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Eimerians from Different Karyotypes of the Japanese Wood Mouse (*Apodemus* spp.), with Descriptions of Two New Species and a Redescription of *Eimeria montgomeryae* Lewis and Ball, 1983

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EIMERIANS FROM DIFFERENT KARYOTYPES OF THE JAPANESE WOOD MOUSE (*APODEMUS* SPP.), WITH DESCRIPTIONS OF TWO NEW SPECIES AND A REDESCRIPTION OF *EIMERIA MONTGOMERYAE* LEWIS AND BALL, 1983

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ABSTRACT: Examination of 131 wood mice (*Apodemus* spp.) representing 2 species and 6 subspecies collected from the Japanese islands of Hokkaido, Honshu, Kyushu, and Tsushima showed that 70 mice (53%) had coccidian oocysts in their feces. These included 21 of 42 (50%) *Apodemus argenteus argenteus*; 7 of 14 (50%) *Apodemus argenteus hokkaidi*; 2 of 3 (67%) *Apodemus argenteus sagax*; 3 of 9 (33%) *Apodemus speciosus ainu*; 36 of 61 (59%) *Apodemus speciosus speciosus*; and 1 of 2 (50%) *Apodemus speciosus tusimaensis*. Four distinct coccidians were identified: *Eimeria argenteus* n. sp. from *A. a. argenteus, A. a. hokkaidi, A. a. sagax,* and *A. s. speciosus*; *Eimeria inuyamensis* n. sp. from *A. a. argenteus, A. s. speciosus,* and *A. s. tusimaensis*; *Eimeria montgomeryae* Lewis and Ball, 1983, from *A. a. argenteus, A. a. hokkaidi, A. a. sagax, A. s. ainu,* and *A. s. speciosus*; and *Eimeria uptoni* Lewis and Ball, 1983, from *A. a. argenteus, A. a. hokkaidi,* and *A. s. speciosus.*

Standard karyotypes were prepared from selected specimens of each host subspecies. All 3 subspecies of *A. argenteus* and *A. s. tusimaensis* have a 2n = 46; *A. s. ainu,* from Hokkaido, has a 2n = 48; and *A. s. speciosus* has at least 2 chromosomal races, 1 on northern (2n = 48) and 1 on southern (2n = 46) Honshu. Both chromosomal races of *A. s. speciosus,* as well as the other subspecies of *Apodemus* examined, shared their coccidian parasites freely.

As part of a long-term study at the University of New Mexico, we are using parasite burdens and other host measurements (e.g., pelage, skeleton, chromosomes, enzymes, etc.) as tools to better understand the genetic relatedness of many groups of small mammals throughout North America, Mexico, and other parts of the world and to investigate the degree of correlation between host variability and parasite host-specificity. During the summer of 1981, we collected small mammals from throughout Japan in a study to look at zoogeographic distribution patterns of Asian insectivores and their possible genetic relationship to their North American counterparts. In addition to moles and shrews, we also collected 131 *Apodemus* Kaup, 1829, representing 2 species and 6 subspecies. Since the genetics of Japanese wood mice had been well studied (Tsuchiya and Yosida, 1971; Tsuchiya et al., 1973; Tsuchiya, 1974) and since these hosts had not been examined for coccidia in Japan, it was of interest to look at this host–parasite system as a potential model to study the subtleties of coccidian host-specificity in rodents.

MATERIALS AND METHODS

All hosts were live-trapped and killed in the field. The intestinal tract was removed and feces from the cecum and colon were placed in vials containing 2% aqueous (v/v) H_2SO_4.

Upon return to the United States (≅60 days) the vials were refrigerated (4 C) until they could be examined. Techniques used to process and examine feces and to measure and photograph oocysts are described elsewhere (Duszynski et al., 1982). All measurements are in μm with ranges given in parentheses following the means.

One or more mice of each subspecies, collected from each locality, were karyotyped according to the procedure described by Baker (1970). Stained karyotypes were examined and chromosomes counted and photographed upon return to The University of New Mexico.

RESULTS

The hosts, their collection localities, the coccidian species with which the hosts were infected, and the representative karyotypes of each host subspecies are presented in Table I.

Eimeria montgomeryae Lewis and Ball, 1983
(Figs. 1–4, 10)

Description

Oocyst subspheroid to ellipsoid with wall ≅1.5 consisting of 2 layers: outer layer pigmented and roughly pitted ≅⅔ of total thickness; inner layer smooth, colorless; micropyle and oocyst residuum absent; 1–3 polar bodies present; sporulated oocysts (n = 167) 21.3 × 18.0 (16–29 × 15–24) with L:W ratio 1.18 (1.00–1.59); sporocysts ovoid, (n = 182) 12.7 × 7.2 (9–16 × 6–8) with L:W ratio 1.78 (1.28–2.21); large Stieda and substieda bodies present, both approximately the same

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TABLE I. Eimeria *spp. found in and diploid numbers of* Apodemus *spp. collected in Japan.*

Apodemus spp.	Collection locality	No. hosts infected/ examined (%)	*Eimeria* spp. identified from hosts	No. hosts karyo-typed	Diploid chromo-some no.
argenteus argenteus	Honshu, Aichi, Inuyama City, near Jyakkoin Temple	6/15 (40)	*argenteus, inuyamensis, uptoni,* sp.*	4	46
	Honshu, Negano 2.4 km E of Haramu-ra	14/22 (64)	*argenteus, inuyamensis, montgom-eryae, uptoni,* sp.*	5	46
	Honshu, Yamagata Pref., 3.5 km N of Zao-Onsen Mt. Lodge	1/4 (25)	*montgomeryae*	1	46
	Kyushu, Oita Pref., mole farm near Ogicho	0/1		1	46
a. hokkaidi	Hokkaido, Univ. of Hokkaido Experi-mental Research Station	7/14 (50)	*argenteus, montgomeryae, uptoni,* sp.*	2	46
a. sagax	Tsushima Island, Nagasaki Pref., Mine	2/3 (67)	*argenteus, montgomeryae,* sp.*	2	46
speciosus ainu	Hokkaido, Univ. of Hokkaido Experi-mental Research Station	3/9 (33)	*montgomeryae,* sp.*	2	48
s. speciosus	Honshu, Aichi, Inuyama City, near Jyakkoin Temple	10/23 (43)	*argenteus, montgomeryae, uptoni,* sp.*	2	46
	Honshu, Negano 2.4 km E of Haramu-ra	4/7 (57)	*argenteus, montgomeryae, uptoni,* sp.*	1	48
	Honshu, Niigata Pref., Shuinji T., Shuin Country Club	10/12 (83)	*inuyamensis, montgomeryae,* sp.*	3	48
	Honshu, Yamagata Pref., 3.5 km N of Zao-Onsen Mt. Lodge	5/11 (45)	*argenteus, montgomeryae, uptoni,* sp.*	1	48
	Kyushu, Oita Pref., mole farm near Ogicho	7/8 (88)	*argenteus, inuyamensis, montgom-eryae, uptoni,* sp.*	4	46
s. tusimaensis	Tsushima Island, Nagasaki Pref., Mine	1/2 (50)	*inuyamensis*	1	46
6	7	70/131 (53)	4	29	2

* Oocysts were unsporulated/deteriorated.

width (≅2.5); sporocyst residuum a compact mass of granules enfolded by sporozoites. Our oocysts were 271 days old when measured.

Taxonomic summary

Diagnosis: Pellérdy (1954) described *Eimeria apodemi,* from *Apodemus sylvaticus* Linnaeus, 1758, and *Apodemus flavicollis* Melchior, 1834, in Hungary, to have asymmetrically ellipsoid oocysts that measured 24 × 20 (21–27 × 15–22) with a smooth brown outer oocyst wall and no polar bodies; the sporocysts were ellipsoid, 12 × 7, with no Stieda body. Lewis and Ball (1983) saw *E. apodemi* in the same 2 host species from the British Isles, but described the oocysts they saw to have a rough outer wall, 1–3 polar bodies, and ovoid sporocysts with a large Stieda body. Lewis and Ball (1983) also described *E. montgomeryae,* from *A. sylvaticus,* to have oocysts with a roughly-pitted outer wall, 1–3 polar bodies, and ovoid sporocysts with large Stieda bodies; oocysts of *E. montgomeryae* were 22 × 19 (18–24 × 16–23). The shape indices of the oocysts and sporocysts of the *E. apodemi* and *E. montgomeryae* measured and described by Lewis and Ball (1983) are identical, as are their line drawings. We are convinced (1) that Pellérdy (1954) could not have missed such key oocyst characters as a rough oocyst wall, polar bodies, and Stieda bodies in his description of *E. apodemi* and, therefore, (2) that the *E. apodemi* and *E. montgomeryae* described by Lewis and Ball (1983) represent a single species with a very broad range of oocyst sizes; this is not unusual in rodent coccidia (see Duszynski, 1971). In looking at the oocysts from Japanese *Apodemus* spp. it was clear that we had a form that was identical to *E. montgomeryae* except that the sporocysts of our form had a distinct substieda body (Figs. 1–4) that was always approximately the same width as the Stieda body (this is an important distinction, see below). In the paper by Lewis and Ball (1983), their figure 3h is a photomicrograph of a sporocyst of *E. montgomeryae*; it shows a distinct substieda body (the same width as the Stieda body) which they omitted in their description and in their line drawing of *E. montgomeryae.* We have included this key character in our redescription (above).

Hosts and localities: See Table I.

Site of infection: Unknown. Oocysts recovered from feces.

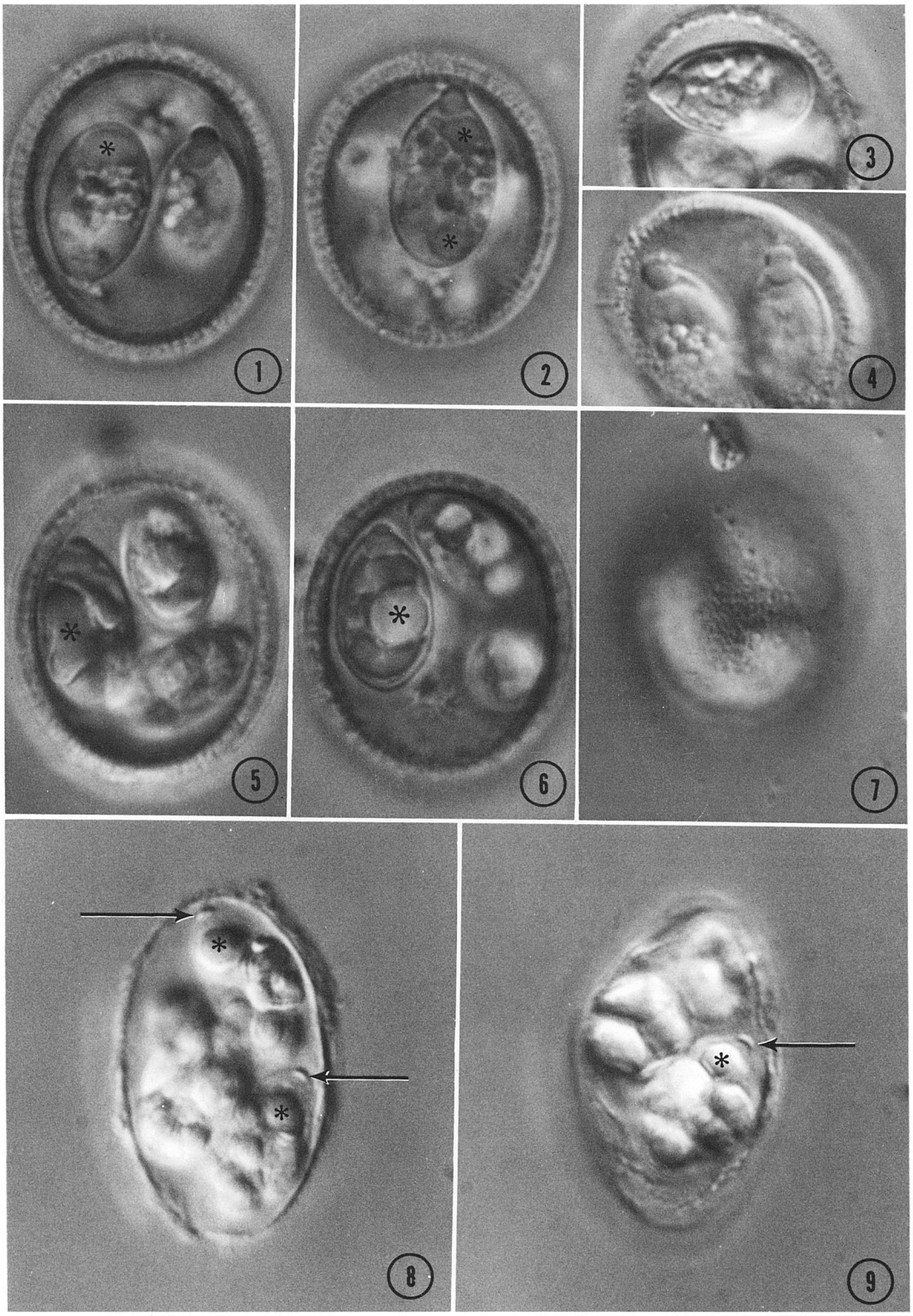
Prevalence: Found in 8 of 21 (38%) infected *Apodemus argenteus argenteus,* 2 of 7 (29%) infected *Apodemus argenteus hokkaidi,* 1 of 2 (50%) infected *Apodemus argenteus sagax,* 2 of 3 (67%) infected *Apodemus speciosus ainu,* and 21 of 36 (58%) infected *Apodemus speciosus speciosus.*

Eimeria argenteus n. sp.

(Figs. 5–7, 11)

Description

Oocyst subspheroid, wall ≅1.5 consisting of 2 layers: outer layer golden, pitted (Fig. 7) ≅¾ of total thickness; micropyle and oocyst residuum absent; 1–2 polar bodies present; sporulated oocysts (n = 87) 22.5 × 19.3 (18–29 × 16–24) with L:W ratio 1.18 (1.00–1.50); sporocysts (n = 92) ovoid, 12.7 × 7.3 (11–14 × 6–9) with L:W ratio 1.74 (1.51–1.99); Stieda and substieda body present with substieda body at least 2 times wider than Stieda body (Figs. 5, 6); sporocyst residuum 1 large spheroid globule ≅4.4; sporozoites with 2 refractile bodies. Oocysts were 340 days old when measured.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Taxonomic summary

Diagnosis: This species most closely resembles *E. montgomeryae,* but differs in that the sporocysts have substieda bodies that are always 2 times wider than their associated Stieda body and the sporocysts have a residuum that is a single, homogeneous body rather than a compact group of granules.

Type host: Apodemus argenteus argenteus Temminck, 1845, Japanese wood mouse, Museum of Southwestern Biology, Division of Mammals, MSB 45248 (female), S. B. George #715, 23 May 1981.

Type locality: Honshu, Aichi, Inuyama City, near Jyakkoin Temple.

Other hosts and localities: See Table I.

Site of infection: Unknown. Oocysts recovered from feces.

Prevalence: Found in 8 of 21 (38%) infected *A. a. argenteus,* 3 of 7 (43%) infected *A. a. hokkaidi,* 1 of 2 (50%) infected *A. a. sagax,* and 5 of 36 (14%) infected *A. s. speciosus.*

Etymology: The specific name is derived from the specific part of the scientific name of the host.

Eimeria inuyamensis n. sp.

(Figs. 8, 9, 12)

Description

Oocyst elongate-ellipsoid, wall ≤1.0 consisting of 2 layers, outer layer slightly sculptured ≅½ of total thickness; micropyle and oocyst residuum absent; 1–2 polar bodies present; sporulated oocysts (n = 14) 25.6 × 16.2 (22–29 × 14–18) with L:W ratio 1.59 (1.50–1.75); sporocysts (n = 14) ovoid 11.3 × 7.4 (10–14 × 6–8) with L:W ratio 1.55 (1.32–1.88); Stieda body present; substieda body absent; sporocyst residuum a large granular mass; sporozoites with at least 1 refractile body present at posterior end. Oocysts were 360 days old when measured.

Taxonomic summary

Diagnosis: Musaev and Veisov (1963) described *Eimeria divichinica,* from *A. sylvaticus* in the USSR, to have smooth-walled oocysts 24 × 19 (16–32 × 10–26), without polar bodies, and usually with ellipsoid sporocysts 11 × 7 (7–13 × 5–11) or spheroid sporocysts ≅9 (8–11), but in either case without a Stieda body. Lewis and Ball (1983) described oocysts which they called *E. divichinica* from *A. sylvaticus* and *A. flavicollis* in the British Isles. These oocysts, with the same general shape of *E. divichinica,* were 25 × 18 (22–28 × 15–20) with 1–3 polar bodies and with ovoid sporocysts, 15 × 9 (13–15 × 8–10), that had definite Stieda bodies. We believe that Lewis and Ball (1983) relied too much on the similarity of shape of these 2 forms and not enough on more definitive characters such as size and the presence or absence of Stieda and polar bodies. *Eimeria inuyamensis* most closely resembles *Eimeria divichinica* sensu Lewis and Ball (1983) which we believe they should have, but did not, name as new. The only difference between their description and ours is that we saw a slightly sculptured outer oocyst wall whereas the oocysts they saw had smooth outer walls. The difference may be attributed to the age and storage medium (2% H_2SO_4) of our oocysts (see Discussion).

Type host: Apodemus sylvaticus Linnaeus, 1758 (from Lewis and Ball, 1983).

Type locality: See Lewis and Ball (1983).

Other hosts and localities: See Lewis and Ball (1983) and Table I.

Site of infection: Unknown. Oocysts recovered from feces.

Prevalence: Found in 4 of 21 (19%) infected *A. a. argenteus,* 2 of 36 (6%) infected *A. s. speciosus* and 1 of 1 *Apodemus speciosus tusimaensis.*

Etymology: The specific name is derived from the locality where the first infected host was collected in our study.

Eimeria uptoni Lewis and Ball, 1983

Description

Oocyst subspheroid, with wall <1; micropyle and oocyst residuum absent; polar body present; sporulated oocysts (n = 5) 13.9 × 11.4 (9–16 × 8–14) with L:W ratio 1.22 (1.11–1.31); sporocysts (n = 5) ovoid, 7.6 × 4.7 (5–10 × 3–6) with L:W ratio 1.61 (1.50–1.70); Stieda body present; substieda body absent; sporocyst residuum a compact mass. Oocysts were 354 days old when measured.

Taxonomic summary

Diagnosis: This species was described by Lewis and Ball (1983) and our observations agree with theirs.

Hosts and localities: See Table I.

Prevalence: Found in 4 of 21 (19%) infected *A. a. argenteus,* 2 of 7 (29%) infected *A. a. hokkaidi* and 11 of 36 (31%) infected *A. s. speciosus.*

Site of infection: Unknown. Oocysts recovered from feces.

DISCUSSION

Apodemus spp. are ubiquitous in Europe and Asia, occupying a similar niche there as *Peromyscus* spp. do in North America. The genetics

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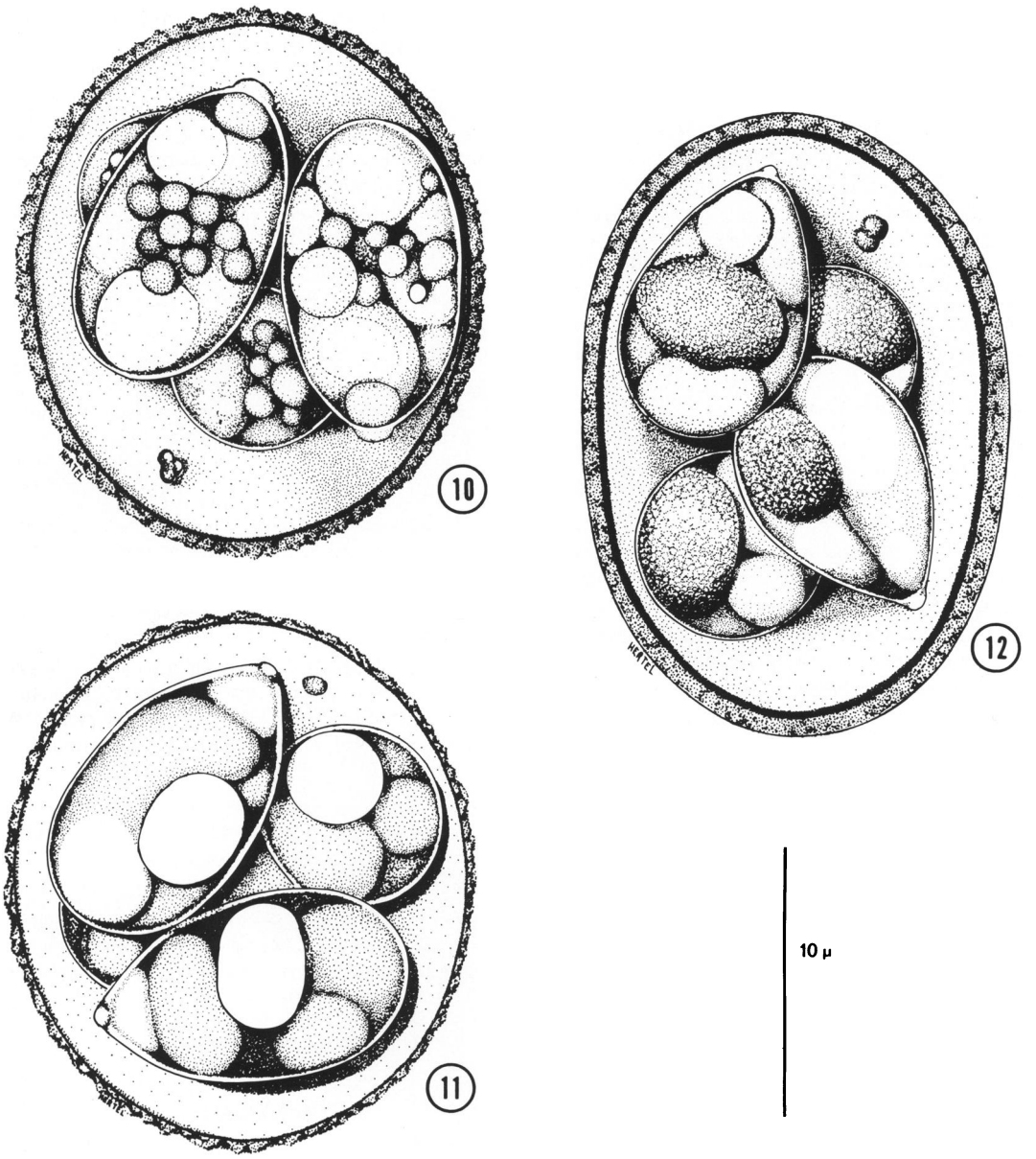
FIGURES 1–9. Photomicrographs of sporulated oocysts of coccidians recovered from the feces of *Apodemus* spp. ×1,800. **1-4.** *Eimeria montgomeryae* Lewis and Ball, 1983. **1, 2.** Note thick outer oocyst wall, refractile bodies of sporozoites (*) and shape and size of Stieda and substieda bodies. **3, 4.** Nomarski Interference Contrast (NIC) photomicrographs that show to better advantage the rough nature of outer oocyst wall and size relationship of Stieda to substieda body. **5-7.** *Eimeria argenteus* n. sp. **5, 6.** Note size relationship of Stieda to substieda body and homogeneous sporocyst residual body (*). **7.** NIC shot of mammillated surface of outer oocyst wall. **8, 9.** *Eimeria inuyamensis* n. sp. Note Stieda bodies (arrows), refractile body of sporozoite (*) and general oocyst shape.



FIGURES 10–12. Line drawings of sporulated oocysts of new and redescribed coccidian species recovered from the feces of *Apodemus* spp.; scale = 10 μm. **10.** *Eimeria montgomeryae.* **11.** *Eimeria argenteus.* **12.** *Eimeria inuyamensis.*

of *Apodemus* spp. has been well studied in Japan. Tsuchiya and Yosida (1971), Tsuchiya et al. (1973), and Tsuchiya (1974) found that the Japanese wood mouse, *A. speciosus,* has 2 chromosomal races which differ by a Robertsonian conversion. One race (2n = 46) is located in southern Japan, while the other race (2n = 48) is located in northern Japan. These 2 groups contact one another along a line extending from Toyama to Hamamatsu on the main island of Honshu in central Japan. Some mice in the areas of contact have been found which have a chromosome number of 2n = 47 (Tsuchiya and Yosida, 1971). The chromosomal races cannot be distinguished by their morphological characteristics, nor is there a discernible difference be-

tween them in several biochemical and immunological characters (Tsuchiya et al., 1973; Tsuchiya, 1974). In addition, 3 subspecies of *A. argenteus* occur sympatrically with the subspecies of *A. speciosus* throughout Japan.

All of the known coccidians from *Apodemus* spp. have been described from Europe and the USSR from *Apodemus agrarius, A. flavicollis,* and/or *A. sylvaticus.* The exact number of valid coccidians is debatable as Levine and Ivens (pers. comm.) list 26 species (eimerians, isosporans), while Lewis and Ball (1983) prefer to synonymize at least 7 of those names from *A. flavicollis* and *A. sylvaticus.* Regardless of the exact number of coccidians described from this host genus, Japanese wood mice had not been examined for Coccidia prior to our study. Thus, it seemed of interest to determine the extent to which variation, if any, might occur between coccidians of the 2 chromosomal races of *A. speciosus* and/or between *A. speciosus* and *A. argenteus* from Japan.

Although 4 different species of *Eimeria* were found infecting Japanese *Apodemus,* specificity for any race or species of host was conspicuously lacking. This is notable in that *A. speciosus* and *A. argenteus* do not appear to be particularly closely related. A recent electrophoretic study of these hosts conducted in our laboratory shows that they are highly divergent genically and their broad sympatric occurrence throughout Japan suggests that they have diverged sufficiently to avoid serious competition. Yet all 6 subspecies and both chromosomal races were found infected with from 1 to 4 species of *Eimeria* and appeared equally susceptible to each.

A variety of possible explanations for this observation exists. Our results are consistent with a growing body of evidence that *Eimeria* infecting most hosts may only be specific at higher taxonomic levels such as the genus and more generalized at the level of the host species/subspecies. From our studies on the genetics of the hosts we examine, we are beginning to see that coccidians which show the former pattern (i.e., specificity at the genus level) are found in hosts characterized by high levels of karyotypic variability and polymorphism. We are just beginning to look at hosts (e.g., moles) that are karyotypically conservative. It should prove informative if their coccidians are tied to the species level of the host. If so, these patterns may be indicative of a general underlying process that will certainly deserve further investigation.

We are beginning to see a consistent pattern emerge in the host–parasite relationship that exists among surface dwelling rodents and their eimerian and isosporan parasites. In previous studies on jumping mice (Duszynski et al., 1982), kangaroo rats (Stout and Duszynski, 1983), woodrats (Reduker and Duszynski, 1985), deermice (Reduker et al., 1985), and voles (Vance and Duszynski, 1985), a very high percentage of infected hosts in all surveys (100%, 85%, 90%, 92%, 91%, respectively) had only 1 coccidian species when examined (i.e., it appears that only 1 parasite will infect a given host at any one time). In this study, 56 of 70 (80%) infected *Apodemus* spp. harbored only 1 coccidian when examined. There appears to be some component of the host–parasite relationship that makes this 1 host–1 coccidian association so dominant in naturally occurring host communities, especially when many host species are known to serve as good hosts for 3, 4, or more coccidians. As we examine other host groups it will be of interest to learn if this pattern remains consistent. If it does, isolation of the causal mechanism could have significant implications for treatment of coccidiosis.

Finally, we must point out that all oocysts were collected and stored in 2% aqueous (v/v) H_2SO_4. This medium, unlike 2% aqueous (w/v) $K_2Cr_2O_7$, seems to be especially harsh on the integrity of oocyst structure and leads to lower sporulation rates than one would expect of this normally resistant coccidian structure.

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