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An eimeriid species (Apicomplexa: Eimeriidae) that parasitises the gall-bladder and bile-duct of three species of *Takydromus* (Sauria: Lacertidae) in eastern and southeastern Asia

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Abstract

Eimeria takydromi n. sp., a coccidian infecting the gall-bladder epithelium and bile-ducts, was found to parasitise three *Takydromus* species: *T. tachydromoides* of Honshu, Japan; *T. smaragdinus* of Amami Oshima, Ryukyu Islands, Japan; and *T. sexlineatus* of Thailand. Although differences were found in the mean oöcyst and sporocyst indices among the different hosts, the considerable overlap in morphometric characters demonstrated conspecificity. *Eimeria takydromi* is an addition to the symbiotic complex associated with *Takydromus* spp. in eastern and southeastern Asia that includes *Plasmodium sasai*, *Trypanosoma takydromi*, *Schellackia* sp. and Lizard Erythrocytic Virus. These host-symbiote associations may have persisted since a lacertid ancestral to the modern species of *Takydromus* dispersed throughout the region from the late Pliocene.

Introduction

The saurian genus *Takydromus* (Lacertidae) has an extensive distribution throughout the mainland of eastern Asia, Japan, the Ryukyu Islands, Taiwan and southeastern Asia into Indonesia (Boulenger, 1921). Its distribution in the Japanese archipelago reflects the eustatic fluctuations of sea level that occurred in the Pleistocene (Telford, 1982a), which contributed to speciation of both lizard populations and their associated symbiotes. During a three-year population study of *T. tachydromoides* and its symbiotes in Honshu a quarter century ago (Telford, in preparation), four coccidian parasites were found in this host. Two species have been described since, *Eimeria rountreei* Bovee, 1971 and *Isospora nagasakiensis* Miyata, 1976, both of which have their endogenous development in the epithelial cells of the small intestine. Two eimeriid species remain undescribed, one intestinal, from which completely sporulated oöcysts were not obtained, and one that parasitises the gall-bladder and bile-duct. Recently, with the examination of biliary coccidia that inhabit *Takydromus smaragdinus* in the Ryukyu Islands and *Takydromus sexlineatus* in Thailand, it has become evident that the same eimeriid parasite is common to these three hosts. Its description is the subject of this report.

Materials and methods

Lizards were collected by hand or noose, brought to the laboratory and kept alive in polyethylene bags in a refrigerator until killed and necropsied within five days of capture. Contents of the large intestine were examined microscopically, without

flotation, in reptilian Ringer's solution. Unsporulated intestinal coccidian oöcysts were placed in 2% potassium dichromate until they sporulated, usually within five days; infected gall-bladders could be distinguished by their cloudy, somewhat opaque appearance. These were removed, together with the liver and fixed in 10% formalin or Bouin's fixative. Sporulated oöcysts from intestinal contents were preserved in vials, containing 10% buffered formalin for 23–25 years. Both living and preserved oöcysts were studied and measured by calibrated ocular micrometer, and preserved oöcysts were photographed at 1,000× under oil-immersion in temporary sealed coverslip preparations. Preserved tissues were processed by standard histological procedure, sectioned at 4–6 μm and stained originally by haematoxylin-eosin or when destained, by a Giemsa technique. Some livers with infected gall-bladders were removed from formalin-preserved *T. smaragdinus* and *T. sexlineatus*, and oöcysts were obtained by puncture of the gall-bladder, following which the tissues were processed as above for histological study. Statistical comparisons were done by Student t-test, with significance chosen at $p \leq 0.05$; the coefficient of variation (CV) used below is defined as SD × 100/$\bar{x}$.

***Eimeria takydromi* n. sp. (Apicomplexa: Eimeriidae)** (Figs 1–4, 17)

The following is the description of a biliary eimeriid that parasitises *Takydromus tackydromoides*, *T. smaragdinus*, and *T. sexlineatus*. Variation in dimensions and indices among hosts is presented in Table I.

Description of oöcysts

Oöcysts without micropyle, elongate ovoid to cylindroid, rounded at ends, with mean length/width (L/W) index (preserved oöcysts) of 1.69 (1.47–2.21). Living oöcysts 27.8 ± 0.4 × 15.8 ± 1.0 μm (27–28 × 14–17 μm, N = 10), with L/W index 1.59–2.0 ($\bar{x}$ 1.79 ± 0.14). Dimensions of oöcysts from same sample, preserved in 10% formalin for 25 years, average 28.2 ± 1.2 × 16.6 ± 0.9 μm (26–32 × 14.5–19.0 μm, N = 50), with L/W index 1.69 ± 0.15 (1.47–2.21). Oöcysts have smooth surfaces and double wall; oöcyst residuum and polar granule absent. Four sporocysts present, with thick single wall and without Stieda body, oval, with mean L/W index 1.28 (1.06–1.50). Sporocyst residuum small and often indistinct, consists of single globule 1–2 μm in diameter, comprised of irregular particles. The dimensions of preserved sporocysts average 9.4 × 7.4 μm (8.5–11.0 × 7.0–8.5 μm, N = 50). Sporozoites within sporocysts usually lie head to tail, and average 7.5 × 2.0 μm; nuclei approximately central, 2 × 1.8 μm; refractile bodies not noted. Vermiform sporozoites from ruptured sporocysts average 12 × 2.8 μm.

Intrinsic development within epithelial cells lining gall-bladder and bile-duct; host cells hypertrophied and protrude from epithelial layer into lumen (Figs 13–14). Sporulation completed usually within gall-bladder, occasionally within lumen of digestive tract.

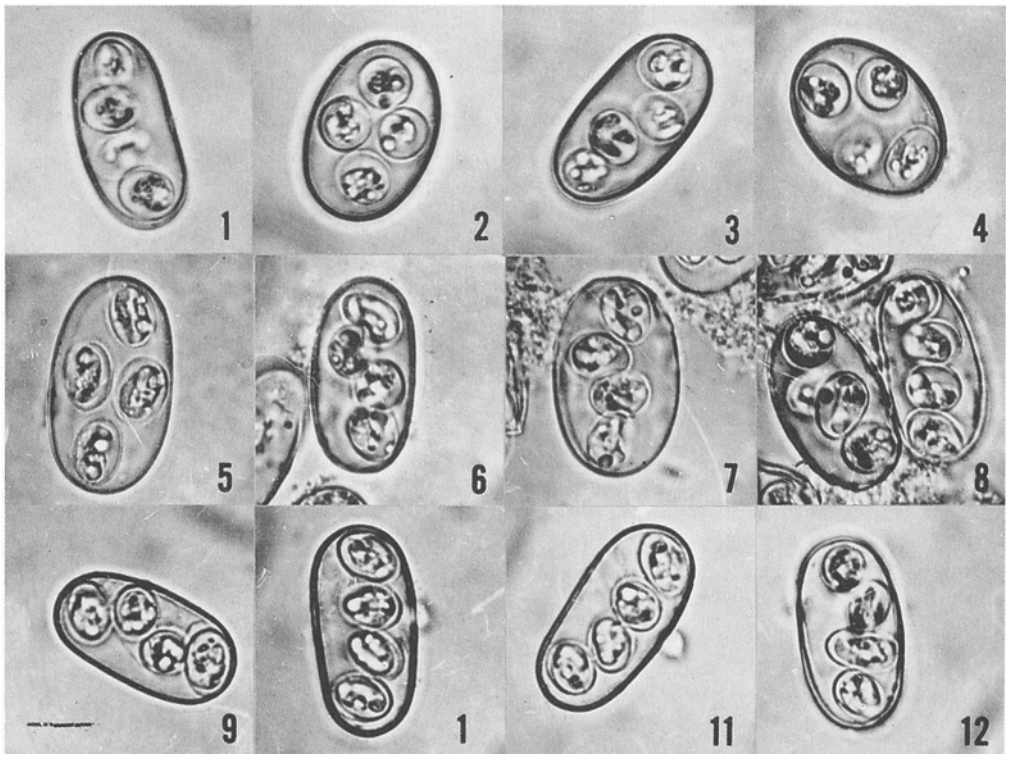
Type-host: *Takydromus tachydromoides* (Schlegel) 1838 (Sauria: Lacertidae), "kanahebi".

Type-locality: Japan, Honshu, Saitama Prefecture, Hanno (35°51′ N, 139°0′ E).

Deposition of types: Hapantotypes (sporulated oöcysts in 10% formalin) and a parahapantotype histological section of liver and gall-bladder deposited in the US National Parasite Collection, Beltsville, Maryland, USA as USNM Helm Coll. Nos 82133 and 82134, respectively. Additional parahapantotypes retained for deposition with the Telford collection.

Other hosts: *Takydromus smaragdinus* from Amami Oshima, Ryukyu Islands (28°09′ N 129°19′ E), and *Takydromus sexlineatus* from Ramintra, near Bangkok, Thailand (13°43′ N, 100°31′ E).

Prevalence: Overall prevalence of *E. takydromi* in the type-host at the type-locality was 17% among 1,107 lizards collected between March 1965 and November 1967; it was not found in 298 *T. tachydromoides* collected from 6 other localities in cen-



Figs 1–12. Eimeria takydromi n. sp. from three species of *Takydromus* (Sauria: Lacertidae). 1–4. Formalin-preserved oöcysts from *T. tachydromoides*, Honshu, Japan. 5–8. Formalin-preserved oöcysts from *T. smaragdinus*, Amami Oshima, Ryukyu Islands, Japan. 9–12. Formalin-preserved oöcysts from *T. sexlineatus*, Thailand. *Scale-bar*: 10 μm.

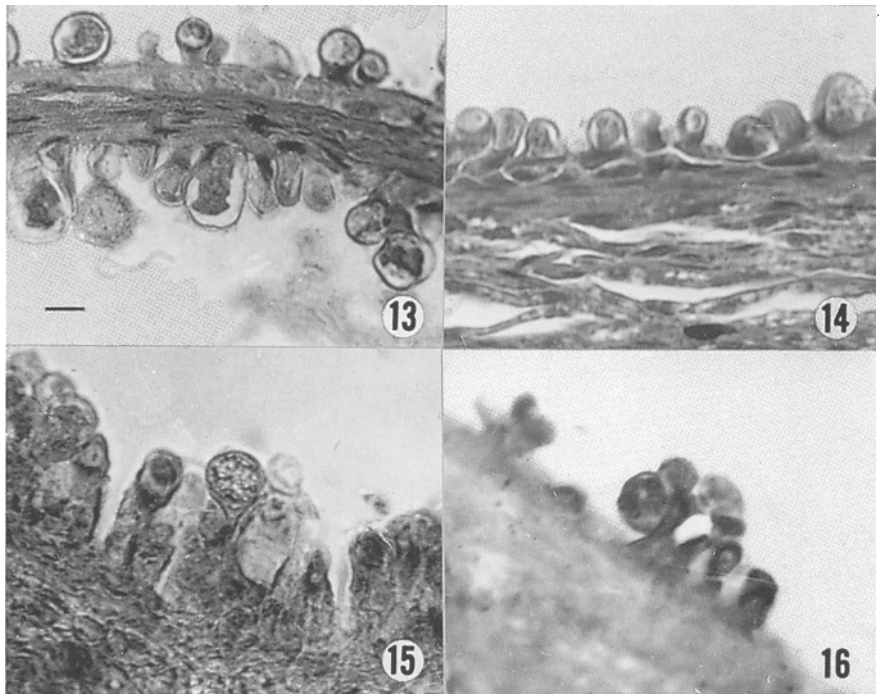
Table I. Variation in the dimensions of sporulated *Eimeria takydromi* oöcysts from three species of *Takydromus*.

	Oöcyst			Sporocyst		
	Length (μm)	Width (μm)	L/W	Length (μm)	Width (μm)	L/W
T. tachydromoides						
mean (N = 50)	28.2 ± 1.21	16.6 ± 0.94	1.69 ± 0.15	9.4 ± 0.6	7.4 ± 0.4	1.28 ± 0.11
range	26–32	14–19	1.47–2.21	8–11	7–9	1.06–1.50
CV	4.3	5.7	8.9	6.4	5.4	8.6
T. smaragdinus						
mean (N = 25)	29.3 ± 1.08	15.4 ± 0.55	1.90 ± 0.09	10.2 ± 0.5	7.8 ± 0.3	1.30 ± 0.08
range	28–32	14–16	1.75–2.04	9–11	7–9	1.18–1.50
CV	3.7	3.6	4.7	4.9	3.9	6.2
T. sexlineatus						
mean (N = 25)	29.7 ± 1.49	15.3 ± 1.02	1.95 ± 0.16	10.2 ± 0.7	6.9 ± 0.4	1.49 ± 0.12
range	26–32	14–17	1.70–2.29	9–11	6–8	1.27–1.69
CV	5.0	6.7	8.2	6.9	5.8	8.1

tral Honshu during the same period. Of 42 *T. smaragdinus* collected in May–June 1965 and May 1967, 7.1% were infected; infected gall-bladders were found in 4 *T. sexlineatus* collected in 1976, but no prevalence data are available.

Discussion

Significant differences in the mean values of most characters exist among the populations sampled: oöcysts from *T. tachydromoides* (Figs 1–4) are



Figs 13–16. Histological sections from gall-bladder lining of hosts to three *Eimeria* species. H&E stain. 13–14. *E. takydromi* n. sp. in *T. tachydromoides*, Honshu, Japan. Epithelial lining doubled back upon itself. 15. *E. japaluris* in *Japalura polygonota*, Amami Oshima, Ryukyu Islands, Japan. 16. *E. pellopleuris* in *Ateuchosaurus pellopleuris*, Amami Oshima, Ryukyu Islands, Japan. *Scale-bar*: 10 μm.

less elongate, on average ($p = <0.01$ for each comparison), than are those from *T. smaragdinus* (Figs 5–8) and *T. sexlineatus* (Figs 9–12), as indicated by the oöcyst L/W (length/width) index, but the indices of the latter two hosts do not differ. As shown by the CV (coefficient of variation) for the L/W indices, the oöcysts from *T. tachydromoides* (8.9) and *T. sexlineatus* (8.2) are more variable in shape than are those of *T. smaragdinus* (4.7). The sporocyst L/W indices of oöcysts from *T. tachydromoides* and *T. smaragdinus* do not differ, but the index of those from *T. sexlineatus* indicate a significantly longer sporocyst than in samples of either of the other hosts ($p = <0.1$ for each comparison). Differences in mean values from samples taken over such a great range (>4,000 km) are not surprising, given the probable isolation of the host populations since the Pliocene. Conspecificity is indicated, in the author's opinion, by the very considerable overlap among all three populations for each of the six characters compared (Table I) and the adherence of the samples from *T. smaragdinus* and *T. sexlineatus* to the other characters described for *E. takydromi* n. sp. from *T. tachydromoides*.

Bovee (1971) described three species of *Eimeria* from the gall-bladder of Japanese lizards with which *E. takydromi* must be compared: *E. japonicis* from *Gekko japonicus* (Gekkonidae), *E. pellopleuris* from *Ateuchosaurus* (=*Lygosoma*) *pellopleurum* (Scincidae) and *E. japaluris* from *Japalura polygonota* (Agamidae). The material studied by Bovee had been preserved by the present author in 10% formalin between 1965–67, and therefore valid comparisons can be made between *E. takydromi* described from preserved oöcysts and the species described by Bovee. Histological sections in the Telford collection demonstrate that in at least the latter two species (Figs 15, 16), as in *E. takydromi* (Figs 13, 14), the intrinsic stages occur in hypertrophied epithelial cells that protrude from the lining of the gall-

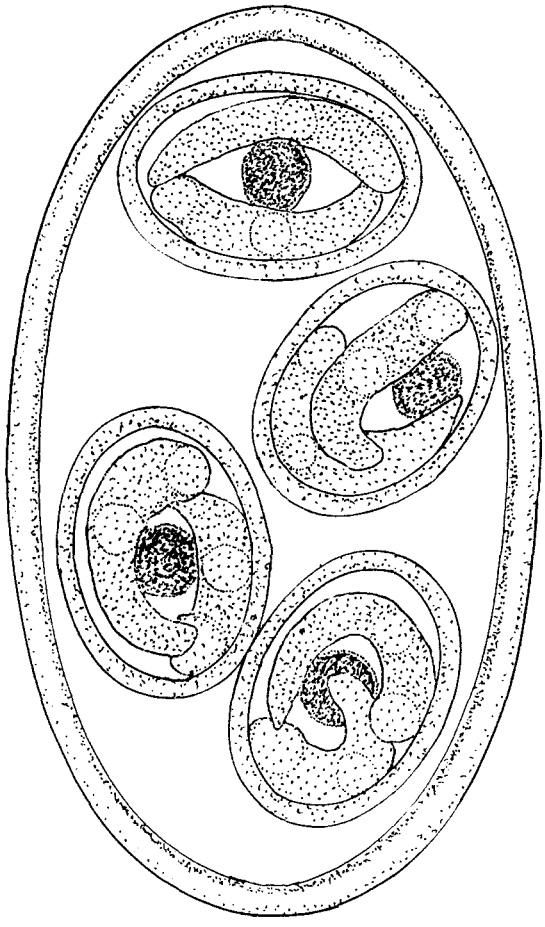


Fig. 17. Composite drawing of a sporulated oöcyst of *Eimeria takydromi*. n. sp.

bladder. This character was used by Paperna & Landsberg (1989) to distinguish their genus *Choleoeimeria* from *Eimeria*, but this distinction is not recognized here, nor has it been accepted by other workers (McAllister *et al.*, 1991). All of these biliary species from East Asian lizards are similar to *E. takydromi* in the absence of a micropyle, oöcyst residuum, polar granule and Stieda body. The oöcysts of *E. japonicis* are 28–35 × 14–19 μm (x̄ 31 × 15 μm), with oöcyst index averaging 2.0 (1.81–2.33), not dissimilar to *E. takydromi*. The sporocysts are, however, considerably longer though of similar width, 11–14 × 7–10 μm (x̄ 12 × 7 μm), with a greater L/W index, 1.5–2.0 (x̄ 1.71). *E. pellopleuris* forms more slender oöcysts, as indicated by a higher average oöcyst index, 2.21, in comparison to average indices of 1.70–1.95 for the three populations of *E. takydromi*. The mean sporocyst index of *E. pellopleuris* is similar to that of *E. takydromi*, 1.26 vs. 1.28 and 1.30 for samples from *T. tachydromoides* and *T. smaragdinus*, respectively, but much lower than the sporocyst index of the sample from *T. sexlineatus*, 1.49. *E. japaluris* similarly forms more slender oöcysts and sporocysts than does *E. takydromi*, with average indices of 2.27 and 1.43, again with greater similarity to the sample from *T. sexlineatus* than to *T. tachydromoides* and *T. smaragdinus*.

Although coccidian species are usually described from fresh, presumably viable oöcysts, there is insufficient evidence, in this author's opinion, that fixation in 10% buffered formalin so alters taxonomic characters that descriptions both adequate and accurate cannot be made from preserved oöcysts. Duszynski & Gardner (1991) have provided the best evidence that some fixatives alter oöcyst morphology in an unacceptable manner. They concluded that oöcysts preserved for 115 days in 10% formalin retained their structural characteristics better than did those in other fixatives, and indeed, upon examining their morphometric data, there is no significant effect upon the dimensions of oöcysts. Their principal objections appear to lie in the presence of 11% fewer sporulated oöcysts and 18% crenation in comparison to unfixed controls, effects which are easily offset by the large numbers of oöcysts that can be included in a reference sample of preserved oöcysts. Weighed against the desirability of deposition of preserved type-material in museum collections, these objections seem of minor importance. The long term storage of preserved oöcysts from oriental lizards for three to five years (Bovee, 1971), and for 23–25 years (this study) did not hinder an accurate description of the several species.

Previously, it was reported that one parasite, *Plasmodium sasai* Telford & Ball, 1969, occurs in

three *Takydromus* species of Honshu (*T. tachydromoides*), Amami Island in the Ryukyus (*T. smaragdinus*) and Thailand (*T. sexlineatus*), a range that exceeds 4,000 km (Telford, 1982a). Another haemoparasite, *Trypanosoma takydromi* Telford, 1982 was described from both *T. smaragdinus* and *T. sexlineatus* (see Telford, 1982b), while both *T. tachydromoides* and *T. sexlineatus* are parasitised by a *Schellackia* species that may be conspecific and a Lizard Erythrocytic Virus (pirhemocyton) as well (Telford, 1982a). It was postulated that these associations represent a host-parasite complex that has persisted since the dispersal of an ancestral lacertid stock that subsequently diversified into the modern *Takydromus* species (Telford, 1982a). The description here of *Eimeria takydromi* n. sp. provides yet another member of this symbiotic complex that testifies to the common origin of the saurian hosts and the persistence of these host-parasite associations for a period that may exceed two million years.

Acknowledgements

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References

Boulenger, G.A. (1920) *Monograph of the Lacertidae*. London. Vol. I, 352 pp., Vol. II, 451 pp.

Bovee, E.C. (1971) New species of *Eimeria* from lizards of Japan. *Transactions of the American Microscopical Society*, **90**, 336–343.

Duszynski, D.W. & Gardner, S.L. (1991) Fixing coccidian oöcysts is not an adequate solution to the problem of preserving protozoan type material. *Journal of Parasitology*, **77**, 52–57.

McAllister, C.T., Freed, P.S., Upton, S.J., Burdick, D.A. & Wilson, N.A. (1991) Parasites of the roughtail gecko, *Cyrtopodion scabrum* (Sauria: Gekkonidae), from Galveston, Texas. *Texas Journal of Science*, **43** 199–204.

Paperna, I. & Landsberg, J.H. (1990) Description and taxonomic discussion of eimerian coccidia from African and Levantine geckoes. *South African Journal of Zoology*, **24**, 345–355.

Telford, S.R., Jr (1982a) Interpopulation variation of a saurian malaria, *Plasmodium sasai* Telford & Ball, 1969, in three host species distributed within a range of 24° north latitude. *International Journal for Parasitology*, **12**, 17–22.

Telford, S.R., Jr (1982b) New saurian trypanosomes from southeast Asian skinks and lacertids. *Journal of Parasitology*, **68**, 319–324.