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Original investigations

Life cycle and ultrastructure of *Adelina tenebrionis* (Sporozoea: Adeleidae) from *Heteronychus arator* (Coleoptera: Scarabaeidae)

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Abstract. The coccidian from the black beetle, *Heteronychus arator* (Fabricius), in New Zealand was identified as *Adelina tenebrionis* Sautet 1930. Its development occurs in the fat body of the host. Merogony produces bundles of 8–19 vermiform merozoites, which range in length from 12.0 to 24.1 μm. Spherical macrogametocytes and small, vermiform microgametocytes fuse to form a zygote. Sporogony produces an oocyst 29.2–45.0 μm in diameter, containing 3–13 sporocysts, 12.3–14.0 μm in diameter. The life cycle takes about 46 days in an alternative host, *Planotortrix excessana* (Lepidoptera: Tortricidae), at 22° C. Electron micrographs of merozoites and gametocytes are presented.

The black beetle, *Heteronychus arator* (Fabricius) (Coleoptera: Scarabaeidae: Dynastidae), is a sporadic but devastating pest of pastures in the northern regions of the North Island of New Zealand. A variety of pathogens have been found to infect black beetles in the field (Longworth and Archibald 1975). The most commonly found pathogen is a coccidian protozoan belonging to the genus *Adelina* (Archibald et al. 1975), which develops in the fat bodies of both adults and larvae. It causes a reduction in the levels of fat in the fat bodies of infected female beetles, which are less likely to survive until oviposition than healthy beetles (King and Mercer 1979). A practical method has been devised for transmitting this disease to healthy black beetles in the field as a means of pest control (King et al. 1985).

Recent laboratory tests of the biocontrol potential of an array of insect pathogens (Wigley et al. 1986) have shown that the black beetle *Adelina* will infect the greenheaded leaf roller, *Planotortrix excessana* (Lepidoptera: Tortricidae). The black beetle is exceedingly difficult to culture in the laboratory, so the discovery that a small, easily reared insect was a host provided an opportunity for time-course infection experiments. Thus, the life cycle and ultrastructure of this coccidian could be described, and its identification as *Adelina tenebrionis* Sautet 1930 made.

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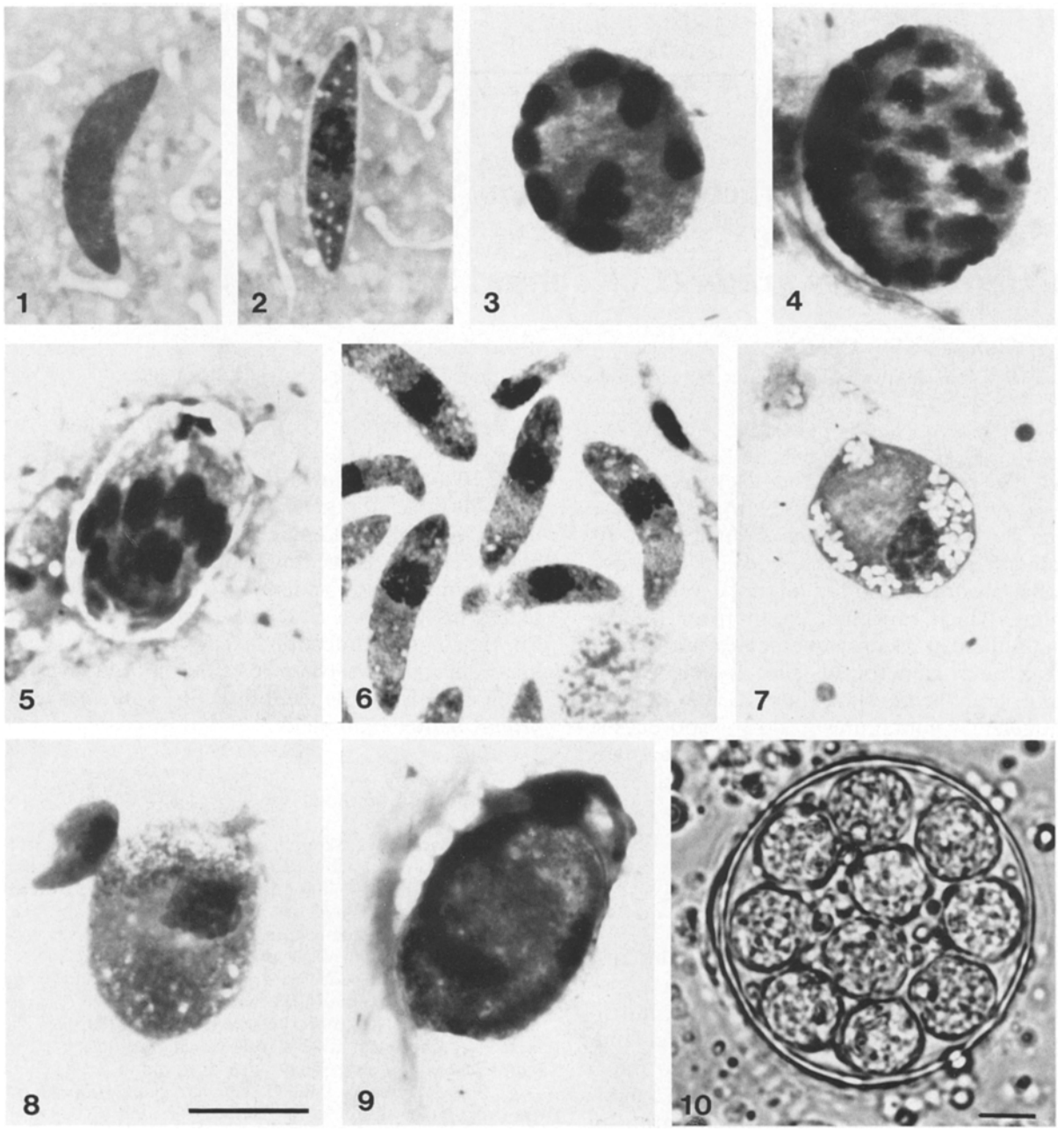
Materials and methods

Adelina oocysts black were obtained from an adult beetle collected in April, 1986, from a suburban garden in Auckland, New Zealand. The amount of inoculum was increased by feeding these oocysts to *Spodoptera litura* (tropical armyworm, Lepidoptera) larvae, which had previously proved to be susceptible. A suspension of oocysts and sporocysts from three infected *S. litura* larvae was added to an artificial diet (Malone and Wigley, in press) on which newly hatched, disease-free *P. excessana* (greenheaded leaf roller, Lepidoptera) larvae were fed. The larvae were incubated at 22° C. Individuals were examined by light microscopy 7 and 19 days after exposure to *Adelina*, and every 2 days thereafter until 37 days had elapsed. Specimens were then taken at about 10-day intervals until 67 days had passed and all the remaining larvae were dead and full of oocysts. Specimens were taken for electron microscopy at 32, 39, and 55 days, when they contained an abundance of different stages of *Adelina*.

For light microscopy, air-dried smears of gut and fat body were fixed in Carnoy's fixative and stained for 45 min in 10% (v/v) Giemsa stain (Gurrs R66 Improved) in 0.02 *M* phosphate buffer at pH 6.9. Measurements were made using a calibrated micrometer eyepiece. For electron microscopy, pieces of tissue were dissected into Karnovsky's fixative, left at room temperature for 10 min, then transferred to fresh fixative at 4° C for 1 h. Specimens were washed twice in 0.12 *M* sodium cacodylate buffer, pH 7.4, and then postfixed in 2.5% (w/v) OsO_4 in 0.1 *M* cacodylate buffer for 1 h at 4° C. After two washes in 0.1 *M* sodium acetate, specimens were passed through 50%, 70%, 90%, and 100% ethanol at room temperature and embedded in LR White medium-grade resin. Sections were poststained

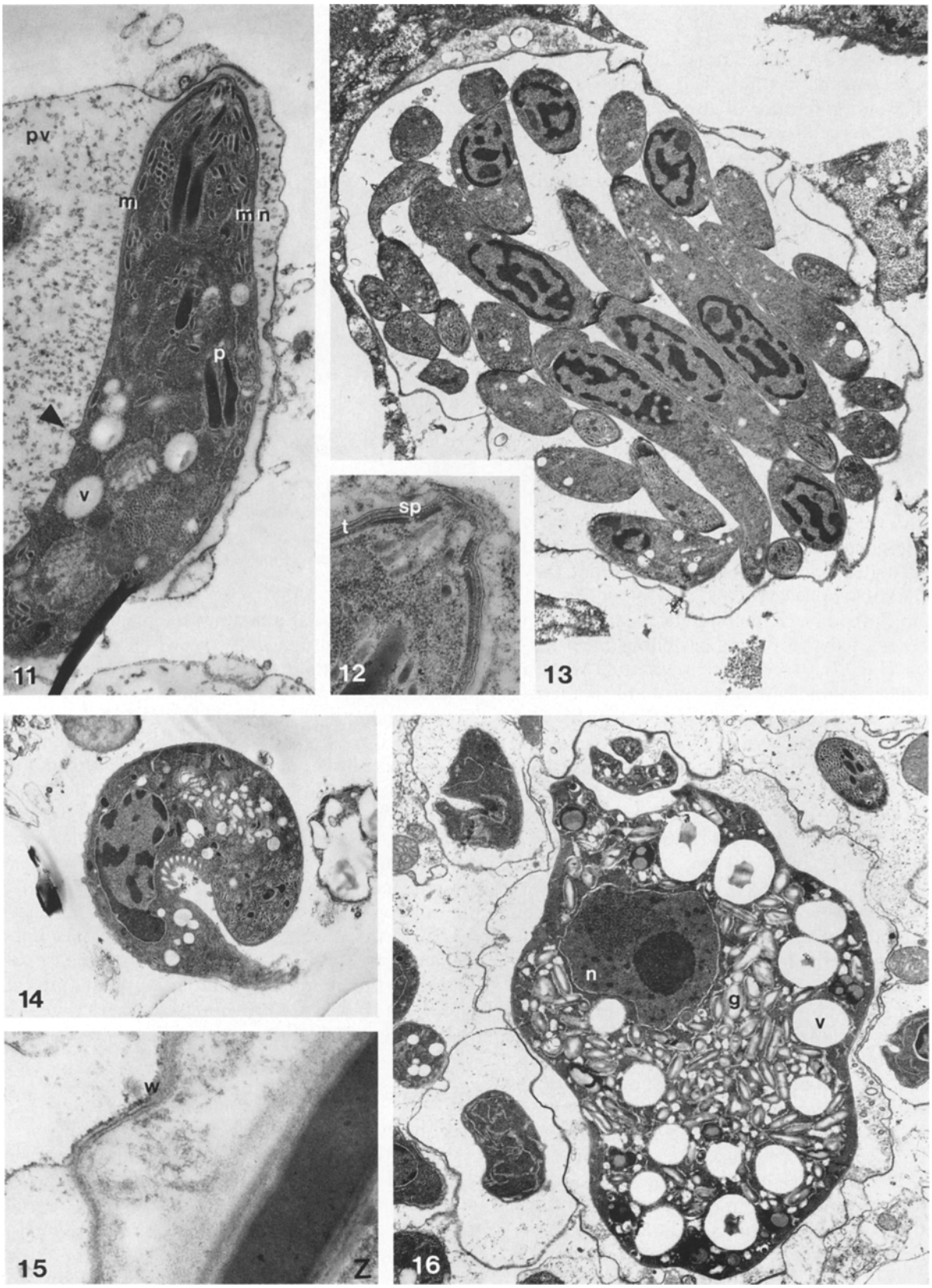


Figs. 1–10. Smears of black beetle *Adelina* (**Figs. 1–9**, Giemsa-stained; **Fig. 10**, fresh). Bars = 10 μm. **Fig. 1.** Sporozoite. **Fig. 2.** Vacuolated sporozoite. **Fig. 3.** Meront with nine nuclei. **Fig. 4.** Multinucleate meront. **Fig. 5.** Bundle of elongate merozoites. **Fig. 6.** Merozoites of various shapes and sizes. **Fig. 7.** Early macrogametocyte with vacuolated cytoplasm. **Fig. 8.** Macrogametocyte with attached microgametocyte. **Fig. 9.** Fused macrogamete and microgamete. **Fig. 10.** Mature oocyst containing sporocysts

in a saturated solution of uranyl acetate in 70% ethanol followed by Venable's lead citrate and viewed at an accelerating voltage of 60 kV.

Results

Broadly vermiform sporozoites (Fig. 1) appeared 7 days after dosing in smears of gut and fat body tissue. Those in the gut stained faintly pink with Giemsa's stain, whereas those in the fat body stained violet-blue and had deep magenta nuclei. Some sporozoites may have had vacuolated cytoplasm (Fig. 2). Sporozoites like those found in the fat body at 7 days were common in all subsequent smears. Spherical meronts with between 4 and 26 nuclei (Figs. 3 and 4) occurred 19 days after dosing in smears of the fat body. Some specimens also contained spherical bundles of elongate, vermi-



Figs. 11–16. Ultrathin sections of black beetle *Adelina*. **Fig. 11.** Motile vermiform stage inside a parasitophorous vacuole (*pv*), showing rhoptries (*p*), micronemes (*mn*), mitochondria (*m*), vacuoles (*v*), a portion of a crystalline-like body (adjacent to the vacuole), and ridges (*arrowhead*) in the outer membrane. ×11661. **Fig. 12.** Apical complex of a motile stage, showing spiral elements of the conoid (*sp*) and layer of microtubules (*t*) just beneath the double outer membrane. ×25000. **Fig. 13.** Bundle of merozoites. ×4615. **Fig. 14.** Folded motile stage, probably a microgametocyte, with concave surface thrown into a series of ridges. ×5829. **Fig. 15.** Detail of three-layered, parasitophorous vacuole wall (*w*) enclosing a zygote with a thick secretion covering its outer membrane (*z*). ×25455. **Fig. 16.** Macrogamete with nucleus (*n*) and prominent nucleolus, polysaccharide granules (*g*), and vacuoles (*v*). ×4322

form merozoites (Fig. 5). These merozoites were generally arranged so that their nuclei line up around the circumference of the sphere and their tapered ends merged together at the poles. In about one out of ten cases, the nuclei were arranged in two rows. The number of merozoites per bundle ranged from 8 to 19 ($n=20$).

By the 23rd day, some of these bundles had broken apart so that long, thin, free merozoites, each with a single, centrally located nucleus, occurred in smears of the fat body. Variable, more or less vermiform stages were apparent at this time (Fig. 6). The length of these stages ranged from 12.04 to 24.12 μm, with a mean of 18.25 ± 0.68 μm ($P<0.05$) ($n=75$, Giemsa-stained). Some were quite broad, almost ovoid in some instances, with finely vacuolated cytoplasm that sometimes had a granular appearance. These were probably developing macrogametocytes. Other stages were much smaller and very elongate and may have been early microgametocytes. These small stages often had their ends coiled back on themselves so that they appeared almost spherical.

By the 26th day, large, spherical stages with highly vacuolated cytoplasm, probably macrogametocytes (Fig. 7), were quite common, with a much smaller, uninucleate stage, probably a microgametocyte, occasionally attached (Fig. 8). By the 27th day, some fused macro- and microgametes were present (Fig. 9). All the above-mentioned stages were abundant in subsequent smears. The first ripe oocysts (Fig. 10) appeared 46 days after dosing. In fresh preparations, oocyst diameters ranged from 29.16 to 45.00 μm, with a mean of 37.31 ± 1.73 μm ($P<0.05$) ($n=25$), and sporocyst diameters ranged from 12.33 to 14.04 μm, with a mean of 12.60 ± 0.35 μm ($P<0.05$) ($n=25$). All larvae were dead by the 67th day.

Ultrathin sections reveal a three-layered membrane forming a parasitophorous vacuole around all stages of the life cycle (Fig. 15). The broad, vermiform stages, which are either sporozoites or merozoites, contain organelles typical of the motile stages of coccidia (Fig. 11). The apical complex consists of a conoid with a canopy layer, five or six spirally coiled elements, and a polar ring (Fig. 12). The outer membrane of the motile stage is lined with a rough, inner membranous complex, which covers a layer of microtubules. Electron-dense rhoptries extend posteriorly from the apical complex (Fig. 11). Micronemes are abundant (Fig. 11), even in merozoites which have not yet separated (Fig. 13). Mitochondria are fairly electron-dense with tubular cristae (Fig. 11). Electron-lucent vacuoles occur in all stages. Micropores

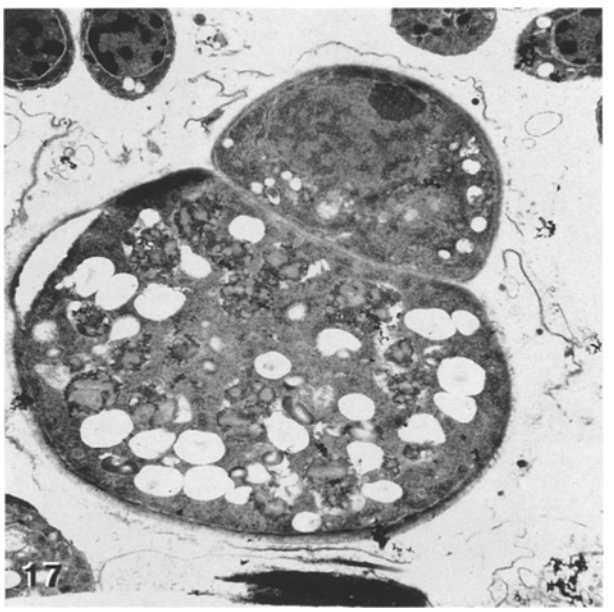


Fig. 17. Ultrathin section of black beetle *Adelina*, showing fused microgamete and macrogamete. × 4912

were not observed. Sometimes the membrane complex is thrown into small ridges (Fig. 11), presumably when the zoite flexes; these are particularly pronounced in some very curved stages, which may be microgametes (Fig. 14). The nuclei of the motile stages occupy almost the full width of the parasite and contain large, irregular patches of chromatin (Figs. 13 and 14). The mature macrogamete (Fig. 16) has a nucleus with a well-defined nucleolus and cytoplasm containing many polysaccharide granules and large vacuoles. Mitochondria are also present, mostly at the periphery of the cell. Once the microgamete and macrogamete have fused (Fig. 17), the outer wall of the parasite becomes thickened by the addition of an electron-dense secretion to the membrane complex surrounding the cytoplasm (Fig. 15). Later life-cycle stages were not observed by electron microscopy.

Discussion

The observations that both the oocysts and sporocysts of this pathogen are spherical, with firm walls, and that each sporocyst contains two sporozoites confirm Archibald's assertion (1976) that this protozoan is an *Adelina* species (Adeleidae).

Light microscopy

The vermiform, motile stages of black beetle *Adelina*, which occasionally have vacuolated cytoplasm, are typical of *Adelina* species, e.g., *A. zonula*

(see Moroff 1907), *A. cryptocerci* (see Yarwood 1937), and *A. sericesthis* (see Weiser and Beard 1959). Roughly spherical plasmodia containing more than ten nuclei are found not only in black beetle *Adelina*, but also in *A. mesnili* (see Pérez 1899) and *A. zonula* (see Moroff 1907). Bundles of elongate merozoites occur in all *Adelina* species. The morphology of the gametocytes, sporocysts, and oocysts of the black beetle *Adelina* is also similar to that of other *Adelina* species.

However, there are small differences in the morphology of life-cycle stages, detectable by light microscopy, that distinguish the black beetle *Adelina* from all but one other *Adelina* species.

Ultrastructure

Electron microscopic studies have been done on only two other *Adelina* species, *A. tribolii* (see Zizka 1969, 1985) and *A. dimidiata* (see Tuzet 1970); thus ultrastructural diagnostic features of species belonging to the genus *Adelina* are not yet clear. Ultrastructural details of the black beetle *Adelina* were generally similar to those of both *A. tribolii* and *A. dimidiata*. The large, paranuclear, crystalline bodies recorded for *A. tribolii* sporozoites (Zizka 1985) were not seen in vermiform stages of the black beetle pathogen, except perhaps in one section (Fig. 11), hence, most of the stages in our material were probably merozoites. Mitochondria, rhoptries, micronemes, and apical complexes of these merozoites were similar to those described for sporozoites of *A. tribolii* (see Zizka 1985) and merozoites of *A. dimidiata* (see Tuzet 1970). As with black beetle *Adelina*, macrogametes of *A. tribolii* have occurred in periparasitic vacuoles bounded by a three-layered wall (Zizka 1969), although such a vacuole was not detected for *A. dimidiata* macrogametes (Tuzet 1970). Internal vacuoles, polysaccharide granules, mitochondria containing short tubules, and a nucleus with a well-defined nucleolus were features of macrogametes common to all three isolates. The complex ridges shown in Fig. 14 have not been recorded for the other *Adelina* species, but similar structures have been noted in *Klossia helicina* (Adeleidae) microgametes (Schulte 1971).

Despite the ultrastructural similarities, the black beetle *Adelina* is not identical to either of the above *Adelina* species. *A. dimidiata* has been found only in a chilopod and is therefore unlikely to be conspecific, and light microscopic studies (Bhatia 1937) show a finger-like process on the macrogametocyte of *A. tribolii*. Such a process was not seen in smears of black beetle *Adelina*.

Identity of Black Beetle Adelina

Since the black beetle *Adelina* infects two lepidopteran species in the laboratory, strict host specificity may not be an attribute of this genus. However, it seems reasonable to assume that a group of related species would infect only insects.

Descriptions of insect-infecting *Adelina* species (Table 1) are all based on light microscopic observations. No preserved material, which would have allowed morphological comparisons to be made, could be located.

Apart from a few early stages, which were probably invading sporozoites, development of the black beetle *Adelina* takes place mostly in the fat body, and possibly in the hemolymph. This feature distinguishes it from *A. simplex* and *A. tipulae*, which develop primarily in the gut (Schneider 1875), and from *A. cryptocerci*, which invades many tissues, including the nerve cord (Yarwood 1937).

Of the remaining *Adelina* species, the black beetle *Adelina* is most similar to *A. tenebrionis*. In *A. tenebrionis*, vermiform merozoites (12–20 μm long) occur in bundles of 8–10. The cycle of merogony may be repeated, or meronts may develop into either microgametocytes or macrogametocytes. Microgametocytes free in the cytoplasm are not illustrated, but microgametes are roughly spherical when attached to the much larger, spherical macrogametes. Oocysts are 20–35 μm in diameter and the 2–12 sporocysts contained therein measure 10–12 μm in diameter (Sautet 1930). In the black beetle-infecting species, merogony produces 8–19 merozoites (12.0–24.1 μm long) bundled together. Oocysts measure 29.2–45.0 μm (present study) or 20.7–46.4 μm (Archibald 1976) and contain 3–13 sporocysts (Archibald 1976), each measuring 12.3–14.0 μm (present study) or 11–13.4 μm (Archibald 1976).

A number of morphological features distinguish the black beetle *Adelina* from the other insect-infecting species. Unlike the black beetle *Adelina*, *A. riouxi* has an ovoid oocyst with a distinct lid that breaks off (Rioux et al. 1972), and *A. collembolae* always has 24 sporocysts in each oocyst (Purrini 1984).

The black beetle *Adelina* has between 3 and 13 sporocysts per oocyst (Archibald 1976), whereas *A. akidium* has between 12 and 20 (Léger 1900) and *A. transita* has between 6 and 20 (Léger 1904). Conversely, *A. mesnili* (see Pérez 1899), *A. zonula* (see Moroff 1907), and *A. sericesthis* (see Weiser and Beard 1959) never have more than eight sporocysts per oocyst.

Table 1. *Adelina* species that infect insects

Species Authority	Tissues infected	Merozoite length	No. merozoites per bundle	Oocyst diameter	No. sporocysts per oocyst	Sporocyst diameter
A. akidium Léger 1900	fat	–	–	30–40 μm	12–20	10 μm
A. collembolae Purrini 1984	fat	–	–	40 μm	24	7.5–8 μm
A. cryptocerci Yarwood 1937	various	11–13 μm	8–40	24–51 μm	5–21	10–12 μm
A. melolonthae Tuzet et al. 1965	fat	15–20 μm 16 μm	20–25 10	30–35 μm	6–14	11 μm
A. mesnili (Pérez 1899)	fat	15 μm	20–30	–	6–8	15 μm
A. riouxi Levine 1977	–	–	–	30–40 μm	8–18	7–10 μm
A. sericesthis Weiser & Beard 1959	fat	16–18 μm 7–8 μm	30–60 8–32	–	4–8	–
A. simplex Schneider 1875	gut	–	–	25–40 μm	8–16	–
A. tenebrionis Sautet 1930	fat	12–20 μm	8–10	20–35 μm	2–12	10–12 μm
A. tipulae Schneider 1875	gut	–	–	35–40 μm	4–10	–
A. transita Léger 1904	various	15 μm	16	30–40 μm	6–20	10–11 μm
A. zonula (Moroff 1907)	fat	15–20 μm	–	–	8	–

Additionally, *A. mesnili* and *A. sericesthis* form bundles of 20–30 and 30–60 merozoites, respectively, in contrast to the bundles of 8–19 produced by the black beetle *Adelina*. Two types of merogony occur in *A. sericesthis*, the second of which produces ovoid merozoites only 7–8 μm long, whereas the merozoites of the black beetle *Adelina* were all fusiform or vermiform. *Adelina melolonthae* also has more than one cycle of merogony (Tuzet et al. 1965), resulting in two different size classes of vermiform merozoites, some 15–20 μm long, in bundles of 20, 25, or more, and some 10 μm long, in bundles of 16. It is not clear from our study whether or not there is only a single merogony in the black beetle pathogen: merozoites range in length from 12.0 to 24.1 μm but do not fall into any obvious morphological categories that might identify them as products of a particular merogony. Since the black beetle pathogen takes 6–7 weeks to complete its life cycle at 22° C in *P. excessana*, and given the large number of merozoites that are found from the 28th day onward, it seems likely that more than one cycle of merogony occurs, but without any clear differences among the merozoites produced.

We conclude that the coccidian from the black beetle, *Heteronychus arator*, is *Adelina tenebrionis* Sautet 1930 for the following reasons: the host species are insects; the fat body is the main site of parasite development; only one type of merogony occurs, producing bundles of 8–19 vermiform merozoites, 12.0–24.1 μm long; the merozoites give rise to microgametocytes and macrogametocytes; the microgamete is small and spherical at first but becomes lenticular when it attaches to the large, spherical macrogamete; the oocyst is roughly spherical, 20.7–46.4 μm in diameter; the oocyst contains 3–13 sporocysts; and the sporocysts are spherical, 11.0–14.0 μm in diameter.

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