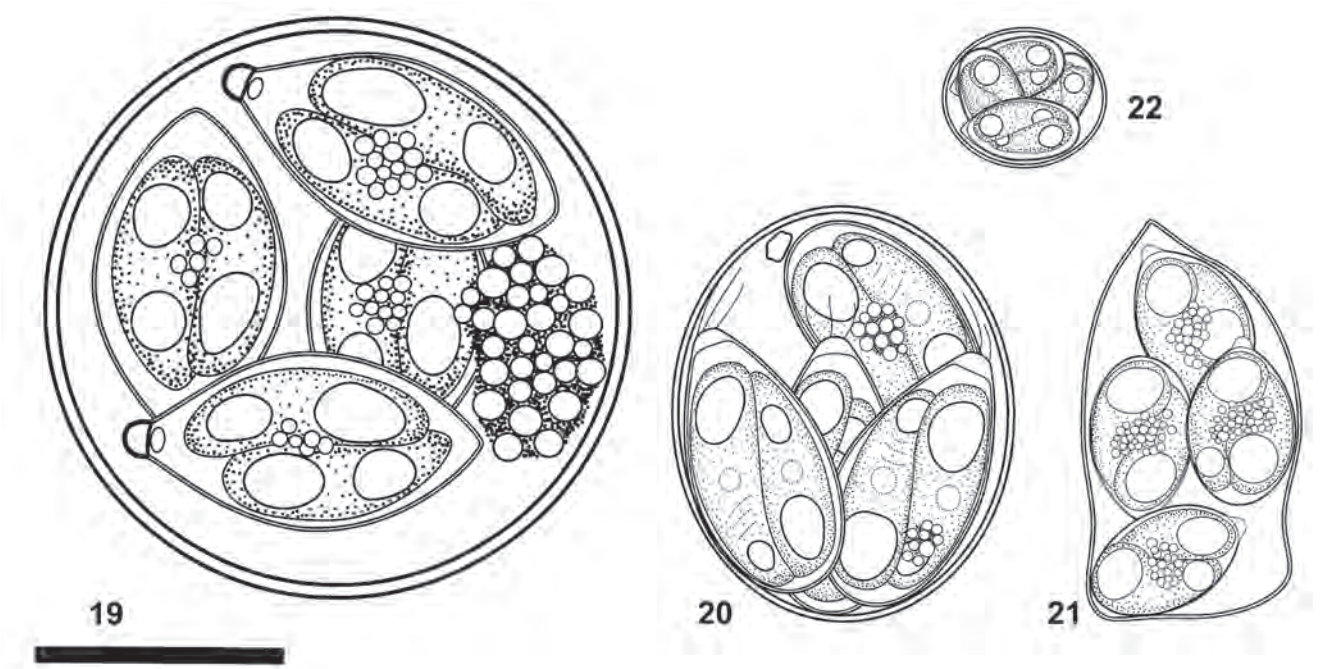


Figs 10–12. DIC photographs of sporulated oocysts of *Eimeria petrasi* sp. n., all depicted at the same scale. **10 –** oocyst showing the Stieda body (black arrow), and one of the polar granules (black arrowhead). Scale bar represents 10 µm; **11 –** outer surface of the oocyst wall with pointed waves marked by white arrows; **12 –** two oocysts showing the wavy outer surface of the oocyst wall (white arrow), Stieda bodies (black arrows), and oocyst residuum (black arrowhead). **Figs 13–15.** DIC photographs of oocysts of *Eimeria palawanensis* sp. n., at the same scale. **13 –** sporulated oocyst showing presence of the Stieda body (black arrow), oocyst residuum (black arrowhead), and refractile body (white arrow). Scale bar: 10 µm; **14 –** general view of the spherical shape of a sporulated oocyst, a white arrow marks the refractile body; **15 –** sporocyst in a collapsed oocyst, the Stieda body is marked by a black arrow, refractile body is marked by a white arrow. **Figs 16–18.** DIC photographs of sporulated oocysts of *Eimeria arakanensis* Široký *et* Modrý, 2006 from the new host *Cuora flavomarginata*, shown at the same scale. **16 –** well-outlined sporocyst showing the Stieda body (black arrow) and substieda body (black arrowhead). Scale bar: 10 µm; **17 –** sporocyst possessing a Stieda body (black arrow) and covered with a fine, membranous, cup-like structure (white arrow); **18 –** fully sporulated, slightly collapsed oocyst showing the typical opposing positions of the sporocysts and large oocyst residuum (white arrowhead). A fine, membranous, cup-like structure covering the Stieda body is marked by a white arrow.



Figs 19–22. Composite line drawings of four new species of *Eimeria* from geoemydid turtles, all depicted at the same scale. Scale bar represents 10 µm. **19 –** *Eimeria surinensis* sp. n.; **20 –** *Eimeria pangshurae* sp. n.; **21 –** *Eimeria hynekprokopi* sp. n.; **22 –** *Eimeria zbatagura* sp. n.

Segade *et al.* 2006). Oocysts of *Eimeria megalostiedai* Wacha *et* Christiansen, 1974 have similar shape, but are smaller (13.9 × 12.8 vs. 16.5 × 13.2) with membrane bounded oocyst residuum. Further, Stieda body of *E. pangshurae* is flat, frequently with filamentous projections compared to large, elongated Stieda body of *E. megalostiedai* (Wacha and Christiansen 1974). *Eimeria ornata* McAllister *et* Upton, 1989 has bigger oocysts (17.9 × 15.7 vs. 16.5 × 13.2) possessing oocyst residuum (McAllister and Upton 1989b).

***Eimeria hynekprokopi* sp. n. (Figs 7, 21)**

Description of oocysts: Sporulated oocyst is elongated, having irregular shape, it tends to be pointed at both poles, 15.6 (14–18) × 8.7 (8–10); oocyst SI 1.8 (1.4–2.25) (n = 30). Oocyst usually possess one point at one pole and two more blunt points at opposite pole, but oocysts bearing two points at both poles were also found. Oocyst wall smooth, colorless, single-layered, and very thin. Because of thin and fragile oocyst wall, the overall shape of the oocysts is variable, influenced to some degree by position of sporocysts. Micropyle, polar granule, and oocyst residuum absent. Sporocysts are ellipsoidal to broadly oval, 6.5 (5–8) × 4.3 (4–5); with smooth, colorless, single-layered very thin sporocyst wall; sporocyst SI 1.54 (1.25–1.88). Stieda body present as small projection, substieda body not discernible. Sporozoites elongated, laying head to tail or encircled one another within the sporocyst. Each sporozoit bears spherical to sub-spherical refractile bodies at both ends, usually one bigger (2–2.5) and one smaller (1.5–2), nucleus was not detected. Sporocyst residuum present as numerous little granules scattered among sporozoites.

Type-host: Indochinese box turtle *Cuora galbinifrons* Bourret, 1939 (Testudines: Geoemydidae).

Type-locality: Central Vietnam.

Type-specimens: Photosyntypes are deposited in collection of Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. IP ProtColl P11.

Site of infection: Unknown; oocysts were collected from the feces of live animals.

Sporulation: Exogenous. Examined samples rarely contained also not fully sporulated oocysts.

Prevalence: Ten of 34 examined *Cuora galbinifrons* were infected (29.4%).

Etymology: The specific epithet *hynekprokopi* is given in honour of Hynek Prokop, for his unselfish tem-

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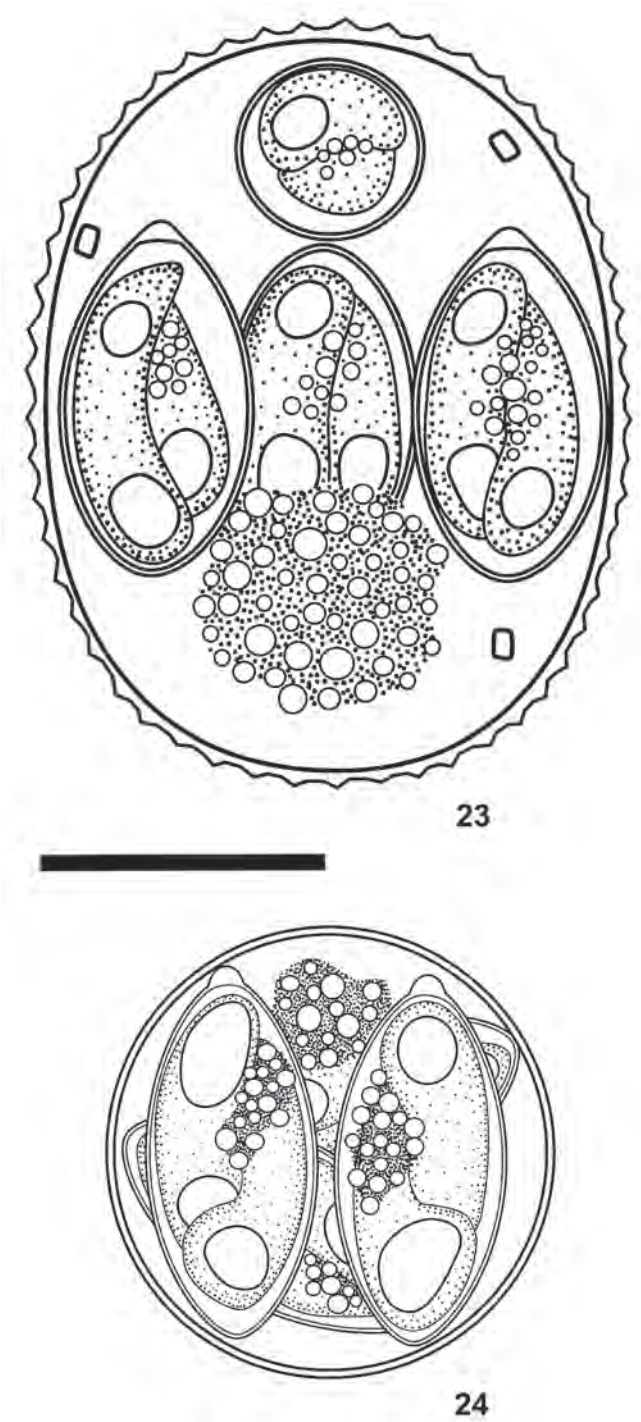

Figs 23–24. Composite line drawings of two new *Eimeria* spp. from geoemydid turtles, both shown at the scale. Scale bar: 10 µm. **23 –** *Eimeria petrasi* sp. n.; **24 –** *Eimeria palawanensis* sp. n.

poral keeping of turtles and for all the assistance with sampling during the study.

Remarks: *Eimeria hynekprokopi* belongs to the group of extremely thin-walled turtles' coccidia together with *E. patta* Široký *et* Modrý, 2005 and *E. motelo* Hůrková, Modrý, Koudela *et* Šlapeta, 2000. Contrary to *E. patta*, *E. hynekprokopi* has pointed shape of oocyst and more elongated oocysts and sporocysts (Široký and Modrý 2005). *E. motelo* has similar oocyst shape as *E. hynekprokopi*, but its sporocysts are more elongated than those of latter species (SI 1.7–2.5 vs. 1.25–1.88). Also the geographic distance between type localities of *E. motelo* and *E. hynekprokopi* tells for *E. hynekprokopi* to be considered as new species (Hůrková *et al.* 2000).

Eimeria zbatagura sp. n. (Figs 8–9, 22)

Description of oocysts: Sporulated oocyst tiny, subspherical to slightly ellipsoidal, stuffed by sporocysts, 7.4 (6–8) × 6.3 (5–7) (n = 30); SI = 1.19 (1–1.6). Oocyst wall smooth, colorless, very thin, single layered. Micropyle and polar granule absent; oocyst residuum was not detected. Sporocysts dizoic, elongate, almost regularly oval, 5.5 (5–6) × 3 (2.5–3) (n = 15), SI = 1.86 (1.67–2), with little pointed Stieda body. Substieda body not discernible. Sporozoites elongate, lying head to tail within sporocyst, each bears spherical to subspherical refractile bodies at both ends (*c.* 1.5 wide), nucleus small (less than 1 wide if visible). Sporocyst residuum not discernible.

Type-host: River terrapin *Batagur baska* (Gray, 1830) (Testudines: Geoemydidae).

Type-locality: Singapore.

Type-specimens: Photosyntypes are deposited in collection of Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. IP ProtColl P12.

Site of infection: Unknown; oocysts were collected from living animals.

Sporulation: Exogenous. Examined samples contained also unsporulated oocysts.

Prevalence: Not studied; examined mixed samples originated from a group of five turtles.

Etymology: The specific epithet *zbatagura* is given, in accordance with International Code of Zoological Nomenclature (Article 31.1) as a noun in apposition (ICZN 1999). It means "originating from *Batagur*" in Czech language.

Remarks: None of *Eimeria* spp. described from any chelonian species possess such unique tiny oocysts like *Eimeria zbatagura*.

Eimeria petrasi sp. n. (Figs 10–12, 23)

Description of oocysts: Oocyst broadly ellipsoidal, 22.9 (20–25) × 18.6 (16–20) (n = 20); oocyst SI 1.22

(1.1–1.4). Oocyst wall colorless, optically single-layered, around 1 thick, fragile, having wavy outer surface covered densely with little pointed knob-like projections. Micropyle absent, up to three polar granules present, subspherical to ellipsoidal (1 × 1–2). Oocyst residuum present, variable in both size and morphology – scattered, irregular to globular (up to 8 wide), composed of fine granular matter, without membranous covering. Sporocyst dizoic, elongate, oval to ellipsoidal, bit pointed at both ends, single-layered, colorless, 12.3 (11–13) × 6.5 (6–7); sporocyst SI 1.87 (1.7–2). Plain, bit pointed, Stieda body present, substieda body not discernible. Sporozoites elongate, laying longitudinally head to tail within sporocyst, with refractile bodies (2–3 wide) at both ends. Sporocyst residuum in a form of small granules scattered among sporozoites. Nucleus not discernible.

Type-host: Asian leaf turtle *Cyclemys dentata* (Gray, 1831) (Testudines: Geoemydidae).

Type-locality: Malinao River, circa 35 km south of Puerto Princessa, Palawan Island, Philippines.

Type-specimens: Photosyntypes are deposited in collection of Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. IP ProtColl P13.

Symbiotype: An alcohol preserved voucher specimen of the host is deposited in Zoological collection of the National Museum Prague under collection number NMP6V 74113.

Site of infection: Unknown; oocysts were collected from feces of living animals.

Sporulation: Exogenous; examined samples contained also unsporulated oocysts.

Prevalence: One of six *Cyclemys dentata* was infected (16.7%). It was the same host as for the following *Eimeria* species.

Etymology: The specific epithet *petrasi* is given in honour of Petr Petrás, for his long lasting and generous help with sampling and field work.

Remarks: There is not described *Eimeria* from any turtle species having oocyst resembling those of *Eimeria petrasi*, particularly its wavy outer surface of oocyst wall, and numerous polar granules.

Eimeria palawanensis sp. n. (Figs 13–15, 24)

Description of oocysts: Oocyst spherical, 14–17 wide; oocyst SI 1–1.1. Oocyst wall smooth, optically single-layered, very thin (~ 1) and fragile, colorless. Micropyle and polar granule absent. Oocyst residuum present, 3 × 3–4, composed of fine granular matter, without membranous covering. Sporocyst elongated, ellipsoidal 12.6 (11–13) × 5.8 (5–6); sporocyst SI 2.2 (1.84–2.4). Sporocyst wall smooth, single-layered, thin. Stieda body present, knob-like, 1–1.5 wide and up to 1 high, substieda body not discernible. Sporocyst residuum present, consists of several granules scattered among sporozoites. Sporozoites vermiform, with one larger (2 × 2–3.5) and one smaller (1–2 wide) refractile body each, laying head to tail longitudinally within sporocyst. Nucleus not discernible.

Type-host: Asian leaf turtle *Cyclemys dentata* (Gray, 1831) (Testudines: Geoemydidae).

Type-locality: Malinao River, circa 35 km south of Puerto Princessa, Palawan Island, Philippines.

Type-specimens: Photosyntypes are deposited in collection of Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, No. IP ProtColl P14.

Symbiotype: An alcohol preserved voucher specimen of the host is deposited in Zoological collection of the National Museum Prague under collection number NMP6V 74113.

Site of infection: Unknown; oocysts were collected from living animals.

Sporulation: Exogenous; examined samples contained also unsporulated oocysts.

Prevalence: One of six *Cyclemys dentata* was infected (16.7%). It was the same host as for *E. petrasi*.

Etymology: The specific epithet *palawanensis* is derived from the name of Palawan Island where the type locality of this species is located.

Remarks: Fragile spherical oocyst of *E. palawanensis* resembles particularly those of *E. pseudemydis* Lainson, 1968, *E. tetradacrutata* Wacha *et* Christiansen, 1976, *E. carri* Ernst *et* Forrester, 1973, *E. lainsoni* Hůrková, Modrý, Koudela *et* Šlapeta, 2000, and *E. ornata* McAllister *et* Upton, 1989. Oocysts of *E. pseudemydis* are in average slightly bigger (19 × 17.5 vs. 14–17), broadly ellipsoidal with pear-shaped sporocysts (Lainson 1968). *Eimeria tetradacrutata* has bigger (19.5 × 19.2 vs. 14–17), thick-walled oocyst, resistant to hypertonic Sheather's solution (oocysts of *E. palawanensis* are destroyed within few minutes), its oocyst wall is more or less striated, oocyst residuum is membrane-bounded, and sporocysts are tear-shaped (Wacha and Christiansen 1976). Oocysts of *E. carri* are smaller (15.9 × 14.5 vs. 14–17), more elongated with membrane-bounded oocyst residuum. Its sporocysts possess, compared to *E. palawanensis*, much smaller Stieda body (Ernst and Forrester 1973). Oocysts of

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Eimeria lainsoni are bigger (19.2 × 18.6 vs. 14–17), have thicker oocyst wall, sporocysts are much broader and sporocysts lack visible Stieda body (Lainson *et al.* 1990). *Eimeria ornata* is most similar to *E. palawanensis*. However, it differs from latter species by more elongated oocysts, presence of polar granule and smaller Stieda body (McAllister and Upton 1989b).

***Eimeria arakanensis* Široký *et* Modrý, 2006 (Figs 16–18)**

Description of oocysts: Sporulated oocysts are broadly oval to sub-spherical 28.5 (25–31) × 23.6 (21–27); oocyst SI 1.21 (1.07–1.36), oocyst wall optically single-layered, colorless, smooth, very thin (~ 0.6). Micropyle and polar granule absent. Oocyst residuum is large, globular, 10–16 wide, composed by granular matter. Sporocysts are ellipsoidal to oval, 12.2 (10–14) × 7.2 (6.5–8), sporocyst SI 1.69 (1.33–1.86), with smooth, colorless and ~ 0.5 thin sporocyst wall. Stieda body present, knob-like, ~ 1 high and 1–2 wide (n = 10). Substieda body sub-spherical to lentil-shaped, 1–1.5 high and 1.5–2 wide, homogenous (n = 8). Thin, membranous, cup-like cover over-layers Stieda body. Sporocyst residuum composed of fine granules is scattered among sporozoites. Cucumber-shaped elongated sporozoites lay head to tail within sporocyst. Each sporozoite bears one sub-spherical to spherical (~ 1.5–2 × 1–2) refractile body at one end, small nucleus (~ 1–2) when visible is located sub-centrally.

New host: Yellow-margined box turtle *Cuora flavomarginata* (Gray, 1863) (Testudines: Geoemydidae).

Locality: Qing Ping Market, Kanton City, Guangzhou Province, China.

Prevalence: Three of six examined *C. flavomarginata* were infected (50%).

Remarks: *Eimeria arakanensis* Široký *et* Modrý, 2006 was originally discovered from *Heosemys depressa* (Anderson, 1875), another member of the family Geoemydidae. Morphology of the oocysts isolated from this new host fit well the traits of this species from original description (Široký and Modrý 2006).

DISCUSSION

In the past decade, we examined more than 150 specimens of turtles and tortoises belonging to 23 species. Eleven out of 12 species of *Eimeria* we have found in these hosts are classified as new (Široký and Modrý 2005, 2006; Široký *et al.* 2006a, b; and this study), proving the diversity of eimeriids in chelonians comparable to that in traditionally studied groups of vertebrates.

So far, the morphological species concept is the predominant method in the taxonomy of eimeriids. However, supportive data about host species and geographic origin are traditionally used, since a high level of host specificity within the genus *Eimeria* is assumed (Duszynski 1986, Lainson *et al.* 2008). Although more than hundred years have passed since the first description of coccidia from chelonians (Labbé 1893), we know virtually nothing about the host specificity of coccidia of chelonians and about their possible cross-transmissions. Recent intensive pet-trade has led to the spread of numerous chelonian species around the globe. Since associated parasites are also distributed in this manner, the importance of host's geographic origin has been radically diminished. To avoid unnecessary synonymy, we extended our differential diagnosis to all *Eimeria* spp. described from hosts belonging to the related chelonian families Geoemydidae, Testudinidae, and Emydidae, which collectively represent the superfamily Testudinoidea sensu Gaffney and Meylan (1988).

According to our recent studies, the Oriental zoogeographic area represents a hot-spot of turtle eimeriid biodiversity. Beside the six new species described above, we have recorded oocysts of several other coccidia in amounts insufficient to be described. Many of the chelonian species are listed by IUCN as endangered or critically endangered (IUCN 2009). Thus the unexplored biodiversity of their parasites can potentially be lost following the collapse of natural populations of hosts, which are often hunted as a food source or source of traditional medicine, as well as exported abroad as pets (Klemens 2000).

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