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Observations on *Eimeria* from *Ambystoma tigrinum*, with Descriptions of Four New Species*

By L. H. Saxe

Introduction

There appear to be only three records of coccidia of the genus *Eimeria* from North American Amphibia. Rankin (1937) reported *Eimeria ranarum* from *Ambystoma opacum*. Walton (personal communication) says that his record (1942) of *Eimeria* from *Ambystoma* is based upon a letter from Dr. Harold Kirby who stated that he had seen *E. ranarum* from *A. opacum*. Doran (1953) reported *Eimeria grobbeni* from *Taricha torosa*.

The author, on several occasions, has noted the presence of coccidian oöcysts in the rectal contents of salamanders. Four species of *Eimeria* have been recognized in material from *Ambystoma tigrinum* (Green) and will be described as new.

Methods

The present observations are based upon material that was removed from the hosts very shortly after their death, placed in vials (diameter = 18 mm.), mixed with 3% potassium dichromate (depth = 8-10 mm.), kept at room temperature (19-23°C), and examined at intervals. Line drawings were made with the aid of a camera lucida. Sections of tissues were cut and stained by Dr. Everett Anderson. Dr. E. R. Becker has read the manuscript, provided advice and made available a translation of an otherwise inaccessible paper.

Observations

Four new species of *Eimeria* from *Ambystoma tigrinum* are characterized as follows:

Eimeria distorta sp. nov. The oöcyst was without a micropyle and in shape was, at least initially, an elongated ellipsoid (figure 1). There was a tendency, however, for the ends to become distorted (figures 2, 3). Deformation was noted in approximately 17% of the unsporulated and 60% of the sporulated oöcysts observed. Fourteen, undeformed, sporulated oöcysts ranged in length from 27.2-31.2 μ and in width from 14.4-16.0 μ. The average size of these oöcysts was 29.2 x 15.5 μ. The average size of 20 undeformed,

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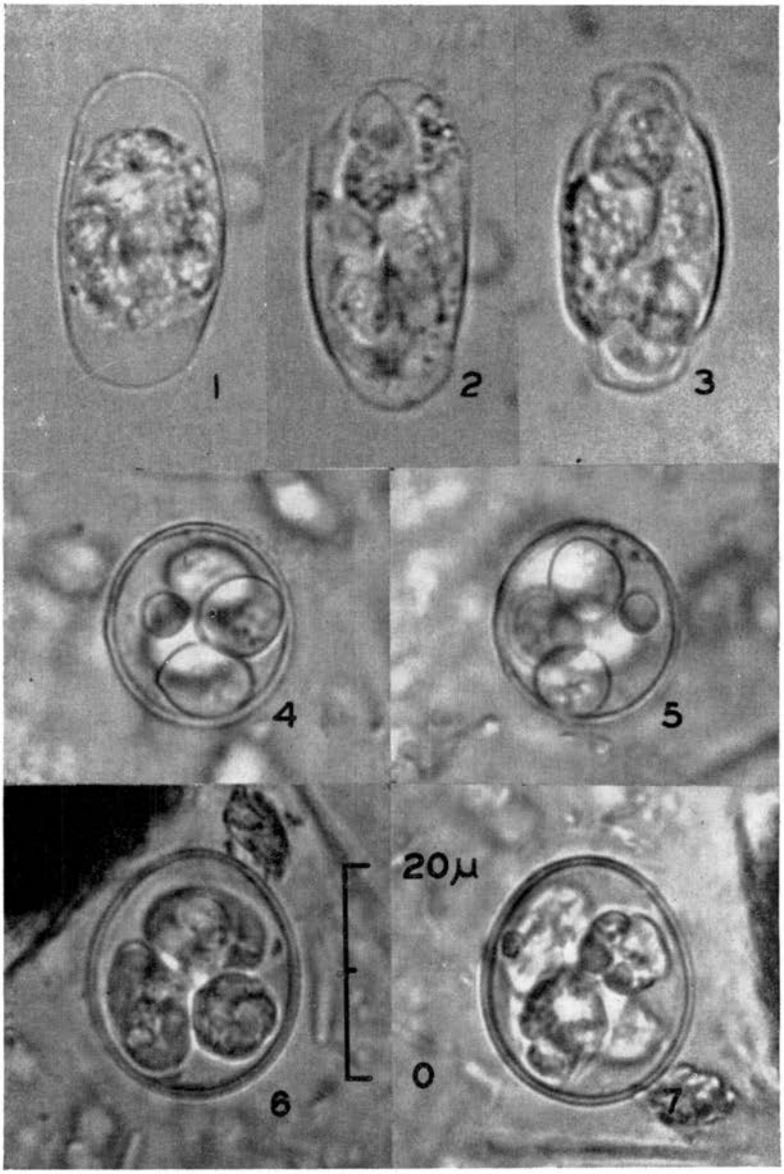


PLATE I

Figs. 1-3. *Eimeria distorta.*
Fig. 1. Oöcyst with contracted but unsegmented oöplasm.

Figs. 2-3. Sporulated oöcysts.

Figs. 4-5. *Eimeria kingi,* sporulated oöcysts.

Figs. 6-7. *Eimeria waltoni,* sporulated oöcysts.

unsporulated oöcysts with contracted oöplasm was 27.5 x 15.5 μ [1]. Oöcystic residual material was present in the form of scattered refractile granules (Text figure A). Spores were elongate ellipsoids with average dimensions of 12.4 x 7.3 μ. The sporocyst was thin and apparently easily deformed by crowding of the spores one against the other. A compact sporocystic residual body was present. One-third of the spores observed at 76 hours were sporulated, as were two-thirds of the spores observed after five days. The host was a larval *Ambystoma tigrinum* with a snout-vent measurement of 77 mm. It was examined on August 1, 1951, a number of days after its collection in Dickinson County, Iowa.

Eimeria kingi sp. nov. Oöcysts were subspherical, without a micropyle, and before sporulation indistinguishable from those of the species next to be described. Twenty-five sporulated oöcysts ranged in length from 16.1-23.3 μ and in width from 14.5-20.7 μ with average dimensions of 20.4 x 18.3 μ. Oöcystic residual material when first seen on the fourth and fifth days of observation was in the form of a compact mass of granules. On the sixth day of observation and thereafter oöcystic residual material commonly consisted of a single refractile sphere (Text figure B). Such spheres had an average diameter of 5.2 μ. Spores were ovoid and 44 averaged 8.8 x 6.5 μ with a range of 7.2-9.9 x 4.8-7.7 μ. The sporocyst was thin and had a small knob at one end. Scattered refractile granules presumable represented sporocystic residual material. Mature spores of this species were first noted on the fifth day of observation. Figures 4 and 5 are photographs of sporulated oöcysts of *E. kingi*. The host was an adult specimen of *Ambystoma tigrinum* taken by Dr. R. L. King from under a stone on the grounds of the Iowa Lakeside Laboratory, Dickinson County, Iowa, on August 4, 1952. *Eimeria kingi* is named in honor of Dr. R. L. King, Professor of Zoology, State University of Iowa, and Director, Iowa Lakeside Laboratory.

Eimeria waltoni sp. nov. Oöcysts were subspherical and without a micropyle. Oöcysts of this species, though on the average somewhat larger than those of the preceeding species, could not be distinguished from oöcysts of *E. kingi* unless sporulated. Twenty-five sporulated oöcysts ranged in length from 20.0-24.2 μ and in width from 16.9-20.9 μ with average dimensions of 22.2 x 19.2 μ, Oöcystic residual material consisted of a relatively small disc

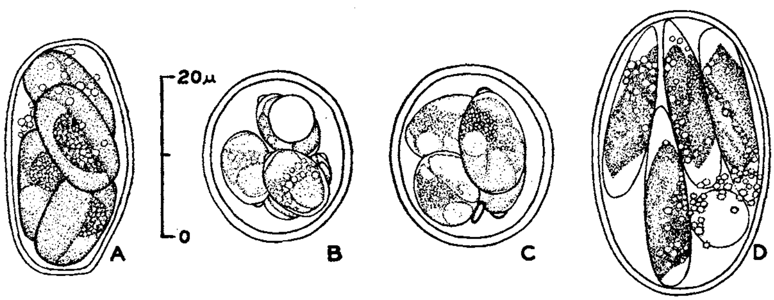
[1] Lavier (1936) reports that with the passage of time there occurs an increase in oöcyst size that may exceed 5 μ; his measurements of oöcyst size (Table I) are those of unsegmented oöcysts.

Explanation of Plates

All figures are photographs of living oöcysts. Photographs for figures 1-3 were taken with facilities provided by Mr. F. P. Ralston. Photographs for figures 4-7 were taken by Mr. F. W. Kent.

shaped, refractile granule (shown in side view in Text figure C). Spores were ovoid and 39 averaged 12.2 x 7.2 μ with a range of 10.8-13.7 x 6.2-8.1 μ. The sporocyst was thin and had a knob at one end. Sporocystic residual material was present in the form of a rather loose aggregration of refractile granules. Spores of this species were first seen 66 hours after the death of the host. Figures 6 and 7 are photographs of sporulated oöcysts of *E. waltoni.* The host was the same specimen as that which harbored the coccidian described above as *E. kingi. Eimeria waltoni* is named in honor of Dr. A. C. Walton, retired Professor of Biology, Knox College, and bibliographer of the parasites of Amphibia.

Eimeria ambystomae sp. nov. Oöcysts were ellipsoidal and lacked a micropyle (Text figure D). Measurements of 64 oöcysts, made over a ten day period, ranged in length from 24.5-35.9 μ and in width from 14.7-19.6 μ with average dimensions of 31.2 x 17.7 μ. There is evidence of a slight increase in oöcyst size with the



Text figures A-D Sporulated oöcysts. A. *Eimeria distorta;* B. *E. kingi;* C. *E. waltoni;* D. *E. ambystomae.*

passage of time: after 24 hours in culture, 26 sporulated or sporulating oöcysts averaged 30.5 x 17.4 μ while after ten days in culture, 25 sporulated oöcysts averaged 32.0 x 17.9 μ. Oöcystic residual material was in the form of a spherical to subspherical body that consisted of a hyalin sphere, making up about two-thirds of the diameter of the residuum surrounded by small refractile granules, that exhibited a tendency to scatter. Measurements of 16 oöcystic residua averaged 12.7 x 11.4 μ. Spores were lanceolate. Measurements of 53 spores averaged 21.9 x 5.1 μ with a range of 17.1-24.5 x 4.1-5.7 μ. Sporocystic residual material was present in the form of refractile granules that were usually scattered but sometimes aggregated in two groups. Almost all oöcysts when removed from the rectum exhibited the oöplasm in the contracted state (Figure 8). Oöcysts (46 to 50) were examined at each of the indicated intervals and the following percentages of sporulated oöcysts were found: 10 hours, 10%; 22 hours, 33%; 36 hours, 76%; 48 hours, 80%. The host was a larval *Ambystoma tigrinum* with a snout-vent length of 90 mm., killed and examined on August 2, 1954, 3 days after its collection in Dickinson County, Iowa.

Eimeria ambystomae was found in a total of six *Ambystoma tigrinum*: four were larvae with snout-vent measurements ranging from 80-90 mm. (collected July 30, 1954) and two were almost completely metamorphosed individuals with snout-vent measurements of 80 and 95 mm. (collected August 11, 1954).

Observations of sporulation time were made on oöcysts from several hosts. One population of oöcysts, examined on August 2 along with the population described above, showed sporulated oöcysts as follows: 10 hours, 18%; 24 hours, 48%; 36 hours 68%; 48 hours, 80%. Two populations examined at 12 hours showed 76% and 57% of their oöcysts to be sporulated. Another population first examined at 25 hours showed 96% of the oöcysts to be sporulated. Sporulation time might be said to range between 12 and 36 hours. Figures 8 to 17 present a photographic record of the process of sporulation as observed in material from several hosts.

From three larvae, merozoites, macro-and microgametocytes of *E. ambystomae* were found in cytoplasm of epithelial cells in stained sections of small intestine taken from two to ten centimeters above the rectum.

On July 21 and 27, 1953 six *Ambystoma tigrinum* larvae were taken from the same pond that provided the 1954 material. Though careful studies were not carried out it is considered very likely that oöcysts found in five of these 1953 larvae were those of *E. ambystomae*.

Sperical or subspherical oöcysts were observed in two of 44 adult *Ambystoma tigrinum* examined in November, 1951. One of these hosts harbored a very small number of oöcysts that were ellipsoidal. Eight specimens of *Desmognathus quadramaculatus* and two of *D. monticola* were also examined and oöcysts were noted in one individual of each species. These 54 hosts were obtained from commercial sources and made available by Dr. E. Witschi and his collaborators.

Discussion

Usually, it is held "that coccidia of the genus *Eimeria* manifest a rather rigid host-specificity" (Becker, 1934). That this generalization may not necessarily apply to the *Eimeria* of Amphibia was pointed out by Lavier (1936). Matubayasi (1937) has reported

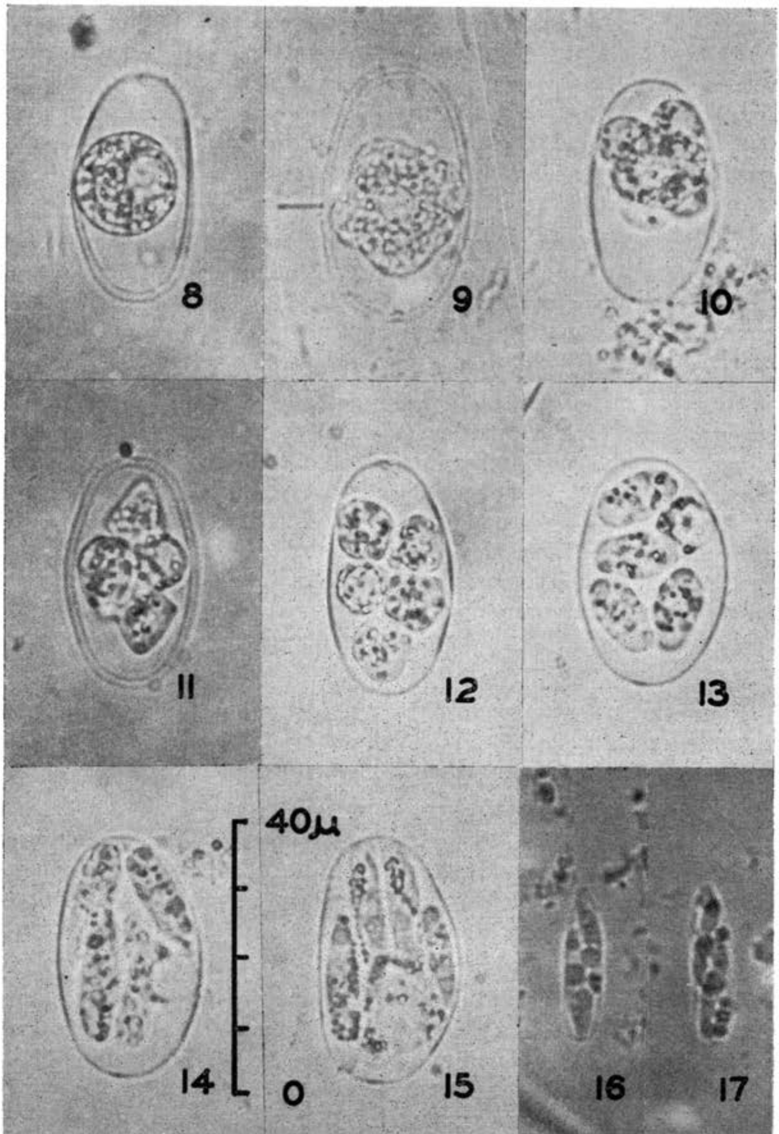


PLATE II

Figs. 8-17. *Eimeria ambystoma.*

Fig. 8 Oöcyst with contracted oöplasm.

Fig. 9. Oöcyst with oöplasm showing bulge formation, the beginning of segmentation.

Fig. 10. Sporulating oöcyst showing segmentation of oöplasm to form four sporoblasts and an oöcystic residuum.

Fig. 11. Sporulating oöcyst; sporoblasts pyramidal; oöcystic residuum at the right.

Fig. 12. Sporulating oöcyst; sporoblasts rounded; oöcystic residuum at the left.

Fig. 13. Sporulating oöcyst; sporoblasts ellipsoidal; oöcystic residuum at upper right.

Fig. 14. Sporulating oöcyst; sporoblasts more elongated; oöcystic residuum at the right.

Fig. 15. Sporulated oöcysts; spores mature, lanceolate; oöcystic residuum at lower right.

Figs. 16-17. Mature, free spores.

E. propria and *E. spherica* from *Triturus pyrrhogaster.* The observations of Lavier; Matubayasi; Rankin (1937); Kirby (*in litt.*); Doran (1953) indicate that certain of the *Eimeria* of Amphibia are widely distributed geographically and not rigidly host-specific.

Hardcastle (1943) in his check list and host index of the genus *Eimeria* cites eight species described from old world species of *Triturus* and *Salamandra.* No new species of *Eimeria* appear to have been described from urodeles since Hardcastle edited his list. The author has reviewed the *Eimeria* described from caudate Amphibia and prepared a summary table that lists the presumably valid and recognizable species (Table I). For *Eimeria tritonis* (Steinhaus, 1891) Walton, 1941, cited by Hardcastle (1943) as a synonym of *E. salamandrae* (for what reason, I do not know), there is no description of the sporulated oöcyst; the organism, thus, cannot be given a characterization that would permit its identification with or distinction from other species of *Eimeria* as currently recognized. *Eimeria labbei* Hardcastle, 1943 (=*Pfeifferia tritonis* Labbé, 1896) is omitted from the table because there is nothing in Labbé's description which would permit a modern specific characterization. Labbé (1899) considered that his material might represent stages in the life cycle of *E. propria.* Siedlecki (1898) identified *P. tritonis* with *E. propria.*

Though there is no certainty, it is not unlikely that the organisms described by Steinhaus and Labbé are of the genus *Eimeria.* If this be true, it is possible that *E. tritonis* (Steinhaus), by the rules, has priority over a subsequently described species. The identification of *E. tritonis* (Steinhaus) or *E. labbei* Hardcastle with any of the more clearly characterized species would require considerable labor and until such studies are carried out these species must be considered as unrecognizable.

Table II lists the species of *Eimeria* reported from Salientia that are considered to be valid. *Eimeria pylori* (Gebhardt, 1897) Levine and Becker, 1933 has been considered to be helminth egg (Braun, 1908) and was not listed by Hardcastle (1943) as a valid species. A translation of Yakimoff and Gousseff's description (1936) of *Eimeria transcaucasica* by Dr. C. A. Hoare (kindly made available by Dr. E. R. Becker) makes it clear that this name is a synonym of *E. mazzai* Yakimoff and Gousseff, 1934.

Eimeria kingi and *E. waltoni* appear to be closley related species. The largest spores of *E. kingi* approach the size of the smallest spores of *E. waltoni,* but in each species oöcysts that might be confused on basis of spore size are exceptional and distinguished readily by the markedly different sizes of the oöcystic residual bodies. As may be seen from Tables I and II these two subspherical species from *Ambystoma* may be distinguished easily from previously

Table I.

Eimeria of Caudata

Name	Author or [Authority]	Oöcyst Form	Oöcyst Size in μ	Oöcyst Residuum	Spores Form	Spores Size in μ	Spores Residuum	Locality (1)	Host (1)
E. ambystomae	Saxe	Elongate ellipsoid	24.5-35.9 × 14.7-19.6 31.2 × 17.7 (mean)	+	Lanceolate	21.9 × 5.1	+	U.S.A.: Dickinson County, Iowa	*Ambystoma tigrinum* (larvae)
E. canaliculata	Lavier, 1936	Ellipsoid	36-42 × 20-27 39-40 × 23-25	+	Lanceolate	25-30 × 6	+	France: Saint-Jean-de-Losne, Côte d'Or	*Triturus alpestris, T. cristatus, T. palmatus, T. vulgaris*
E. distorta	Saxe	Elongate ellipsoid	27.2-31.2 × 14.4-16.0 29.2 × 15.5 (mean)	+	Ellipsoidal	12.4 × 7.3	+	U.S.A.: Dickinson County, Iowa	*Ambystoma tigrinum* (larva)
E. grobbeni	Rudovsky, 1925	Ellipsoid to subsphere	12 × 9.0-10.5(2)	0	Ovoid	5-6 × 4	±	Southern and Western Austria	*Salamandra atra*
E. kingi	Saxe	Subsphere	16.1-23.3 × 14.5-20.7 20.4 × 18.3 (mean)	+	Ovoid	8.8 × 6.5	+	U.S.A.: Dickinson County, Iowa	*Ambystoma tigrinum* (adult)
E. propria	(Schneider, 1881) emend. Lavier, 1936	Ellipsoid	36-43 × 20-27 38-41 × 22-24	+	Fusiform	18-22 × 7-8	+	France: Poitiers, Vienne; Saint-Jean-de-Losne, Côte d'Or	*Triturus alpestris, T. cristatus, T. vulgaris*
E. salamandrae	Steinhaus, 1889 [Simond, 1897]	Sphere or subsphere	18-25 20-30	0	Spherical or subspherical	not given	+	Austria: Vienna Germany: Berlin, Stuttgard France: Brittany	*Salamandra maculata*
E. salamandrae-atrae	(Phisalix, 1927)	Subsphere or shortened ellipsoid	27.5 × 23	?	Spherical	10.5	?	France: La Monta, Hautes-Alpes	*Salamandra atra*
E. spherica	(Schneider, 1887) [Lavier, 1936]	Sphere	22-38 35 (most commonly)	+	Fusiform	12-15 × 6-7	+	France: Poitiers, Vienne; Saint-Jean-de-Losne, Côte d'Or	*Triturus alpestris*
E. tertia	Lavier, 1936	Ovoid	22-23 × 18-25 26 × 21 (mean)	+	Fusiform	12-15 × 6-7	+	France: Saint-Jean-de-Losne, Côte d'Or	*Triturus alpestris*
E. waltoni	Saxe	Subsphere	20.0-24.2 × 16.9-20.9 22.2 × 19.2 (mean)	+	Ovoid	12.2 × 7.2	+	U.S.A.: Dickinson County, Iowa	*Ambystoma tigrinum* (adult)
Eimeria sp.	Rudovsky, 1925	Subsphere with depressed area	9-10 × 10-11	+	Ovoid	3.3 × 2.5 (2)	0	Southern and Western Austria	*Salamandra atra*

(1) = cited by author or authority.
(2) = calculated from figures.

Table II.
***Eimeria* of Salientia**

Name	Author or [Authority]	Oöcyst			Spores			Locality (1)	Host (1)
		Form	Size in μ	Residuum	Form	Size in μ	Residuum		
E. belawini	Yakimoff, 1930	Sphere	12.21	0	Spherical (2)	4.44	?	U.S.S.R.: Pjatigorsk, North Caucasia	*Hyla arborea*
E. cyanophlyctis	Chakravarty and Kar, 1944, 1952	Ellipsoid to subsphere	15.4-19.8 × 15.4-17.6	+	Fusiform	11 × 4.4-6.6	+(1944) 0(1952)	India: Calcutta	*Rana cyanophlyctis*
E. himalayanum	Ray and Misra, 1942, 1943	Subsphere	7-10	0	Fusiform	4.9 × 2.8 (1942) 5.2 × 2.8 (1943)	+	India: Mukteswar-Kumaun U. P.	*Bufo himalayanus*
E. laminata	Ray, 1935	Sphere	8-11	0	Fusiform	4.5-6 × 3	+	India: Calcutta	*Bufo melanostictus*
E. leptodactyli	Carini, 1931a, 1931b	Ellipsoid	23 × 17 (mean)(1931a) 22 × 16 (mean)(1931b)	+	Ovoid	9 × 6.5	+	Brazil: São Paulo	*Leptodactylus ocellatus*
E. mazzai	Yakimoff and Gousseff, 1934, 1936	Sphere	16-18 16.5 (mean)	0	Ovoid	6-8 × 4	+	U.S.S.R. Zurnabad, Azerbaidjan	*Bufo vulgaris*
E. neglecta	Nöller, 1920	Sphere	9-10	0	Ellipsoidal	7 × 3.5-4	+	Germany: Hamburg; Thüringia	*Rama esculenta* *Rana temporaria* (larvae)
E. prevoti	(Laveran and Mesnil, 1902)	Generally, ellipsoid Also, subsphere	20-22 × 12-15 18 × 16	+	Fusiform	8.5 × 3-4 (3)	+	France: Bellevue, Garches, Seine et Oise	*Rana esculenta*
E. ranae	Dobell, 1908, 1909	Sphere or subsphere, tends to collapse over spores	18-22	+	Fusiform	14 × 7	+	England: Cambridge Germany: Munich	*Rana esculenta* *Rana temporaria*
E. ranarum	(Labbé, 1894) [Laveran and Mesnil, 1902]	Ellipsoid	17 × 12	0	Fusiform	7 × 4	+	France: Bellevue, Garches, Seine et Oise	*Rana esculenta* *Rana temporaria*

(1) = cited by author or authority.
(2) = perhaps only sporoblasts [Yakimoff and Gousseff, 1934, 1936].
(3) = calculated from figures.

described species by oöcyst shape and size, the presence of an oöcystic residuum and shape and size of spores.

Eimeria distorta and *E. ambystomae* can be distinguished from previously described amphibian *Eimeria* on the basis of oöcyst shape and size alone but other characters (see Tables I and II) corroborate that they are new species. Both *E. ambystomae* and *E. canaliculata* have lanceolate spores but the oöcysts and spores of the latter are larger. Also the oöcystic residuum of *E. canaliculata* consists of a large mass of large granules. The oöcystic membrane of *E. ambystomae* lacks the fine "canalicules" that are found in the equatorial zone of the oöcystic membrane of *E. canaliculata.*

Summary

1. Coccidia of the genus *Eimeria* reported from Amphibia have been reviewed and their characterizations are summarized in tabular form.
2. *Eimeria distorta, E. kingi, E. waltoni* and *E. ambystomae,* found in *Ambystoma tigrinum,* are described as new species.

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