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REVISION OF MICROSPORIDA (PROTOZOA) CLOSE TO THELOHANIA WITH
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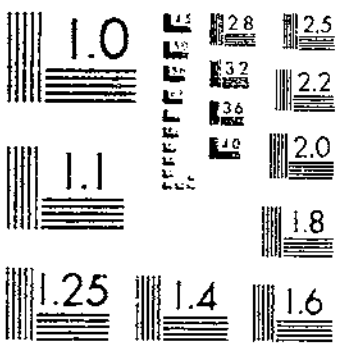

MICROCOPY RESOLUTION TEST CHART
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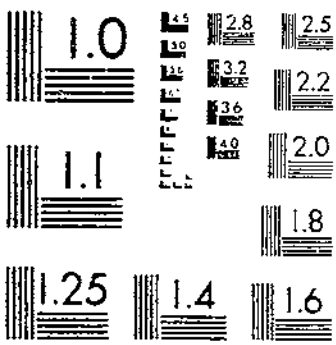

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Revision of Microsporida (Protozoa) Close to Thelohania, With Descriptions of One New Family, Eight New Genera, and Thirteen New Species

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CONTENTS

Revision of Microsporida (Protozoa) Close to *Thelohania*, With Descriptions of One New Family, Eight New Genera, and Thirteen New Species

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ABSTRACT

Thelohaniidae fam. n. is erected to include *Agmasoma* gen. n., *Amblyospora* gen. n., *Chapmanium* gen. n., *Cryptosporina* gen. n., *Hyalinocysta* gen. n., *Inodosporus* Overstreet and Weidner, *Parathelohania* Codreanu, *Pegmatheca* gen. n., *Pilosporella* gen. n., *Systenostrema* gen. n., and *Thelohania* Henneguy. Species previously placed in the genus *Thelohania* are discussed and 13 new species (*Amblyospora amphipodae*, *A. callosa*, *A. keenani*, *A. khalulini*, *A. mojungensis*, *Chapmanium cirritus*, *Cryptosporina brachyfila*, *Hyalinocysta chapmani*, *Parathelohania chagrasensis*, *Pegmatheca simulii*, *Pilosporella chapmani*, *P. fishi*, and *Systenostrema tabani*) are described. The new classification is based on morphological characteristics seen in preparations made for the light microscope and the scanning and transmission electron microscopes. Additional taxonomic criteria are based on host-parasite relationships. Synonymies are given for previously described species, and a diagnostic key is provided for the separation of genera.

INTRODUCTION

Henneguy in Henneguy and Thélohan (1892b) established the genus *Thelohania* for three new species (*T. contejeani*, *T. giardi*, and *T. octospora*) on the basis of their having eight spores within an envelope. Thelohan (1892) placed this genus in a new family, Glugeidae (Glugeidées), along with its type genus, *Glugea* Thélohan (1892), both of which he believed to have spores enclosed by an envelope.

Gurley (1893) added *T. macrocystis* to the genus *Thelohania*. He also believed the envelope (pansporoblastic membrane) and the number of spores contained by it to be important since he created another new genus, *Pleistophora*, for a species, *P. typicalis*, having a variable number of spores, but more than eight, developing within a membrane. He placed both genera in the family Glugeidae. Labbé (1899) placed these two genera in the family Nosematidae after suppressing Glugeidae by making *Glugea* a synonym of *Nosema* Nägeli (1857).

Stempell (1909) did not consider the presence or absence of a pansporoblastic membrane important as a family character. Rather, he used the form and development of vegetative stages to determine family taxa, thereby creating the family Pleistrophoridae for the genus *Pleistophora*, retaining the family Nosematidae, which included *Thelohania*, and restoring the family Glugeidae for *Glugea*.

Leger and Hesse (1922) also ignored the pansporoblastic membrane enclosing the spores of *Pleistophora* and *Thelohania* as well as the vegetative development described by Stempell when they proposed a completely new classification based solely on spore shape. They were apparently so impressed with the differences in the shapes of the spores of the new species of *Cocconema* and *Mrazekia* that they placed them in the families Cocconemidae and Mrazekidae, having spores spherical and rod shaped, respectively. Also, they placed *Glugea*, *Nosema*, *Pleistophora*, and apparently *Thelohania* in the family Glugeidae, representing species with pyriform spores. We now know that a classification based on spore shape alone, excluding important criteria such as sporogonic stages and pansporoblastic membranes, cannot diagnostically accommodate the tremendous variety of species known today.

Kudo (1924c) used a combination of the classification systems of Stempell and of Léger and Hesse as he also did not consider the pansporoblastic membrane to be an important taxonomic character. This is seen in the manner in which he handled the taxonomic placement of a microsporidium described from the mosquito *Culex pipiens pipiens* Linnaeus. Kudo (1920) first named the microsporidium *Thelohania magna*, believing it had only octonucleate sporonts giving rise to eight spores not enclosed in a pansporoblastic membrane. Later (1924c and 1925b), he transferred this species to the genus *Stempellia* after studying additional infected mosquitoes having sporonts with two, four, six, and eight nuclei giving rise to two, four, six, and eight spores. Hazard and Fukuda (1974) confirmed through electron microscopy that *S. magna* has no pansporoblastic membrane surrounding the spores. In his monograph on the microsporidia Kudo (1924c) retained many species in the genus

Thelohania having sporonts forming eight spores not enclosed within a pansporoblastic membrane. Weiser (1961) used essentially the same taxonomic criteria for *Thelohania*.

Tuzet et al. (1971) reestablished the importance of the pansporoblastic membrane by using it as the diagnostic character for a new family, Polysporidae, for genera having two or more spores enclosed within a pansporoblastic membrane. The family is not valid, however, because they did not derive its name from one of the genera included in the family. Our studies of the ultrastructure of species formerly placed in the genus *Thelohania* reveal that some of them represent several new genera and consequently a new family. The criteria used to delineate our new genera and the new family are partly those used by Stempell (nature of vegetative stages), Léger and Hesse (spore shape), and Henneguy, Gurley, and Tuzet et al. (the pansporoblastic membrane). However, we also used new information from our electron microscopy studies concerning the ultrastructure of the sporonts, pansporoblasts, and spores. Thirteen new species are also described, and a key for the separation of genera is presented.

METHODS

Light Microscopy

Giemsa-stained smears were prepared by smearing infected tissue on a microscope slide, air drying, fixing in 95% methanol for 4 minutes, staining with a 1:9 mixture of Giemsa and pH 7.41, buffered, distilled water for 10 minutes, and rinsing with tapwater. Heidenhain's-hematoxylin-stained wet smears were prepared by smearing infected tissue on a glass coverslip and immersing before drying in aqueous Bouin's fixative for 6 hours, washing in 70% ethanol overnight, mordanting in iron alum for 4 hours, staining with Heidenhain's hematoxylin overnight, destaining with iron alum to desired intensity, and mounting on a microscope slide. To determine infected host tissues, some specimens were fixed in Carnoy's fluid, embedded in paraffin, sectioned, stained with Heidenhain's hematoxylin, counterstained with Eosin Y, and covered with a glass coverslip sealed with Histoclad. Fresh and fixed spores were measured with an A.E.I. Cook image-splitting micrometer at a magnification of 1,000 ×.

Scanning Electron Microscopy

Infected larvae were either smeared in distilled water on glass-covered metal specimen stubs or smeared directly on the stub and fixed in the vapors of osmium tetroxide. The stubs were placed in a

vacuum evaporator, and the spores coated with 200 to 300 Å of gold at a vacuum of 2×10^{-5} torr. In some species, spore suspensions were made from infected larvae. These spores were fixed in 4% glutaraldehyde or 1% osmium tetroxide or both in 0.1 *M* cacodylate buffer, dehydrated in ethanol, and held in absolute ethanol overnight. Spores were dispersed on glass-covered metal specimen stubs and coated with gold as above.

Transmission Electron Microscopy

Small pieces (1 mm^3) of infected specimens were fixed in 4% glutaraldehyde in 0.1 *M* phosphate or cacodylate buffer for either 4 hours at room temperature or overnight at 8° C, then postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in either Epon (Luft 1961) or Epon-Araldite (Mollenhauer 1964). Sections of 600 to 900 Å were stained with saturated aqueous uranyl acetate, followed by lead citrate (Venable and Coggeshall 1965). Photomicrographs were taken at an accelerated voltage of either 50 or 75 kV.

THELOHANIIDAE fam. n.

The family represents species having sporonts that, after three sporogonic divisions, give rise to eight uninucleate microspores (octospores) enclosed by a pansporoblastic membrane. Occasionally, a few sporonts of some species fail to complete the normal number of sporogonic divisions, producing macrospores (one, two, three, or four in number) also enclosed by a pansporoblastic membrane and often mixed with normal microspores. Microspores and macrospores have identical ultrastructures, differing only in size. The pansporoblastic membrane surrounding the spores may be persistent or subpersistent, depending on species.

At the time of nuclear division, the cytoplasm of the sporont divides by endogenous budding and constricts around the daughter nuclei, giving the sporont a lobed appearance. The dividing sporonts secrete metabolic substances (extranuclear granules of Kudo, 1924a) which are deposited in the space between the lobed sporont and the pansporoblastic membrane. These metabolic substances can take the form of small granules or larger crystalliform particles. During sporulation these particles are noticeably reduced in size and number and in a few species disappear. At sporulation microtubules are formed (in some species) inside the pansporoblast surrounding the sporoblasts. The microtubules are produced from proteinaceous excesses forming the outer layer (exospore of Vávra, 1966) of the spore wall and persist inside the pansporoblast until the pansporoblastic membrane ruptures.

Some species, in addition to producing octospores, have another kind of sporogony, producing a variable number (6–40) of spores that are binucleate and not enclosed by a pansporoblastic membrane (free spores). This sporogonic sequence usually develops in the adult female host. Sporulation usually occurs in the oenocytes, reducing the fecundity and longevity of the female host, but not causing sudden death. All of the sporonts, however, do not sporulate. Instead, they invade the ovaries, where they divide and produce binucleate stages that enter the ovarioles and later cause patent infections in the larval progeny.

Octospores usually produce gross symptomology (white discoloration of muscle, hepatopancreas, or fat body) and mortality in young female or male hosts or both (depending on the microsporidian species). However, free spores associated with the transovarial sequence in adult female hosts do not produce gross symptomology.

The species of this family are common parasites of a variety of aquatic and semiaquatic invertebrate animals. The type genus is *Thelohania* Henneguy.

KEY TO THE GENERA OF THELOHANIIDAE

1. Polar filament abruptly constricts near the middle to form a much narrower distal portion 2
1′. Polar filament of nearly uniform diameter from base to distal end or only gradually narrowing to the distal end 7
2. Octospores (fresh or preserved) oval or oblong 3
2′. Octospores (fresh or preserved) pyriform 4
3. Octospores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin have a constricted posterior end ..... *Parathelohania* Codreanu, 1966 (p. 56)
3′. Octospores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin have one or both ends truncate; the posterior end is often somewhat invaginated ..... *Amblyospora* gen. n. (p. 9)
4. Pansporoblasts fusiform; the pansporoblastic membrane persistent, retaining the spores ..*Chapmanium* gen. n. (p. 45)
4′. Pansporoblasts oval; the pansporoblastic membrane subpersistent, breaking and freeing spores readily 5
5. Pansporoblastic membrane easily observed in Bouin's-fixed and Heidenhain's-hematoxylin-stained smears; octospores broadly pyriform ..... *Hyalinocysta* gen. n. (p. 52)
5′. Pansporoblastic membrane extremely difficult to observe in Bouin's-fixed and Heidenhain's-hematoxylin-stained smears 6

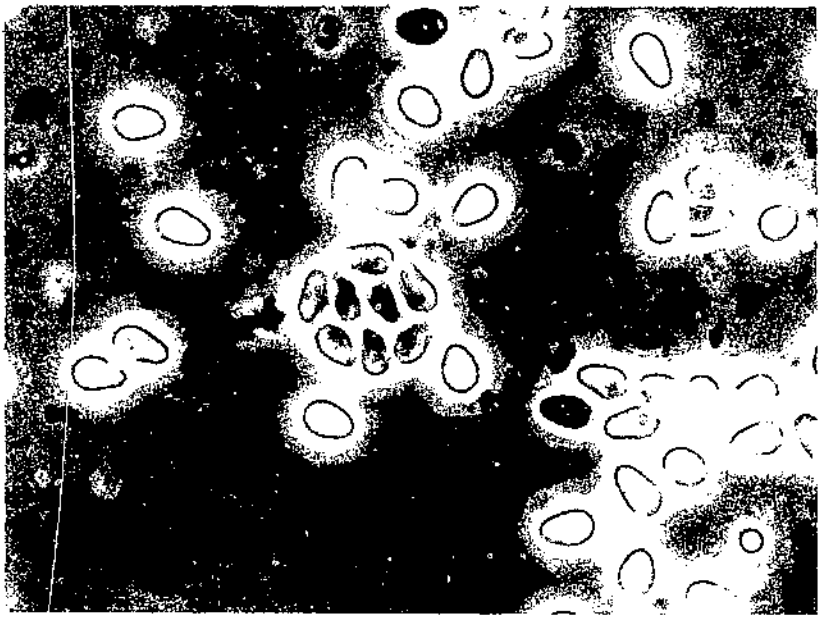
6. Cytoplasm of sporonts not dividing until last nuclear division; parasites of decapod crustaceans ... *Agmasoma* gen. n. (p. 6)
6'. Cytoplasm of sporonts partially dividing by endogenous budding with each nuclear division; parasites of insects ..... *Systenostrema* gen. n. (p. 87)
7. Octospores subspherical or spherical *Pilosporella* gen. n. (p. 82)
7'. Octospores oval or pyriform 8
8. Fresh (nonpreserved) pansporoblasts filled with dense, amber-colored crystalliform particles, obscuring the spores; only one species known, this from an aquatic mite ..... *Cryptosporina* gen. n. (p. 49)
8'. Fresh pansporoblasts not filled with dense crystalliform particles; octospores readily visible in pansporoblasts 9
9. Octospores with four or five acicular appendages *Inodosporus* Overstreet and Weidner, 1974 (p. 55)
9'. Octospores without acicular appendages 10
10. Octospores oval, with both ends equally rounded; parasites of blackfly larvae ..... *Pegmatheca* gen. n. (p. 79)
10'. Octospores oval or pyriform, with the posterior end more broadly oval than the anterior end; parasites of decapod crustaceans ..... *Thelohania* Henneguy, 1892 (p. 90)

AGMASOMA gen. n.

Only one developmental sequence is known, in which sporonts form octospores.

The pansporoblasts are subspherical or oval and have a fragile pansporoblastic membrane that soon ruptures when dissected from host tissues, releasing small oval to pyriform spores (fig. 1). Unlike the other genera of Thelohaniidae, cytoplasmic division in the sporonts is delayed, beginning only after the nuclei have made three divisions forming octonucleate plasmodia. The ultrastructure of the octospores differs from that of the species of *Thelohania* in decapod crustaceans by having a polar filament with a broad basal portion abruptly constricting to form a longer and much narrower distal end and by not having a prominent lamellate polaroplast (fig. 3). The spore wall is smooth and without surface structure. Living octospores are without a mucous envelope.

Agmasoma should perhaps be placed in a new family since it appears that the sporonts divide by a process much different from that in other genera (i.e., not by cytosplasmic budding, fig. 2). Also, these microsporidia do not secrete metabolic substances that form granules or crystalliform particles as do the other members of the family. We do not erect another family here, however, because our



PX 4450

FIG. 8F 1.—Fresh octospores of *Agmasoma penaei* (Sprague). × 3,000.

PX 1151

FIG. 8F 2.—Sporulating sporont in *A. penaei* pansporoblast. × 22,500. PM, pansporoblastic membrane; SW, spore wall.

information came from electron photomicrographs of tissues taken from a shrimp packed in ice for many days, and this treatment may have produced abnormalities in the sporonts. We hope to make a sounder case for including *Agmasoma* in Thelohaniidae after study of material from infected live shrimp. We do not hesitate to establish a new genus, however, since the polar filament of the spores is structurally very different from that in spores of other species in decapod crustaceans. We name the genus *Agmasoma*, meaning "fragmenting body," referring to the type of cytoplasmic division at the time of sporulation. The only known species of this genus is the type, *A. penaei* (Sprague).

Agmasoma penaei (Sprague, 1950) comb. n.

Thelohania penaei Sprague, 1950a, Occas. Pap. Mar. Lab. La. State Univ. 5: 4; Sprague, 1965, J. Protozool. 12: 66; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sprague and Couch, 1971, J. Protozool. 18: 530.

Spore size.—Living octospores measure 4.0×2.2 μm [6.0–7.0 ×3.5–4.2 μm, macrospores] (Sprague 1950a); 3.82–5.25×2.33–3.34 μm (authors).

Host.—*Penaeus setiferus* (Linnaeus), a shrimp, collected in Florida and Louisiana, U.S.A.

Infection site.—Reproductive organs.

Living and preserved octospores are pyriform and have a small visible vacuole in the broader posterior end (fig. 1), the vacuole appearing larger and being more prominent in Heidenhain's-hematoxylin-stained smears. The living spore has no mucous envelope.

The cytoplasm of the sporonts does not divide by budding, but instead, by a delayed aggregation of protoplasm after three nuclear divisions to form eight spores (fig. 2). During cytoplasmic division no metabolic products (granules or crystalliform particles) are deposited inside the pansporoblastic membrane, and during sporulation no microtubules are formed, leaving the pansporoblasts void of these materials.

The octospores (fig. 3) have a thin exospore, an abruptly constricting polar filament, and an indistinct polaroplast. The large basal portion of the polar filament is unusually short, forming only 2½ coils inside the spore, whereas the longer and narrower distal portion forms 6 coils.

We know nothing of the host-parasite relationship or whether or not this microsporidium causes death of its hosts. We assume that fecundity is reduced in female hosts since their reproductive glands are heavily infected. This microsporidium is not only unusual be-

cause of the method in which its sporonts divide, but also because sporonts develop in the reproductive organs (a tissue not supporting sporonts producing octospores in other species of Thelohaniidae).

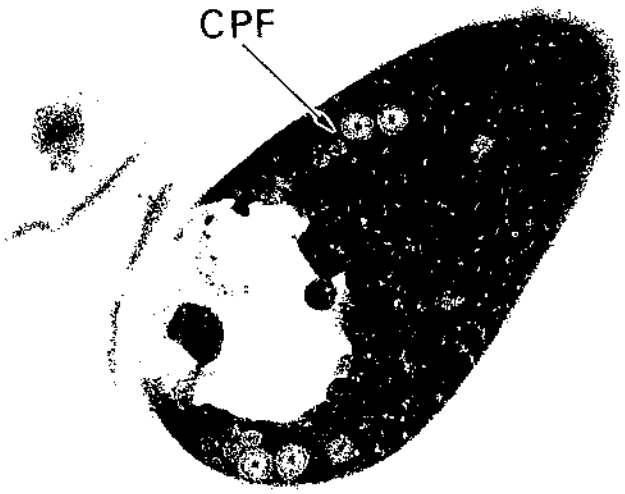


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FIGURE 3.—Ultrastructure of *A. penaei* octospore. × 30,750. CPF, constricted polar filament.

AMBLYOSPORA gen. n.

Most, if not all, species of this genus are dimorphic and have two developmental sequences in their hosts: one only in immature males or in both immature males and females, producing octospores enclosed by a pansporoblastic membrane; and another in adult females, producing a variable number of free spores.

The pansporoblasts are oval. Living octospores are oval, having broadly rounded posterior ends and somewhat narrow anterior ends (fig. 31). Preserved with aqueous Bouin's fixative and stained with Heidenhain's hematoxylin, the octospores are truncate on one or both ends, depending on the species (figs. 9, 12, and 33). The pansporoblastic membrane remains intact for a short time when removed from the body of the host. The sporonts producing octospores secrete many dense granules (metabolic products) of various size, depending on the species, that are retained by the pansporoblastic membrane. These granules are readily discernible in transmission electron photomicrographs (figs. 14, 22, 28, and 36) and are

also prominent, in some species, in both Giemsa-stained and Heidenhain's-hematoxylin-stained smears. The chromosomes in the dividing sporonts of this genus are also readily stained with Heidenhain's hematoxylin in wet fixed smears, and the ease with which they can be observed is characteristic of all *Amblyospora* species (fig. 34). The octospores have a thick exospore, a long polar filament consisting of a broad basal portion abruptly constricting near the middle to a narrow distal portion, and a prominent lamellated polaroplast. The living octospores of most species are covered by a mucous envelope (fig. 8), which is readily resolved in dilute india ink preparations (Lom and Vávra, 1961 and 1963).

The multinucleate sporonts associated with female hosts and the transovarial transmission of these microsporidia to their host progeny produce free spores (4 to 12 in number) which develop in oenocytes, are thin walled, and have a large prominent vacuole and a polar filament of uniform diameter. We have seen great morphological differences in free spores among adult female mosquito hosts. For instance, *Aedes punctor* (Kirby) females have cylindrical spores developing in the ovaries; *Coquillettidia perturbans* (Walker) females have elongate spores developing in oenocytes; *Culex salinarius* Coquillett females have larger elongate spores developing in oenocytes; *Culiseta impatiens* (Walker) females have spherical spores developing in the ovaries; and *Psorophora columbiae* (Dyar and Knab) females have short oval spores developing in the ovaries. These new species are not described here because we do not have information concerning the ultrastructure of the octospores in larvae. When nothing is known about the free spores associated with adult females, a species can be distinguished by the size of the octospores in larvae, the number of coils of its polar filament, the ratio of coils formed by the broad basal portion of the polar filament to its narrower distal portion, and the pathology (benign or fatal) produced in the host. Other characters often useful in distinguishing species are the shape of fixed octospores (having one or both ends truncate) and the type of metabolic products (granules, large or small, or crystalliform particles) secreted by sporonts. Even though we suspect that most *Amblyospora* species are transmitted to their host only through the ovaries (Kellen et al. 1965 and Chapman et al. 1966), we hesitate to describe new species without having some information concerning the ultrastructure of either the octospores or free spores. We present six new species here, however, because we do have information concerning the ultrastructure of octospores or because they have been given status as possible species by designation as a subspecies or variety by preceding authors.

This genus represents a large number of closely related species

known from a large variety of aquatic invertebrate hosts such as amphipods, blackflies, caddisflies, and mosquitoes. The name *Amblyospora* means "blunt spore," referring to the characteristic shape of the octospores fixed in aqueous Bouin's fluid as wet smears and stained with Heidenhain's hematoxylin. We designate *Amblyospora californica* (Kellen and Lipa) as the type species since more is known concerning both sporogonic sequences in this species than in the others.

Amblyospora amphipodae sp. n.

Spore size.—Living octospores measure 4.35–5.46×3.02–3.39 μm; suspected free spores measure 9.06–10.3×3.55–3.76 μm.

Host.—*Crangonyx richmondensis* Ellis, an amphipod.

Infection site.—Hepatopancreas.

Holotype.—Swamp east of Trenton, Fla., U.S.A., on State Road 26, Jan. 18, 1973, USNM No. 24377 (Hazard, Knell, and Oldacre).

Paratype.—USNM No. 24378 (Hazard, Knell, and Oldacre).

Fresh octospores are broadly oval (fig. 4) and are not surrounded by a mucous envelope. The octospore fixed in Bouin's fluid and stained with Heidenhain's hematoxylin is equally truncate on both ends and somewhat invaginated on the posterior end (fig. 5). The

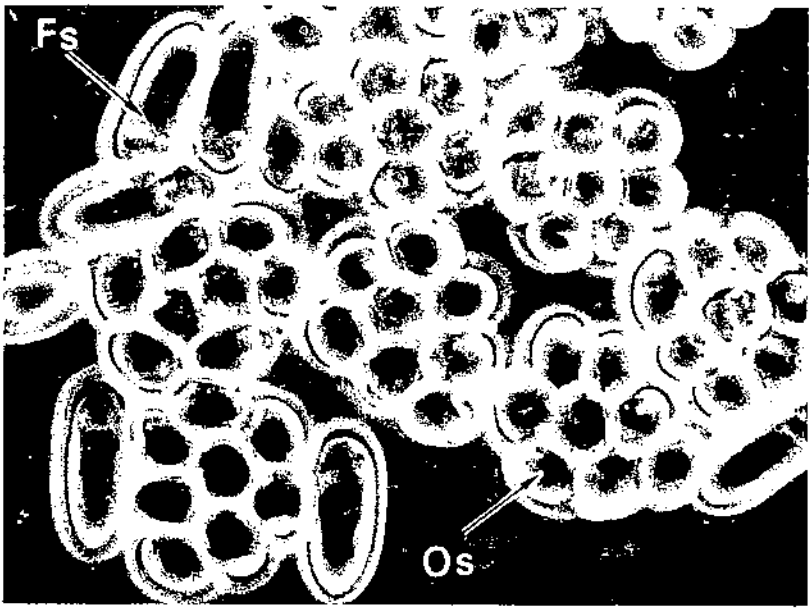

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FIGURE 4.—Fresh spores of *Amblyospora amphipodae* sp. n. × 2,000. Fs, free spore; Os, Octospore.

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FIGURE 5.—Heidenhain's-hematoxylin-stained octospores and free spores of *A. amphipodae*. × 2,000.

living spores suspected of being free spores look superficially like the spores of *Stempellia magna* (see Hazard and Fukuda 1974) in that they are coniform (fig. 4).

The octospore has a very thick endospore and exospore and a large polaroplast consisting of many tightly compressed lamellae (fig. 6). The polar filament makes 9 coils inside the spore: 3½ to 4 coils in the broad basal portion and 5 coils in the narrow distal portion. The posterior end does not collapse into the area of the vacuole as readily as do other species in preparations of tissue for electron microscopy. The pansporoblasts contain a mixture of small and moderately large granules, these persisting after the spores mature, at which time a few tubules can also be seen.

The large coniform spores, suspected as being free spores, have thin exospores; very large polaroplasts, consisting of many tightly compressed lamellae; and long, gradually tapering polar filaments, making nearly 40 coils (many overlapping coils in the posterior end) inside the spores (fig. 7). These free spores (?) are binucleate.

The animals infected with octospores display gross symptoms (whitish coloration of the hepatopancreas); however, the discoloration is not as evident in these animals as are the *Amblyospora* infections in aquatic insects. Little is known concerning host pathology, host-parasite relationship, or parasite biology. We have seen a

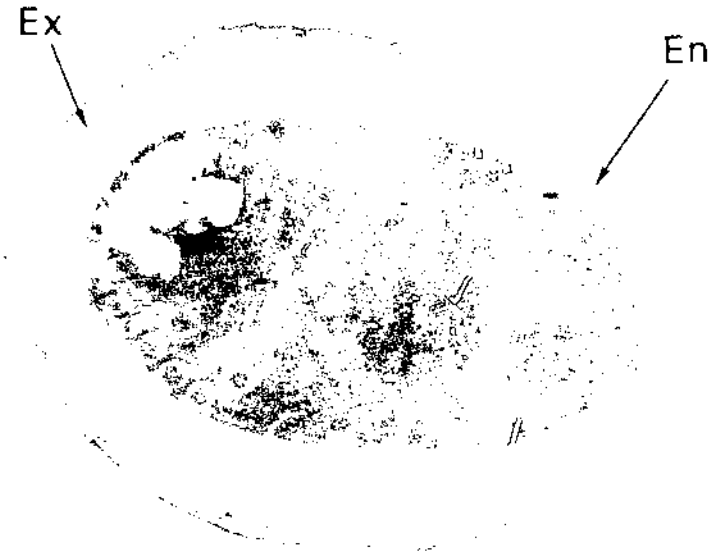

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FIGURE 6.—Ultrastructure of *A. amphipodae* octospore. × 22,500. Ex, exospore; En, endospore.

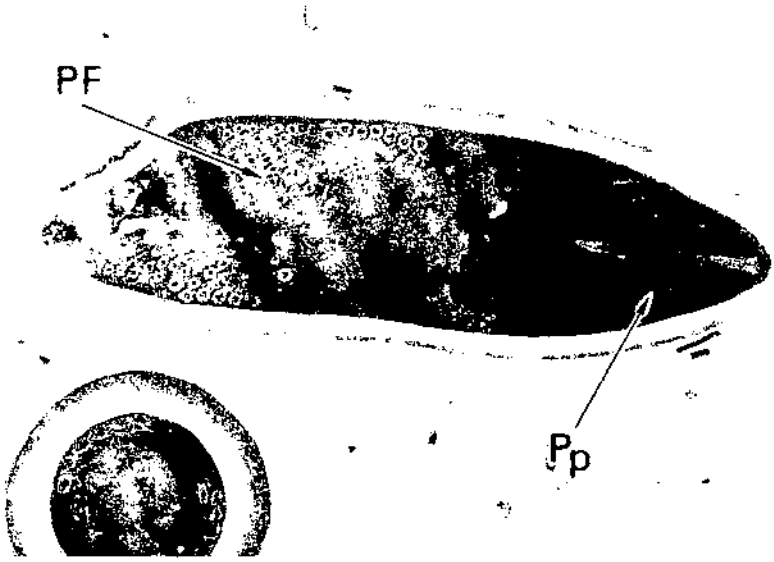

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FIGURE 7.—Ultrastructure of *A. amphipodae* free spore. × 12,000. PF, polar filament; Pp, polaroplast.

number of species in a variety of amphipod species, this one named after the class of its host (Amphipoda).

Amblyospora benigna (Kellen and Wills, 1962) comb. n.

Thelohania benigna Kellen and Wills, 1962a. J. Insect Pathol. 4: 55, Kellen et al., 1965. J. Invertebr. Pathol. 7: 161, Weiser, 1966. Nomen. Invert. p. 460, Kellen et al., 1966a. J. Invertebr. Pathol. 8: 355.

Spore size.—Living octospores measure $4.15 \pm 0.05 \times 2.68 \pm 0.02$ μm; preserved octospores measure $4.02 \pm 0.04 \times 2.95 \pm 0.03$ μm.

Host.—*Culex apicalis* Adams, a mosquito, collected in California, U.S.A.

Infection site.—Adipose tissue of male and female larvae (Kellen et al. 1965).

Nothing is known of the sporogonic stages in adult females or of the ultrastructure of the octospores. Infected male and female larvae survive to become adult mosquitoes (Kellen et al. 1965). We have seen only Giemsa-stained spores of this species; therefore, we tentatively place it in *Amblyospora*, pending study of material prepared for Heidenhain's hematoxylin staining or new information from electron microscopy.

Amblyospora bicortex (Baudoin, 1969) comb. n.

Thelohania bicortex Baudoin, 1969. Protistologica 5: 441.

Spore size.—Octospores measure 7.5×5.5 μm.

Host.—*Phryganea grandis* Linnaeus, a caddisfly, collected in France.

Infection site.—Adipose tissue of larvae.

Baudoin (1969) found only octospores associated with larvae of *P. grandis*. He describes the octospores as having thick walls. The thick basal portion of the polar filament is easily observed in his photograph of extruded fresh spores. He claims to have seen the same species in *Trichostegia minor* (Curtis), mixed with another *Thelohania* species. (See *Amblyospora trichostegiae*.) In our opinion, these spores represent two distinct species in the two hosts, the smaller and oval spores in *Trichostegia minor* being the octospores of *A. trichostegiae*.

This microsporidium was poorly prepared (perhaps improper spore fixation) for electron microscopy, and only a few details can be seen in Baudoin's electron photomicrographs. The polaroplast and the thick exospore is typical of *Amblyospora*, but the ultrastructure of the polar filament cannot be resolved.

We place this species in the genus *Amblyospora* based on the structure seen in Baudoin's electron photomicrographs. Better electron photomicrographs and additional information concerning the free spores will undoubtedly illustrate other differences. Nothing is known concerning host-parasite relationship.

Amblyospora bolinasae (Kellen and Wills, 1962) comb. n.

Thelohania bolinasae Kellen and Wills, 1962a, J. Insect Pathol. 4: 48; Kellen et al., 1965, J. Invertebr. Pathol. 7: 161; Weiser, 1966, Nemoci hmyzu, p. 460; Weiser, 1969, An atlas of insect diseases, p. 254.

Spore size.—Living octospores measure 6.89±0.08×4.85±0.04 μm; preserved octospores measure 5.70±0.07×4.45±0.08 μm.

Host.—*Aedes squamiger* (Coquillett), a mosquito, collected in California, U.S.A.

Infection site.—Oenocytes of male larvae (Kellen et al. 1965).

Nothing is known of the sporogonic stages in adult females or of the ultrastructure of the octospores. Infected male larvae die prior to pupation. From the information we have from Giemsa-stained smears, we tentatively place this species in *Amblyospora*, pending additional information from electron microscopy.

Amblyospora bracteata (Strickland, 1913) comb. n.

Glugea bracteata Strickland, 1913, J. Morphol. 24: 90.

Thelohania bracteata Debaisieux and Gastaldi (in part), 1919, La Cellule 30: 203; Kudo (in part), 1924c, Ill. Biol. Monogr. 9 (2-3): 150; Fantham et al. (in part), 1941, Parasitology 33: 202; Weiser (in part), 1947, Pr. Moravské Přír. Spol. 18: 42; Thomson (in part), 1960, J. Insect Pathol. 2: 357; Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 123; Maurand and Manier, 1968, Ann. Parasitol. Hum. Comp. 43: 79.

Spore size.—Preserved (?) octospores measure 3.0–2.5×2.7 μm (Strickland 1913).

Host.—*Simulium aureum* Fries (=*Simulium bracteatum* of Strickland), a blackfly, collected in Massachusetts, U.S.A., and in Canada.

Infection site.—Adipose tissue of larvae.

Strickland (1913) described the octospores as short elliptical bodies, somewhat truncate on the ends, and his illustrations of them are typical of the octospores of *Amblyospora*. He names an additional host, *Simulium hirtipes*, but this is not a nearctic species, and according to Stone et al. (1965) it is the type species of *Prosimulium*. We have studied the ultrastructure of octospores of several *Amblyospora*, not described here, in blackflies from the Southern United States and find diagnostic differences in each form in each host

species. We are reasonably sure, therefore, that the microsporidia in the European blackflies of Debaisieux and Gastaldi (1919) and Weiser (1947, 1961, and 1966) also represent new species of *Amblyospora*. Debaisieux and Gastaldi and Weiser gave the name *T. bracteata* to all of the forms they found in *Simulium maculatum* Meigen, *S. ochraceum* Coquillett, and *S. venustum* Say. Lutz and Splendore (1904) described a microsporidium from a *Simulium* species in Brazil that may also be a new species of *Amblyospora*; however, descriptions of these await new studies of blackflies collected in Brazil and Europe.

Vávra (1965) studied the ultrastructure of what he called *T. bracteata*, presumably found in a *Simulium* species collected in Czechoslovakia, and Liu et al. (1971) and Liu and Davies (1972a, 1972b, and 1973) studied the ultrastructure of a species they called *T. bracteata* in *Simulium vittatum* Zetterstedt collected in Ontario, Canada. In each case they studied species that have structures (granules and tubules, thick walls, and abruptly constricting polar filaments) characteristic of *Amblyospora*, but these are not necessarily identical to *T. bracteata*. Descriptions of new species from our studies of simulids collected in Florida must also wait until the ultrastructure of a neotype of *A. bracteata* from *S. aureum* has been studied.

Amblyospora californica (Kellen and Lipa, 1960) comb. n.

Thelohania californica Kellen and Lipa, 1960, J. Insect Pathol. 2: 1; Thomson, 1960, J. Insect Pathol. 2: 357; Kellen and Wills, 1962b, J. Insect Pathol. 4: 321; Kellen and Wills, 1963, Proc. 1st Int. Congr. Protozool., p. 490; Kudo and Daniels, 1963, J. Protozool. 10: 112–120; Kellen et al., 1965, J. Invertebr. Pathol. 7: 161; Kellen et al., 1966b, Exp. Parasitol. 18: 251; Weiser, 1966, Nemoci hmyzu, p. 460; Kellen et al., 1966a, J. Invertebr. Pathol. 8: 355; Chapman et al., 1967, Proc. N.J. Mosq. Exterm. Assoc. 54: 54–60; Kudo, 1971, Protozoology, p. 816; Khaliulin and Ivanov, 1971, Parazitologiya 5: 100.

Thelohania opacita: Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 113. New synonymy.

Nosema lunatum Kellen et al., 1967, J. Invertebr. Pathol. 9: 22. New synonymy.

Thelohania sp. Chapman, 1966, Univ. Nev. Bull. T-2: 37; Tsai et al., 1969, Mosq. News 29: 103.

Spore size.—Living octospores measure 6.8–10.4×5.0–6.5 μm; preserved octospores measure 5.45–8.72×4.3–5.4 μm; living free spores measure 12.86±0.17×3.75±0.05 μm; preserved free spores measure 11.43±0.15×3.70±0.06 μm.

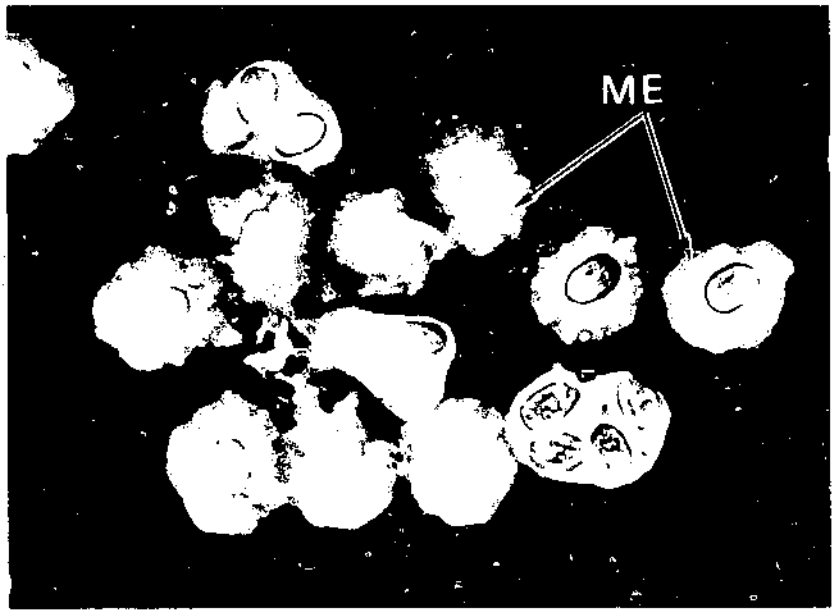
Host.—*Culex tarsalis* Coquillett, a mosquito, collected in California, U.S.A. (Kellen and Lipa 1960); Louisiana, U.S.A. (Chapman et al. 1967); Nevada, U.S.A. (Chapman 1966); and Utah, U.S.A. (Tsai et al. 1969).

Infection site.—Octospores develop in the oenocytes of male larvae

(Kellen et al. 1965), whereas free spores develop in oenocytes of adult females, carrying schizonts via the ovaries.

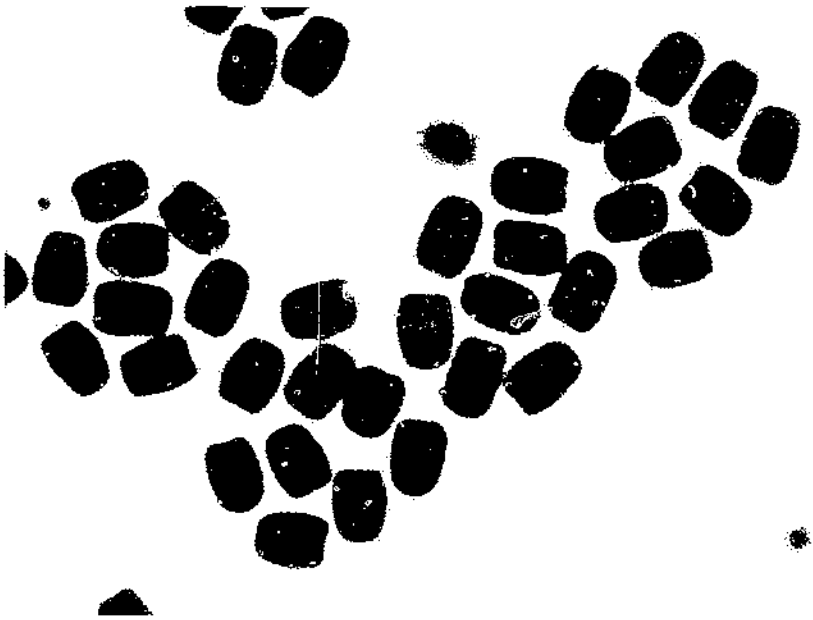
Living octospores are surrounded by a thick mucous envelope (fig. 8). Octospores fixed in aqueous Bouin's fluid as wet smears and stained with Heidenhain's hematoxylin are truncate and obviously somewhat invaginated on their posterior ends (fig. 9). Living free spores are elongate and curved, have a distinct dark vacuole, and readily extrude their polar filament. According to Kellen et al. (1967), they are binucleate.

The octospore has a thick and rugose exospore, an abruptly constricting polar filament, and a large polaroplast consisting of tightly compressed lamellae (fig. 10). The polar filament consists of 12 coils, 5 formed by the broad basal portion and 7 formed by the narrow distal portion. Kudo and Daniels (1963) also studied the octospore ultrastructure, but their results were poor, possibly owing to improper fixation. The sporonts that eventually produce octospores secrete dense granular particles that aggregate in large clumps inside the pansporoblastic membrane (fig. 11). These then disappear during sporulation. We believe that these granules contribute to the formation of the thick exospore. Nothing is known of the ultrastructure of free spores in adult females.



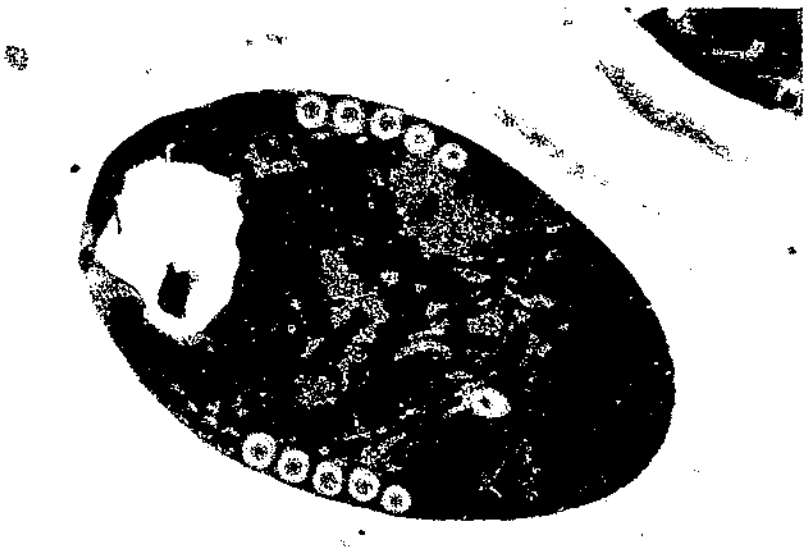
PN 1457

FIGURE 8.—Fresh octospores of *Amblyospora californica* (Kellen and Lipa) in dilute indie ink. × 1,000. ME, mucous envelope.



PN 4458

FIGURE 9.—Heidenhain's-hematoxylin-stained octospores of *A. californica*. × 2,000.



PN 4459

FIGURE 10.—Ultrastructure of *A. californica* octospore. × 17,100. CPF, constricted polar filament; N, nucleus.

PN 4160

FIG. 8.—11. Dividing sporont in *A. californica* pansporoblast. × 4,800. PM, pansporoblastic membrane, MG, metabolic granules.

Infected female larvae and adults do not show gross symptoms, and they survive long enough to reproduce, passing the infection to their progeny. The sporonts in these females remain dormant in the hemolymph of larvae, invading oenocytes at or near the time of pupation. These stages then go through repeated divisions, filling the hypertrophied blood cells with quadrinucleate and binucleate sporonts (Kellen et al. 1967). At sporulation the oenocytes enlarge, becoming 200 μm or more in diameter, and are filled with free spores. Octospores form only in male larvae, all of which succumb to the infection in their last instar.

Amblyospora callosa sp. n.

Spore size.—Preserved octospores measure 2.33–2.92×2.07–2.49 μm; preserved free spores measure 3.02–4.08×1.64–2.60 μm.

Host.—*Rhacophila fuscula* (Walker), a caddisfly.

Infection site.—Both octospores and free spores develop in the adipose tissue of larvae.

Holotype.—Stream near sewage plant east of Belchertown, Mass., U.S.A., May 16, 1972, USNM No. 24379 (Hall and Hazard).

Paratype.—USNM No. 24380 (Hall and Hazard).

Octospores preserved in aqueous Bouin's fluid as wet smears are nearly truncate on both ends (fig. 12). The pansporoblastic membrane that encloses them is subpersistent, but remains intact longer than the membrane in many other species of *Amblyospora*. Conform spores, suspected of being free spores, were found mixed with octospores in the adipose tissue of a larva. Whether or not the conform spores are free spores from another sporogonic sequence of this species has yet to be confirmed. We do not know if the octospores of this species are enclosed by a mucous envelope since these spores have not been observed in dilute india ink.

Octospores have a thick exospore, a somewhat thinner endospore, and a polar filament consisting of 11 coils (fig. 13); the thick basal portion of the polar filament forms the first 5 coils and the narrow distal portion forms 6 coils. The pansporoblasts containing a dividing sporont also contain a few large, dense crystalliform particles (fig. 14); however, after sporulation these large particles become smaller and much less dense. The pansporoblasts are also packed with minute granules that fill the area between the dividing sporont and the pansporoblastic membrane. These small granules diminish, however, during sporulation.

The suspected free spores are very similar in ultrastructure to those of *Amblyospora amphipodae*, but are much smaller, and the polar filament is much shorter, forming only 15 coils inside the spore (fig. 15).

Nothing is known concerning the biology of this species or its host-parasite relationship. According to the pathology observed, we doubt if the host would survive the larval stage. The name *callosa* means "hard skin," referring to the somewhat persistent pansporoblastic membrane.

Amblyospora campbelli (Kellen and Wills, 1962) comb. n.

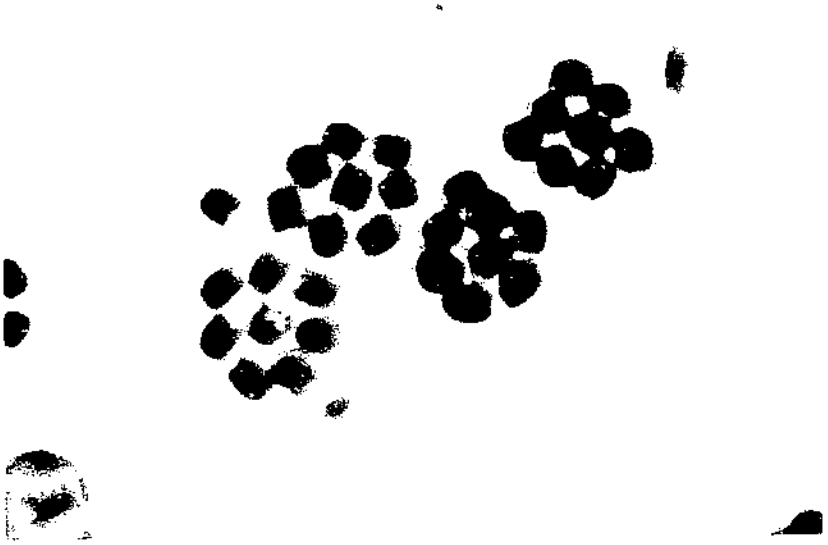
Thelohania campbelli Kellen and Wills, 1962a, J. Insect Pathol. 4: 51; Kellen et al., 1965, J. Invertebr. Pathol. 7: 101; Weiser, 1966, Nemoci hmyzu, p. 460; Kellen et al., 1966a, J. Invertebr. Pathol. 8: 355.

Spore size.—Living octospores measure 6.01±0.05×4.13±0.04 μm; preserved octospores measure 5.47±0.13×3.88±0.02 μm.

Host.—*Culiseta incidens* (Thomson), a mosquito, collected in California, U.S.A.

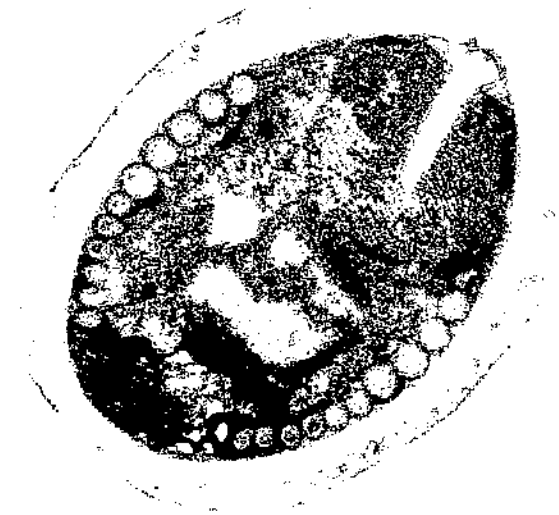
Infection site.—Adipose tissue of larvae (Kellen et al. 1965).

We know nothing of the ultrastructure of this species or of the existence of free spores in adult females; therefore, we tentatively place it in this genus until it has been studied by electron micros-



PN 4461

FIGURE 12.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora callosa* sp. n. × 2,000.



PN 4462

FIGURE 13.—Ultrastructure of *A. callosa* octospore. × 22,500.

copy. According to Kellen et al. (1965) less than half of the progeny larvae acquire patent infections, and they succumb during the fourth stadium.

Amblyospora canadensis (Wills and Beaudoin, 1965) comb. n.

Thelohania inimica canadensis Wills and Beaudoin, 1965, J. Invertebr. Pathol. 7: 11.

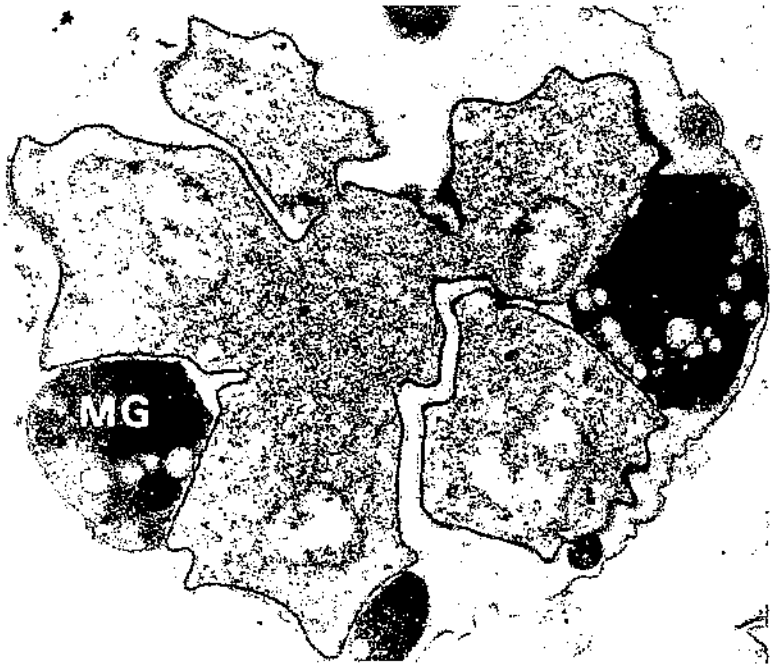
Thelohania inimica: Bailey et al., 1967, J. Invertebr. Pathol. 9: 354. New synonymy.

Thelohania nr *opacita* Anderson, 1968, J. Invertebr. Pathol. 11: 451. New synonymy.

Thelohania opacitor Fulton et al., 1974, Mosq. News 34: 89. New synonymy.

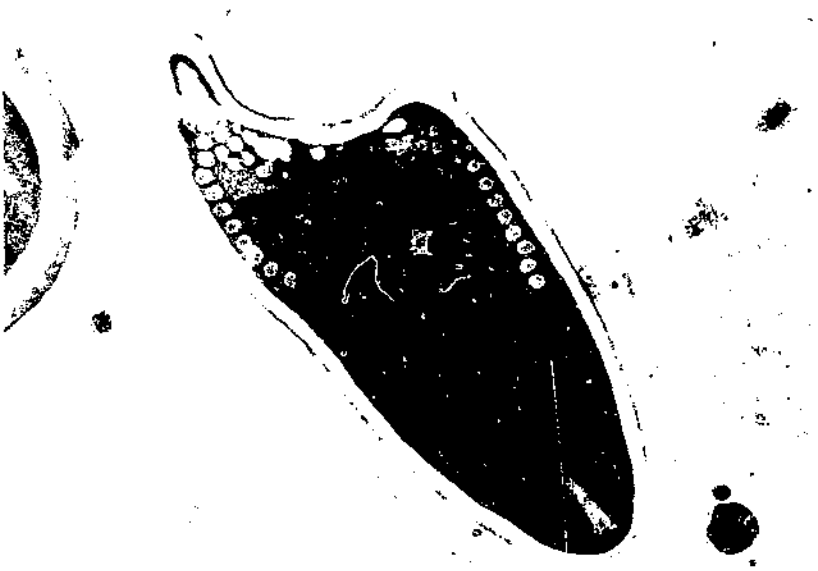
Thelohania sp. Chapman et al., 1966, J. Invertebr. Pathol. 8: 453; Chapman et al., 1967, Proc. N.J. Mosq. Exterm. Assoc. 54: 56; Chapman et al., 1969, Proc. N.J. Mosq. Exterm. Assoc. 56: 205.

Spore size.—Living octospores measure 4.35–7.0×3.5 μm (reported here), 5.42±0.09×4.19±0.08 μm (Wills and Beaudoin 1965), 6.36±0.03×4.64±0.03 μm (Chapman et al. 1966), 6.0±0.03×4.4 ±0.03 μm (Anderson 1968); preserved octospores measure 5.48–4.92



PN 4463

FIGURE 14.—Dividing sporont in *A. callosa* pansporoblast. × 12,000. MG, metabolic granules.



PN 1161

FIGURE 15.—Ultrastructure of *A. calloso* free spore. × 12,000.

× 4.48–4.92 μm (Bailey et al. 1967), 5.3±0.08×4.2±0.09 μm (Anderson 1968).

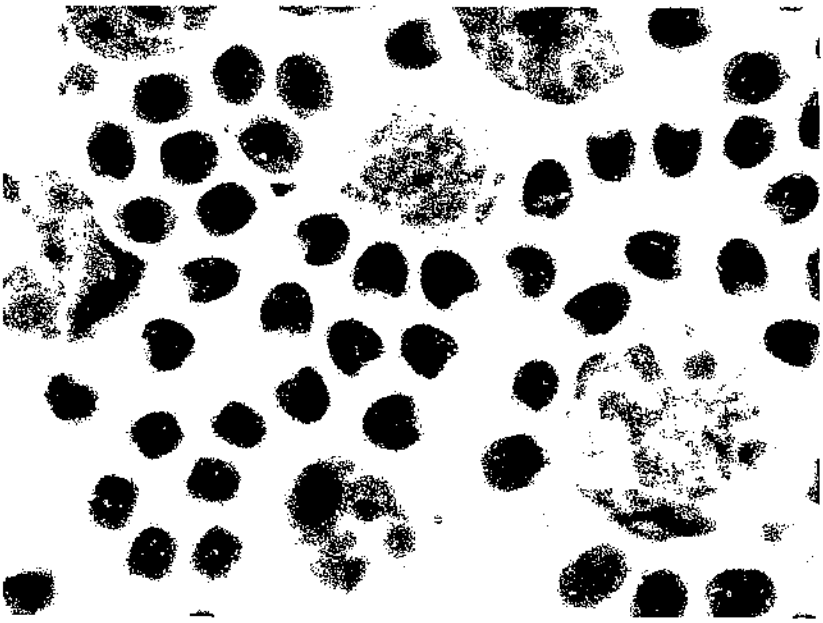
Host.—*Aedes canadensis* (Theobald), a mosquito, collected in Connecticut, U.S.A. (Anderson 1968); Louisiana, U.S.A. (Chapman et al. 1966); Massachusetts, U.S.A. (reported here); and Pennsylvania, U.S.A. (Wills and Beaudoin 1965).

Infection site.—Adipose tissue of larvae.

Nothing is known about the sporonts or free spores in adult females. Octospores, fixed as wet smears with aqueous Bouin's fluid and stained with Heidenhain's hematoxylin, are usually truncate and somewhat invaginated at their posterior ends (fig. 16). The living octospore is thinly covered by a mucous envelope that is difficult to observe in dilute india ink.

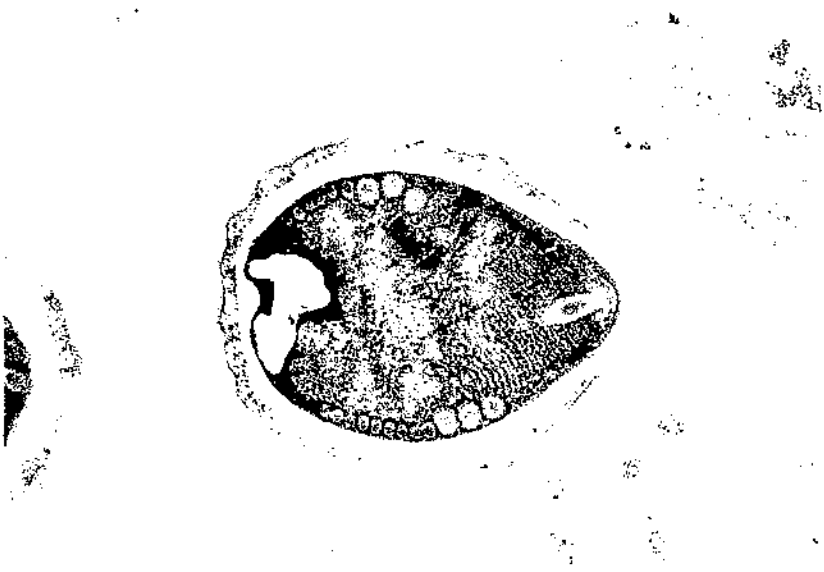
The ultrastructure of the octospore is similar to that of other species; however, the polar filament consists of 11 coils, only 3 formed by the broader basal portion (fig. 17). The young pansporoblast contains many small, loose granules as well as scattered dense clumps of granules (fig. 18).

All progeny male larvae become patently infected and die during the last larval stadium (Chapman et al. 1966). We recognize it as a species since Wills and Beaudoin (1965) proposed its uniqueness by



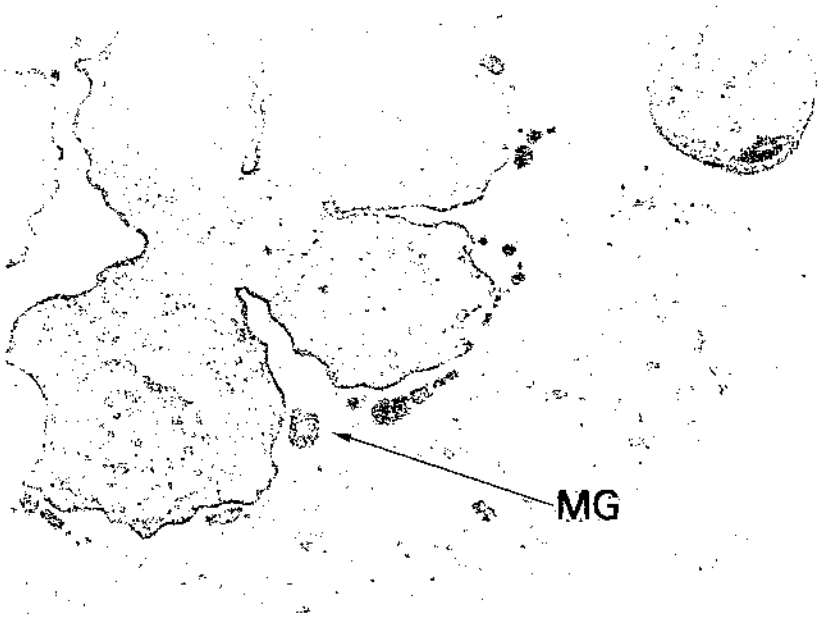
PN-4465

FIGURE 16.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora canadensis* (Wills and Beaudoin). × 2,000.



PN-4467

FIGURE 17.—Ultrastructure of *A. canadensis* octospore. × 12,000.



PN 1166

FIGURE 18.—Dividing sporont in *A. canadensis* pansporoblast. × 7,500. MG, metabolic granules.

making it a subspecies of *Amblyospora inimica* (Kellen and Wills 1962) and because our studies of its ultrastructure support this distinction.

Amblyospora gigantea (Kellen and Wills, 1962) comb. n.

Thelohania gigantea Kellen and Wills, 1962a, J. Insect Pathol. 4: 46; Kellen et al., 1965, J. Invertebr. Pathol. 7: 161; Weiser, 1966, Nemoci hmyzu, p. 460; Khaliulin and Ivanov, 1971, Parazitologiya 5: 98.

Spore size.—Living octospores measure 8.10±0.06×5.48±0.04 μm; preserved octospores measure 6.69±0.07×5.18±0.20 μm.

Host.—*Culex erythrothorax* Dyar, a mosquito, collected in California, U.S.A.

Infection site.—Oenocytes of male larvae (Kellen et al. 1965).

Nothing is known of the sporogonic sequence in adult females or of the ultrastructure of the octospores in males. Male larvae succumb to infection (Kellen et al. 1965). We tentatively place this species in the genus *Amblyospora*, based on observations of sporonts and spores in Giemsa-stained smears.

Amblyospora inimica (Kellen and Wills, 1962) comb. n.

Thelohania inimica Kellen and Wills, 1962a, J. Insect Pathol. 4: 53; Wills and Beaudoin in part, 1965, J. Invertebr. Pathol. 7: 11; Kellen et al., 1965, J. Invertebr. Pathol. 7: 161; Weiser, 1966, Nemoci hmyzu, p. 460; Kellen et al., 1966a, J. Invertebr. Pathol. 8: 355; Chapman et al., 1966, J. Invertebr. Pathol. 8: 452; Chapman et al., 1967, Proc. N.J. Mosq. Exterm. Assoc. 54: 56; Bailey et al., 1967, J. Invertebr. Pathol. 9: 451; Chapman et al., 1969, Proc. N.J. Mosq. Exterm. Assoc. 56: 206.

Perezinana sp. Chapman, 1966, Univ. Nev. Bull. T. 2: 37; Tsai et al., 1969, Mosq. News 29: 103.

Spore size.—Living octospores measure 5.25–7.0 × 3.5–5.25 μm (reported here), 5.73 ± 0.06 × 3.92 ± 0.04 μm (Kellen and Wills 1962a), 6.18 ± 0.04 × 4.41 ± 0.02 μm (Chapman et al. 1966); preserved octospores measure 5.43 ± 0.04 × 4.11 ± 0.05 μm (Kellen and Wills 1962a).

Host.—*Culiseta inornata* (Williston), a mosquito, collected in California, Florida, Louisiana, Nevada, and Utah, U.S.A.

Infection site.—Adipose tissue of male and female larvae (Kellen et al. 1965).

Spores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin are characteristically truncate, usually on both ends (fig. 19). The extranuclear granules are easily observed in quadrinucleate and octonucleate pansporoblasts prepared by the same histological method. Also, the living spore has a mucous covering that is somewhat difficult to detect in dilute india ink.

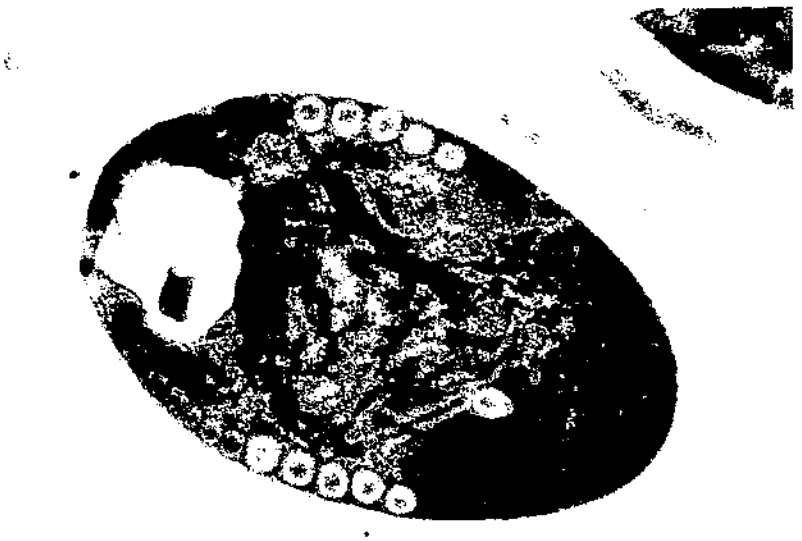
The sporonts secrete dense granules that clump, forming large masses scattered throughout the pansporoblast. These clumps of granules become smaller and lose their continuity during sporulation. The polar filament forms 10 coils inside the spore, the first 5 of which are made by the broad basal portion (fig. 20). The anterior half of the polaroplast contains many tightly compressed parallel lamellae. However, these lamellae become widely separated and divergent in the posterior half, creating cell-like areas.

We have no information concerning the transovarial stages in adult females. According to Kellen et al. (1965), the patently infected larvae die in their last instar.

Simmers (1974b) has reported *Culiseta inornata* also as the host of *Parathelohania opacita* [= *Amblyospora opacita* (Kudo)], based solely on the size of stained spores. We do not understand how he could determine it to be *A. opacita*, however, since the spore size range of *A. inimica* is near that of *A. opacita*, according to our measurements from host specimens collected in Florida and Louisiana. Simmers listed the mean size and standard error of the mean for *A. opacita* stained spores as 4.90 ± 0.06 × 3.80 ± 0.04 μm, which, according to him, was not significantly different from the spores he found in *C.*

PN 1165

FIGURE 19.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora inimica* (Kellen and Wills). × 2,000.



PN 1169

FIGURE 20.—Ultrastructure of *A. inimica* octospore. × 19,000.

mornata. Our measurements show that the range of spore size, excluding macrospores, of *A. inimica* and *A. opacita* is 6.13–6.18×4.38–4.41 μm and 5.35–6.28×4.13–5.02 μm, respectively, and that these ranges overlap in both the spore length and width. It is also important to note that Kellen et al. (1965) list the size of *A. inimica* spores taken from larvae in California as 6.48±0.04×4.41±0.02 μm, while Chapman et al. (1966) list the size of the spores of the same species found in Louisiana as 5.73±0.06×3.92±0.04 μm. We have examined a slide (SIU 0010) which Simmers deposited in the Zoology Research Museum of Southern Illinois University, and we conclude that the larva used to prepare this slide had probably ingested spores from the remains of another infected larva. We came to this conclusion because the stained smear contained many more diatoms than spores and no meronts or sporonts.

Amblyospora keenani sp. n.

Spore size.—Living octospores measure 4.19–4.79×2.76–3.23 μm; preserved octospores measure 2.76–3.29×1.96–2.49 μm; preserved macrospores measure 3.82–6.10×2.49 μm.

Host.—*Aedeomyia squamipennis* (Lynch Arribálzaga), a mosquito.

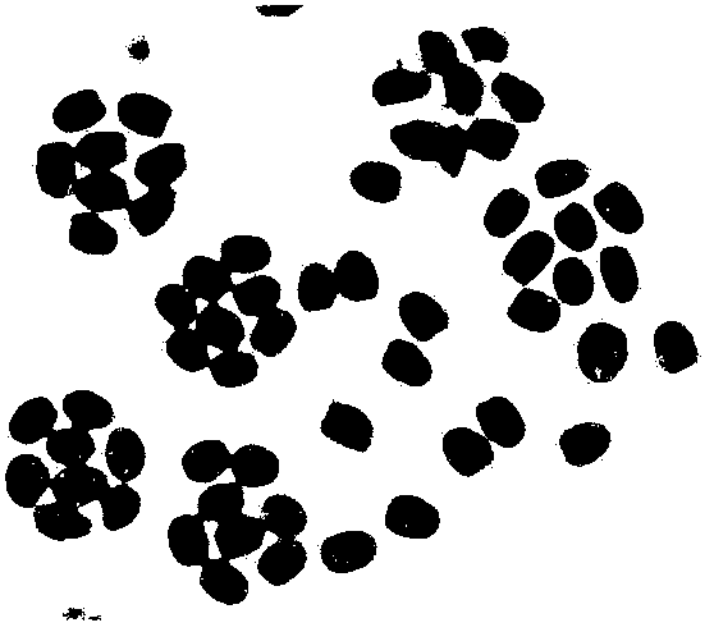
Infection site.—Adipose tissue of larvae.

Holotype.—Chagras River, Canal Zone, Mar. 6, 1973, USNM No. 24381 (Anthony, Hazard, and Keenan).

Paratypes.—USNM No. 24382; World Health Organization Reference Center, Ohio State University, Accession No. 1663 (Anthony, Hazard, and Keenan).

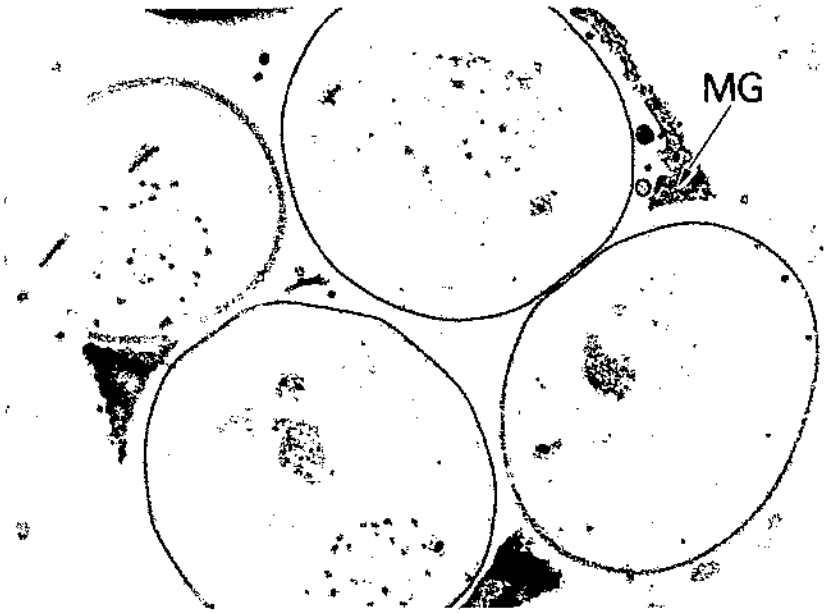
Adult females have not been examined; therefore, nothing is known about transovarial transmission of infection in this species. Living octospores have no visible vacuole, and they are surrounded by a thin mucous envelope. Heidenhain's-hematoxylin-stained octospores are truncate only at their posterior ends (fig. 21). Macrospores are occasionally observed but are few in number. Infected larvae do not become characteristically creamy white throughout the body; instead, the infection is displayed in the cuticle as scattered areas, giving the larvae a spotted appearance.

Sporonts secrete granules into the vacant areas inside the pansporoblastic membrane. Some of these become clumped and fuse together to form scattered large crystalliform particles (fig. 22). When the spores are fully formed, the large particles change considerably in size and structure, most becoming much smaller and others disappearing. It appears that most of the granules in the pansporoblasts are utilized in the formation of the thick exospore



PN 1470

FIG. 21.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora keenani* sp. n. × 2,000.



PN 1471

FIG. 22.—Sporoblasts in *A. keenani* pansporoblast. × 12,000. MG, metabolic granules.

(fig. 23). The polar filament of this species is unusual, as its broad basal portion is very long, forming 6 of the 8 coils in the octospore (fig. 24).

Patently infected larvae succumb. However, we do not know whether or not both sexes of larvae are involved in these patent infections. Finding this species in *A. squamipennis* is very important, as this mosquito is also the host of a *Parathelohania* species. This clearly demonstrates that the criteria used to establish the new genera proposed here are stable morphological characters and not structural changes occurring in different host genera because of biochemical differences among host animals. We name this species after Marvin Keenan, who gave much of his time and energy to help us survey large numbers of mosquitoes for mosquito pathogens during three expeditions into the jungles, lakes, and rivers of the Canal Zone and Panama.

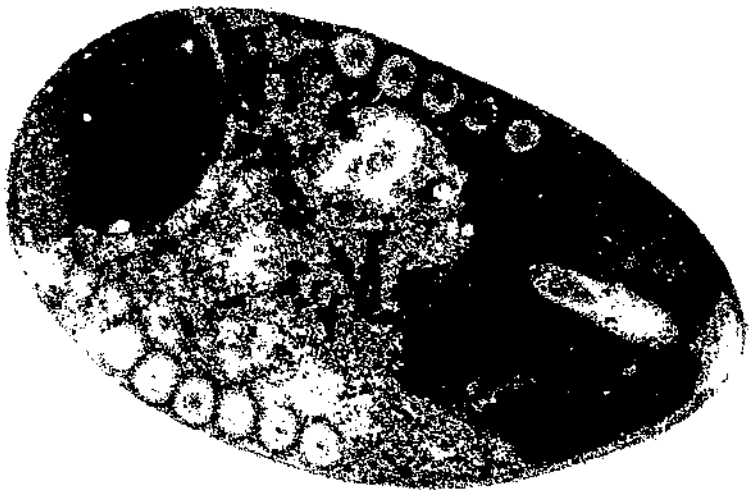
Amblyospora khaliulini sp. n.

Parathelohania opacita var. *mariensis* Khaliulin and Ivanov, 1971, Parazitologiya 5, 98.

Parathelohania opacita Weiser (in part), 1947, Pr. Moravske Pfir. Spol. 18; 37; Weiser (in part), 1961, Monogr. Angew. Entomol. 17, 112; Weiser (in part), 1963c, Bull. WHO 28, 124; Weiser (in part), 1966, Nemoci hmyzu, p. 459; Weiser, 1969, An atlas of insect diseases, p. 244. New synonymy.

PN 1172

FIG. 23. Sporulating pansporoblast of *A. keenani*. × 20,000. Mt, microtubules.



PN 4173

FIG. 21.—Ultrastructure of *Amblyospora* octospore. × 30,750.

Pleistophora sp. Nöller, 1920, Arch. Protistenkd. 41: 187; Kudo, 1924c, Ill. Biol. Monogr. 9:2, 3: 158; Welch, 1960, J. Insect Pathol. 2: 387; Chapman et al., 1973, Mosq. News 33: 165.

Spore size.—Living octospores measure 6.05–7.26 × 4.2–4.8 μm (Khaliulin and Ivanov 1971), 5.6–8.7 × 3.4–5.3 μm (Welch 1960), 7.40 ± 0.17 × 5.56 ± 0.19 μm (Chapman et al. 1973).

Host.—*Aedes communis* (DeGeer) [= *Aedes nemorosus* of Nöller 1920 and Weiser 1947], a mosquito, collected in Manitoba, Canada; Czechoslovakia; Germany; Alaska and Massachusetts, U.S.A.; and Mari, U.S.S.R.

Infection site.—Hemolymph and adipose tissue of larvae.

Holotype.—Winter pool near South Deerfield, Mass., U.S.A., May 14, 1972, USNM No. 24383 (Hall and Hazard).

Paratypes.—USNM No. 24384; World Health Organization Reference Center, Ohio State University, Accession No. 1665 (Hall and Hazard).

Only the sporogonic sequence producing octospores is known in this species. Octospores fixed in aqueous Bouin's fluid as wet smears and stained with Heidenhain's hematoxylin are often truncate at both ends, the posterior ends always being somewhat invaginated

(fig. 25). Living octospores were not examined for the presence of a mucous envelope.

The ultrastructure of the octospores of this species (fig. 26) is very similar to that of *A. californica*, the only difference being in the number of coils formed by the broad basal portion of the polar filament ($3^1/_2$ in this species and $4^1/_2$ in *A. californica*). The sporonts contain large vacuolated aggregates of granules, most of which disappear during sporulation.

Patently infected larvae succumb during the last stadium; however, we do not know whether or not sporulation occurs in both sexes of larvae. We name this species after G. L. Khaliulin of the Soviet Union who, along with Ivanov, attempted to distinguish it from other species by making it a variety of *Thelohania opacita*.

Amblyospora lairdi (Weiser, 1965) comb. n.

Duboscqia lairdi Weiser, 1965, Zool. Anz. 175: 232; Weiser, 1966, Nemoci hmyzu, p. 432; Baudoin, 1969, Protistologica 5: 441; Weiser, 1969, An atlas of insect diseases, p. 214.

Spore size.—Living octospores measure 4.0–4.5×3.0–4.0 μm (Weiser 1965), 6.0–6.5×4.0 μm (Weiser 1966).

Host.—*Polycentropus flavomaculatus* Pictet, a caddisfly, collected in Czechoslovakia.

Infection site.—Adipose tissue of larvae.

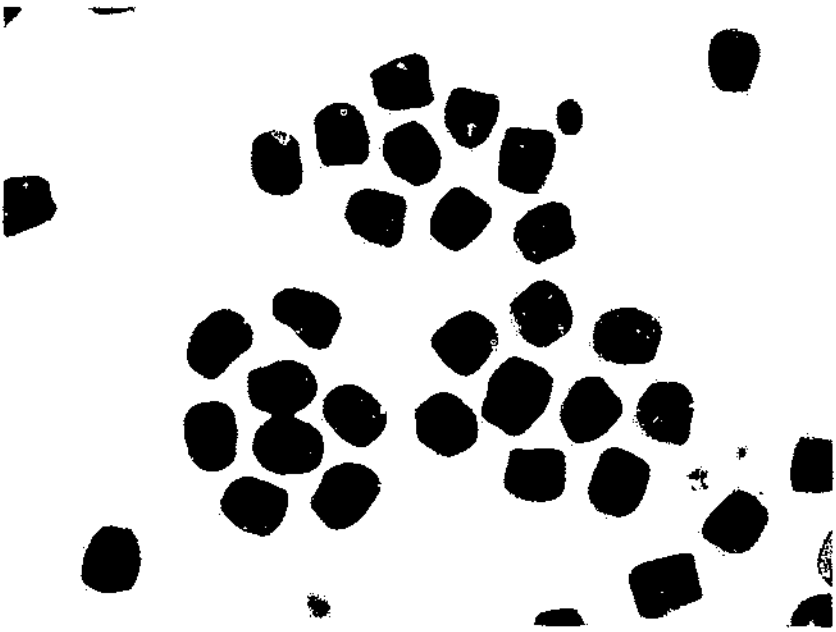
Weiser (1965) makes no mention of a sporogonic sequence in adult females or of free spores. He describes the chromosomes in sporonts producing octospores as being prominent in stained preparations and remarks that the octospores are broadly egg-shaped, with a prominent lens-shaped vacuole. He also reports that these spores are surrounded by a mucous envelope (gelatinous capsule of Weiser).

No studies have been made of the ultrastructure of this species, but Weiser's illustrations of sporonts and spores and his detailed account are sufficient to place it in this genus.

Amblyospora minuta (Kudo, 1924) comb. n.

Thelohania minuta Kudo, 1924c, Ill. Biol. Monogr. 9(2–3): 163; Kudo, 1925a, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1. Orig. 96: 437; Weiser, 1947, Pr. Moravske Přír. Spol. 18: 37; Thomson, 1960, J. Insect Pathol. 2: 359; Weiser, 1961, Monogr. Angew. Entomol. 17: 111; Weiser, 1966, Nemoci hmyzu, p. 452; Fulton et al., 1974, Mosq. News 34: 89.

Thelohania rotunda Kudo, 1924c, Ill. Biol. Monogr. 9(2–3): 162; Kudo, 1925a, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1. Orig. 96: 435; Weiser, 1947, Pr. Moravske Přír. Spol. 18: 36; Thomson, 1960, J. Insect Pathol. 2: 361; Weiser, 1961, Monogr. Angew. Entomol. 17: 111; Weiser, 1966, Nemoci hmyzu, p. 452. New synonymy.



PN 1474

FIGURE 25.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora khaliulini* sp. n. × 2,000.

PN 1475

FIGURE 26.—Ultrastructure of *A. khaliulini* octospore. × 16,000.

Thelohania sp. Chapman et al., 1967, Proc. N.J. Mosq. Exterm. Assoc. 54: 56; Chapman et al., 1969, Proc. N.J. Mosq. Exterm. Assoc. 56: 205.

Spore size.—Preserved oval octospores measure 2.5–3.5×1.5–2.0 μm (Kudo 1924c); preserved subspherical octospores measure 2.5–3.0×2.3–2.7 μm (Kudo 1924c); living free spores average 4.62×2.37 μm (reported here).

Host.—*Culex erraticus* (Dyar and Knab) (=*Culex leprincei* of Kudo), a mosquito, collected in Florida (reported here), Georgia (Kudo 1924c), and Louisiana (Chapman et al. 1967 and 1969), U.S.A.

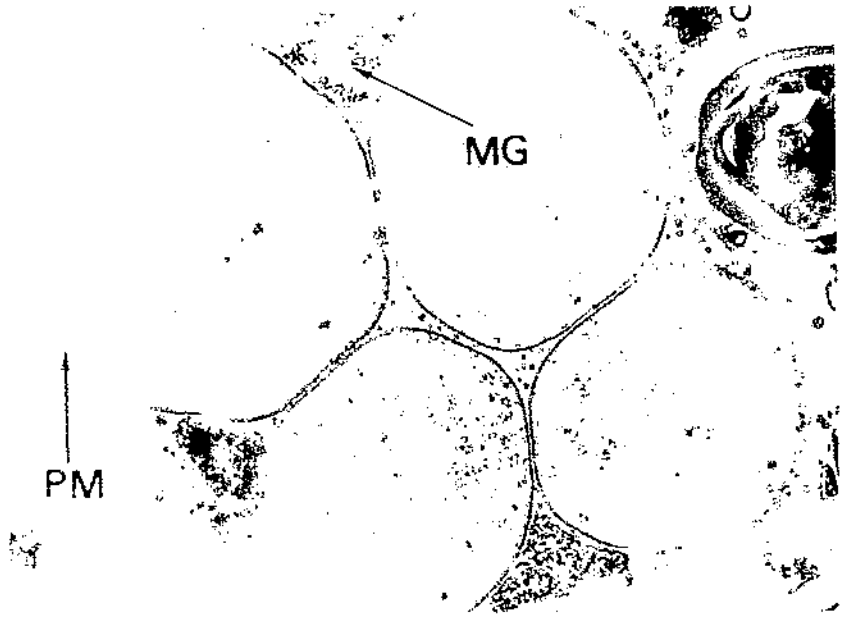
Infection site.—Octospores develop in the adipose tissue of larvae.

Oval octospores stained with Heidenhain's hematoxylin in fixed wet smears are equally truncate at both ends (fig. 27). These octospores have not been examined for the presence or absence of mucous envelopes. We have seen a few oblong spores in adult females, which we believe to be free spores.

The sporonts secrete large granules of uniform size, some aggregating to form a few larger particles (fig. 28). These mostly disappear after the spores are fully developed. The octospore has a short polar filament consisting of only 4 coils, 2½ of which are

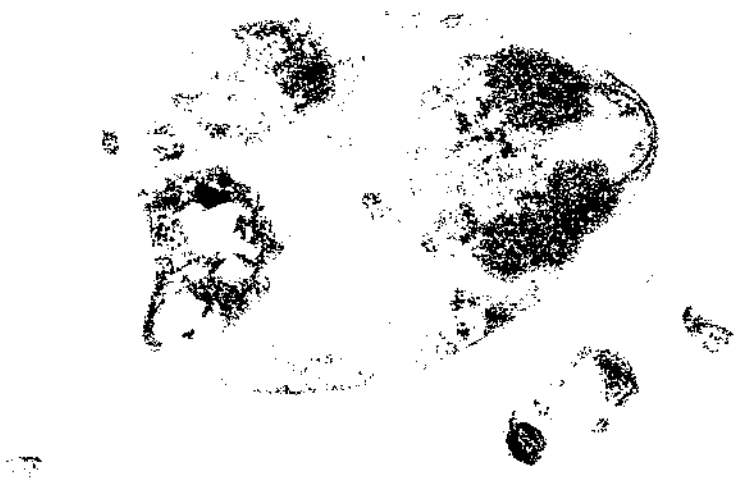
PN 4476

FIGURE 27.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora minuta* (Kudo). × 2,000.



PX 1177

FIG. 28.—Sporoblasts in *A. minuta* pansporoblast. × 10,800. MG, metabolic granules; PM, pansporoblastic membrane



PX 1178

FIG. 29.—Ultrastructure of *A. minuta* octospore. × 30,000.

formed by the broad basal portion (fig. 29). The ultrastructure of subspherical octospores could not be resolved since all of these spores appeared to be aberrant. Nothing is known of the ultrastructure of sporonts or free spores in adult females.

This microsporidium usually produces benign infections in larvae when only isolated fat lobes are invaded by the parasite. However, some larvae probably succumb to disease when the infection is more extensive. This species is unusual because it has aberrant subspherical octospores as well as normal oval octospores. Kudo (1924c and 1925a) believed these two types of spores represented two distinct species, however, we commonly find oval and subspherical spores mixed together in the same pansporoblasts.

Amblyospora mojingensis sp. n.

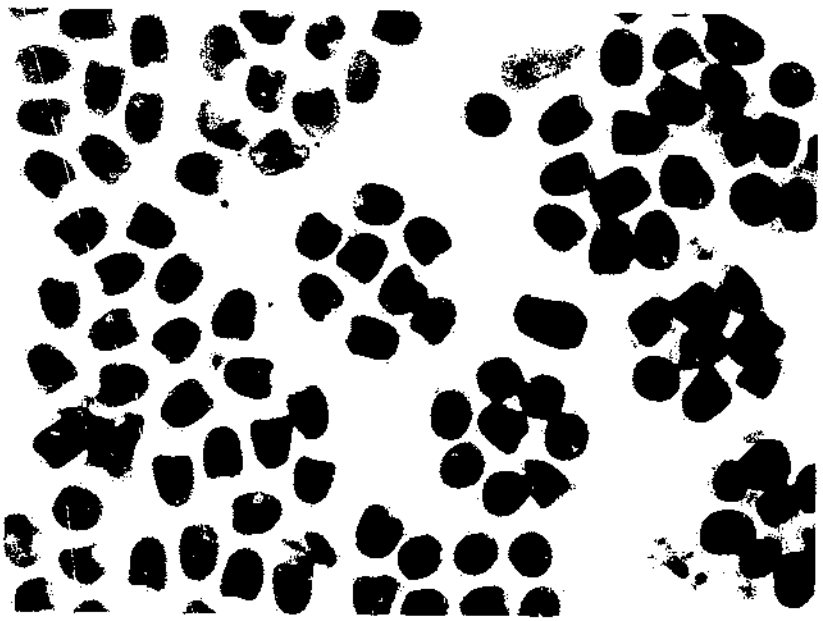
Spore size.—Preserved octospores measure 2.6–3.5×2.4–2.6 μm; preserved macrospores measure 3.7–4.5×2.8–3.3 μm.

Host.—*Anopheles eiseni* Coquillett, a mosquito.

Infection site.—Adipose tissue of larvae.

Holotype.—Mojinga Swamp, Canal Zone, June 14, 1973, USNM No. 24385 (Anthony and Hazard).

Paratypes.—USNM No. 24386; World Health Organization Refer-



PN-4479

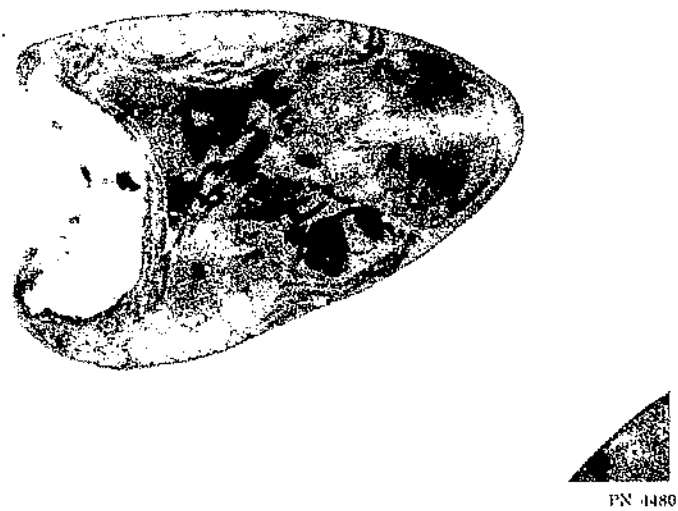
FIGURE 30.—Heidenhain's-hematoxylin-stained octospores of *Amblyospora mojingensis* sp. n. × 2,000.

ence Center, Ohio State University, Accession No. 1657 (Anthony and Hazard).

Only the sporogonic sequence producing octospores is known. Octospores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin are truncate only at the posterior end, appearing more invaginated that truncate (fig. 30). Macrospores, however, stained in the same way often appear truncate at both ends, with a less obviously invaginated posterior end. The octospores were not examined in dilute india ink for the presence or absence of a mucous envelope.

Octospores have a thick, rugose exospore, a polar filament with a broad basal portion abruptly constricting beyond its middle to form a short narrow distal portion, and a prominent lamellated polarplast (fig. 31). The polar filament is unusual in that it forms 7 coils inside the spore, 4 of them by the broad basal portion.

This species is important since it is a parasite of *Anopheles*, a genus previously known only as a host of *Parathelohania*. It is named after the Mojinga Swamp near the eastern coast of the Canal Zone, where the infected host larva was collected.



PN 4480

FIGURE 31.—Ultrastructure of *A. mojingensis* octospore. × 22,800.

Amblyospora noxia (Kellen and Wills, 1962) comb. n.

Thelohania noxia Kellen and Wills, 1962a, J. Insect Pathol. 4: 49; Kellen et al., 1965, J. Invertebr. Pathol. 7: 161; Weiser, 1966, Nemoci hmyzu, p. 460.

Spore size.—Living octospores measure 6.58±0.07×4.47±0.04 μm; preserved octospores measure 5.34±0.02×4.04±0.01 μm.

Host.—*Culex thriambus* Dyar, a mosquito, collected in California, U.S.A.

Infection site.—Oenocytes and adipose tissue of male and female larvae (Kellen et al. 1965).

No studies have been made of the ultrastructure, and nothing has been reported on a second sporogonic sequence in adult females. This microsporidium is transmitted via the egg to about one-half of its larval progeny. Both males and females acquire patent infections and succumb during the fourth larval instar. Because we have seen Giemsa-stained smears of sporonts and spores which appear typical of *Amblyospora*, we tentatively place the species in this genus.

Amblyospora opacita (Kudo, 1922) comb. n.

Thelohania opacita Kudo, 1922, J. Parasitol. 8: 75; Kudo, 1924b, J. Parasitol. 11: 84; Kudo, 1924c, Ill. Biol. Monogr. 9 (2–3): 159; Weiser (in part), 1947, Pr. Moravské Přír. Spol. 18: 37; Poisson, 1953, *in* Traité de zoologie (P. P. Grassé, ed.), p. 1063; Kellen and Lipa, 1960, J. Insect Pathol. 2: 7; Thomson, 1960, J. Insect Pathol. 2: 360; Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 112; Weiser (in part), 1966, Nemoci hmyzu, p. 459; Chapman et al., 1966, J. Invertebr. Pathol. 8: 453; Anderson, 1968, J. Invertebr. Pathol. 11: 442; Kudo, 1971, Protozoology, p. 816; Khaliulin and Ivanov, 1971, Parazitologiya 5: 99.

Parathelohania opacita: Simmers, 1974b, Trans. Ill. State Acad. Sci. 67: 17; Simmers, 1974c, J. Parasitol. 60: 721.

Thelohania opacitor Fulton et al., 1974, Mosq. News 34: 89. New synonymy.

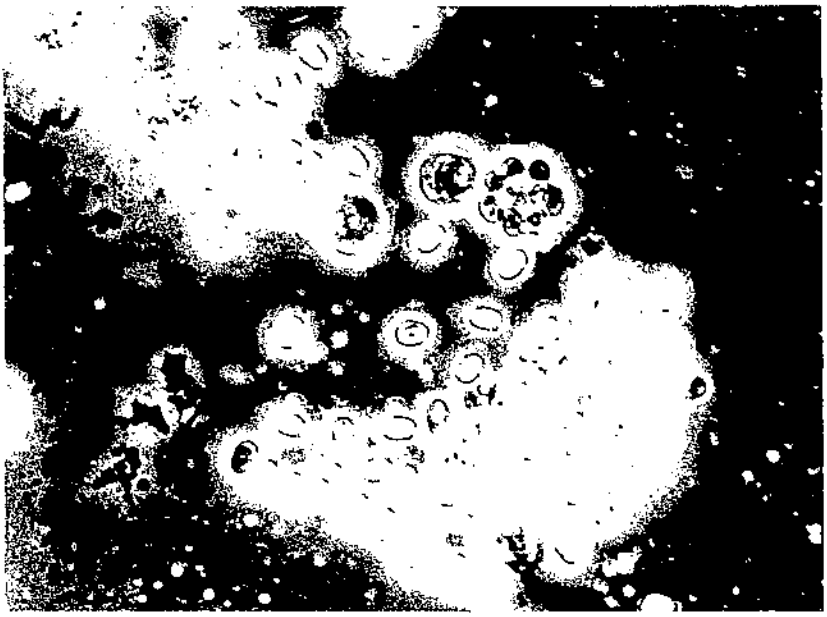
Thelohania sp. Chapman et al., 1967, Proc. N.J. Mosq. Exterm. Assoc. 54: 56; Chapman et al., 1969, Proc. N.J. Mosq. Exterm. Assoc. 56: 205.

Spore size.—Living octospores measure 5.5–6.0×3.5–4.0 μm and macrospores measure 8.0–8.5×4.5–5.5 μm (Kudo 1922); living octospores measure 5.83±0.09×4.19±0.03 μm (Chapman et al. 1966), 5.9±0.03×4.1±0.03 μm (Anderson 1968).

Host.—*Culex territans* Walker (=*Culex testaceus* of Kudo), a mosquito, collected in Connecticut, Florida, Georgia, Louisiana, and New York, U.S.A.

Infection site.—Adipose tissue of larvae.

We have not seen the transovarial stages or the free spores in adult females. The living octospore is oval, having the posterior end more broadly rounded than the anterior end (fig. 32), and has a



PN-4481

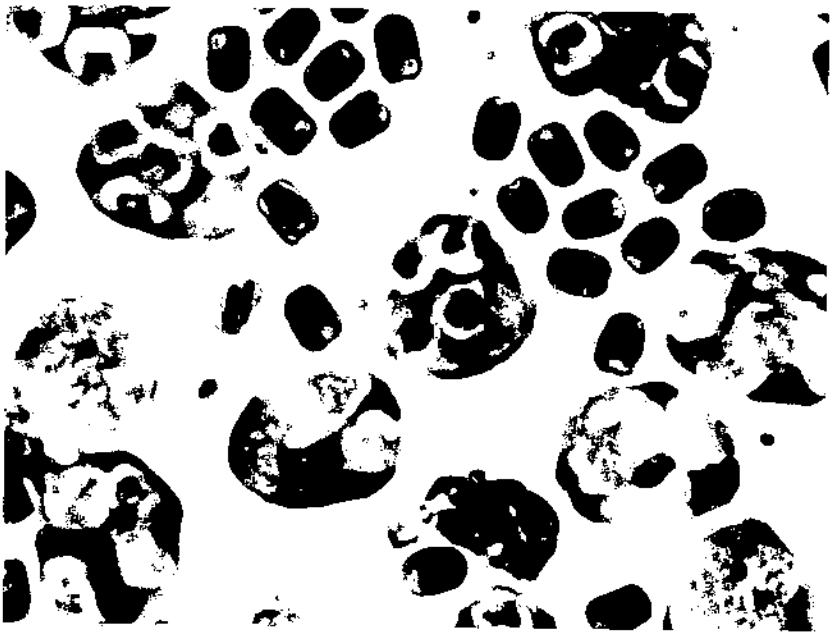
FIGURE 32.—Fresh octospores of *Amblyospora opacita* (Kudo). × 2,000.

thick mucous envelope. Octospores fixed in aqueous Bouin's fluid as wet smears and stained with Heidenhain's hematoxylin are truncate at their posterior ends, which are also somewhat invaginated (fig. 33). In these fixed and stained preparations the granules in the pansporoblasts are deeply colored by the stain, and the chromosomes are readily visible in the dividing sporonts (fig. 34).

The octospores differ from those of other species in having 8 coils of the polar filament, 3½ of which are formed by the broad basal portion (fig. 35). The pansporoblasts contain large clumps of dense granules (fig. 36). Most of these granules diminish during sporulation.

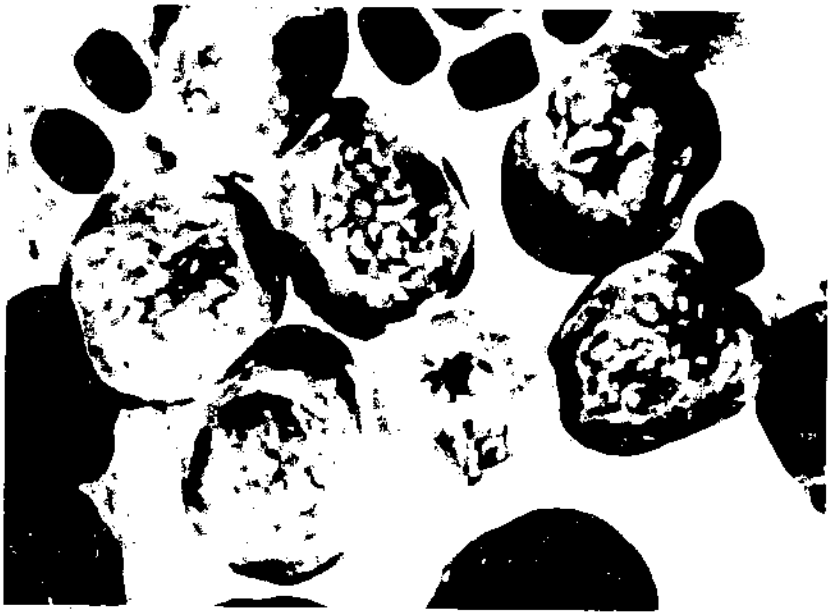
About one-half of male and female progeny are infected, and they succumb to infection during the last larval instar (Chapman et al. 1966).

Weiser (1966) reported *Aedes annulipes* (Meigen), *A. sticticus* (Meigen), *A. vexans* (Meigen), and *Culiseta annulata* (Schrank) as hosts of *A. opacita*. And Tour et al. (1971) reported the larvae of *Aedes caspius* (Pallas) and *A. detritus* (Haliday) as additional hosts of *Amblyospora opacita*. Weiser and Tour et al. obviously listed these additional hosts because diagnostic differences in octospores in host larvae are difficult, if not nearly impossible, to resolve under the light microscope. We believe the microsporidia in these hosts



PN 4482

FIGURE 33.—Heidenhain's-hematoxylin-stained sporonts and octospores of *A. opacita*. × 2,000.

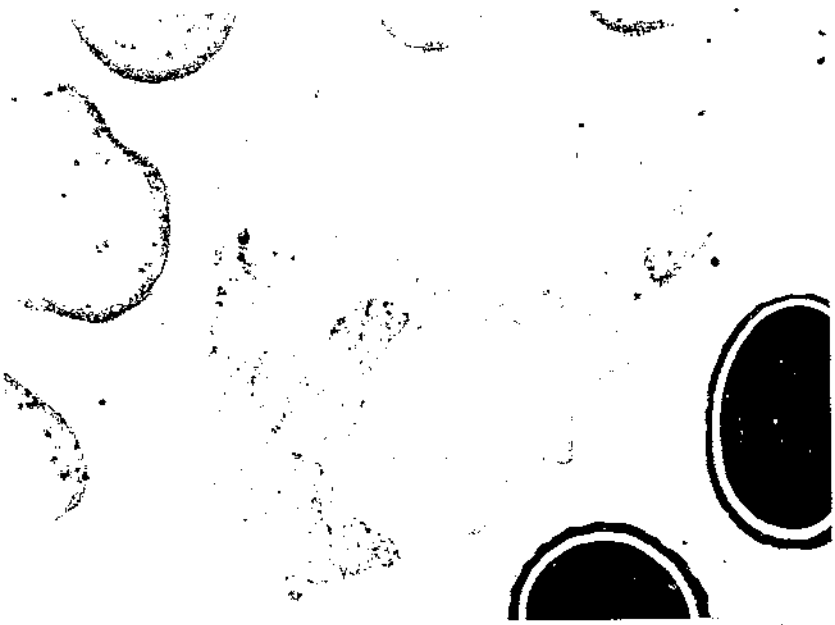


PN 4483

FIGURE 34.—Heidenhain's-hematoxylin-stained sporonts of *A. opacita*, showing chromosomes and nuclear division. × 2,000.

PN 4184

FIGURE 35 Ultrastructure of *A. opacita* octospore × 16,000



PN 4185

FIGURE 36 Pansporoblast of *A. opacita*, showing sporoblasts and metabolic granules.

represent new species of *Amblyospora*, and we are therefore reluctant to list them as hosts of *A. opacita* until the ultrastructure of these forms has been studied.

In his original description of *A. opacita*, Kudo (1922) reported its host as *Culex apicalis*, a mosquito restricted to the Southwestern United States. Kudo included an illustration of the host larvae in the original description that is morphologically similar to *Culex territans* Walker, which is a common mosquito in the eastern half of the United States. Later, Kudo (1924c) referred to the host as *Culex testaceus*, an invalid mosquito name.

Summers (1974b) lists *Culex pipiens pipiens* Linnaeus and *Culiseta inornata* (Williston) as additional hosts of *A. opacita*. We have already discussed the microsporidium in the latter host under *A. inimica*. We do not list *Culex pipiens pipiens* as a host of *A. opacita* until better evidence of microsporidian infectivity is obtained by electron microscopy studies.

Amblyospora trichostegiae (Baudoin, 1969) comb. n.

Thelohania trichostegiae Baudoin, 1969. Protistologica 5: 444.
Thelohania bicortex Baudoin (in part), 1969. Protistologica 5: 444. New synonymy.

Spore size.—Octospore measurements were not given by Baudoin; spores suspected of being free spores measure 8.5×4.0 μm.

Host.—*Trichostegia minor* (Curtis), a caddisfly, collected in France.

Infection site.—Adipose tissue of larvae.

Baudoin (1969) gives no specific information about the oval octospores found in *T. minor* since he assumed they were the same species as *A. bicortex* in *Phryganea grandis*. The coniform spore, described by Baudoin as *Thelohania trichostegiae*, is in our opinion the free spore of the developmental sequence in females since in form and structure it is similar to the free spores of *A. amphipodae* and *A. callosa*. Baudoin does not give descriptions of the sporonts that produce the coniform spores, nor does he mention if they are bound in groups of eight in a pansporoblastic membrane.

Baudoin's electron photomicrographs of coniform spores show ultrastructures similar to those of *A. amphipodae* and *A. callosa*. The spore appears to be binucleate, has a thin, smooth exospore, a large lamellated polaroplast, and a long polar filament (about 20 coils) of uniform diameter.

This microsporidium needs to be reexamined before its taxonomic placement is certain. However, we tentatively place it in *Amblyospora* since the smaller spores pictured by Baudoin are similar in

appearance to the octospores of *Amblyospora* and since the larger spores are ultrastructurally similar to what we believe are free spores in *A. callosa*.

Amblyospora unica (Kellen and Wills, 1962) comb. n.

Perkinsiana unica Kellen and Wills, 1962a, J. Insect Pathol. 4: 49; Kellen et al., 1965 J. Invertebr. Pathol. 7: 161; Weiser, 1966, Nemoci hmyzu, p. 460.

Spore size.—Living octospores measure 6.53 ± 0.12 × 4.97 ± 0.08 μm; preserved octospores measure 5.63 ± 0.07 × 4.42 ± 0.06 μm.

Host.—*Aedes melanimon* Dyar, a mosquito, collected in California, U.S.A.

Infection site.—Oenocytes and adipose tissue of male and female larvae (Kellen et al. 1965).

Nothing is known concerning the stages in adult females or the ultrastructure of the sporonts and octospores in larvae. About one-half of the progeny larvae acquire patent infections and succumb during their last stadium. We have seen Giemsa-stained smears of this species, but since it is difficult to determine spore structure in such preparations, we tentatively place it here until additional information concerning its ultrastructure becomes available.

Other Hosts of Undescribed *Amblyospora* Species

Aedes abserratus (Felt and Young)
  Thelohania nr. *opacita* Anderson, 1968
Aedes annulipes (Meigen)
  Thelohania opacita: Weiser 1961, 1963c, 1966
Aedes cantator (Coquillett)
  Thelohania nr. *opacita* Anderson, 1968; Chapman et al., 1973
Aedes caspius (Pallas)
  Thelohania opacita: Tour et al., 1971
Aedes cataphylla Dyar
  Thelohania sp. Kellen et al., 1965; Chapman et al., 1973
Aedes cinereus (Meigen)
  Thelohania nr. *opacita* Anderson, 1968
  Thelohania sp. Chapman, 1966
Aedes detritus (Haliday)
  Thelohania opacita: Tour et al., 1971
Aedes dorsalis (Meigen)
  Thelohania sp. Kellen et al., 1965; Chapman, 1966; Tsai et al., 1969
Aedes excrucians (Walker)
  Thelohania nr. *opacita* Anderson, 1968

Thelohania sp. Chapman et al., 1973
Aedes fitchii (Felt and Young)
Thelohania sp. Chapman et al., 1973
Aedes grossbecki Dyar and Knab
Thelohania sp. Chapman et al., 1966; Chapman et al., 1967
Aedes hexodontus Dyar
Thelohania sp. Kellen et al., 1965; Chapman et al., 1973
Aedes increpitus Dyar
Thelohania sp. Kellen et al., 1965
Aedes pullatus (Coquillett)
Thelohania sp. Chapman et al., 1973
Aedes punctor (Kirby)
Thelohania sp. Chapman et al., 1973
Aedes riparius Dyar and Knab
Thelohania sp. Chapman et al., 1973
Aedes sollicitans (Walker)
Thelohania sp. Kellen et al., 1966a, Chapman et al., 1966; Chapman et al., 1967; Chapman et al., 1969
Aedes stictieus (Meigen)
Thelohania opacita: Weiser 1963c, 1966
Thelohania sp. Chapman et al., 1966; Chapman et al., 1967; Chapman et al., 1969
Aedes stimulans (Walker)
Thelohania nr. *opacita* Anderson, 1968
Thelohania sp. Franz and Hagmann, 1962
Aedes taeniorhynchus (Wiedemann)
Thelohania sp. Kellen et al., 1966a; Chapman et al., 1966; Chapman et al., 1967; Chapman et al., 1969
Aedes ventrovittis Dyar
Thelohania sp. Kellen et al., 1965
Aedes vexans (Meigen)
Thelohania opacita: Weiser 1961, 1963c, 1966
Coquillettidia perturbans (Walker) [=*Mansonia perturbans*]
Stempellia sp. Chapman et al., 1967
Culex annulirostris Skuse
Thelohania opacita: Laird, 1956
Culex peccator Dyar and Knab
Thelohania sp. Chapman et al., 1969
Culex peus Speiser
Thelohania sp. Kellen et al., 1965
Culex salinarius Coquillett
Thelohania sp. Kellen et al., 1966a; Chapman et al., 1966; Chapman et al., 1967; Chapman et al., 1969
Culiseta annulata (Schrank)

Thelohania opacita: Weiser 1961, 1963c, 1966
Thelohania sp. Bresslau and Buschkiel, 1919
Culiseta impatiens (Walker)
Thelohania sp. Tsai et al., 1969
Culiseta particeps (Adams)
Thelohania sp. Kellen et al., 1965
Mansonia dyari Belkin, Heinemann and Page
Amblyospora sp. Hazard and Oldacre, hoc loco
Mansonia lebeti Borcham
Amblyospora sp. Hazard and Oldacre, hoc loco
Psorophora columbiae (Dyar and Knab)
Thelohania sp. Chapman et al., 1966; Chapman et al., 1967; Chapman et al., 1969

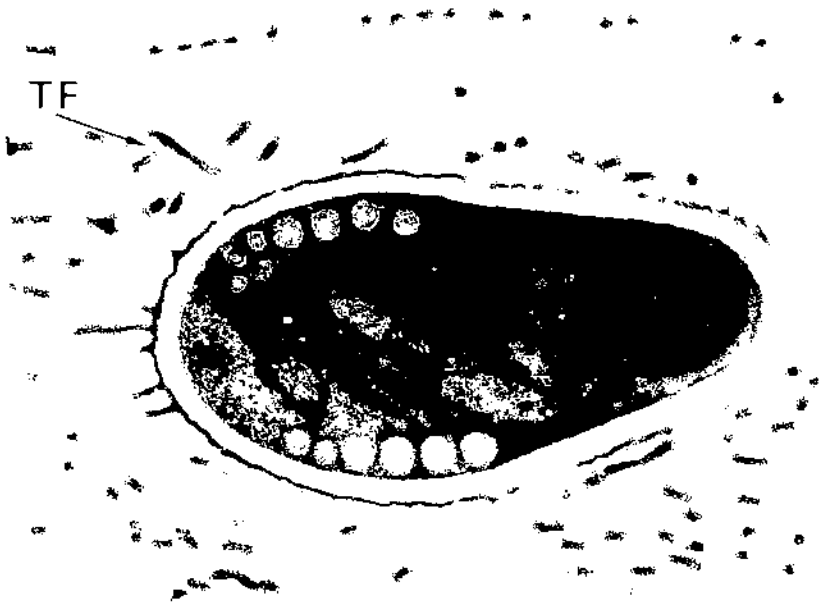
CHAPMANIUM gen. n.

Adult female hosts have not been examined, so we do not know if the species of this genus have sporonts producing free spores and developmental stages transmitting infection to progeny via the ovaries.

The pansporoblasts are fusiform, and each contains small pyriform octospores (fig. 37). The sporonts divide by budding (fig.

PX 1186

FIGURE 37 Fresh pansporoblasts of *Chapmanium cirritus* sp. n. × 2,000



PN-1187

FIGURE 38.—Ultrastructure of *C. cirritus* octospore. × 30,750. TF, threadlike filaments.

39). The pansporoblastic membrane is persistent, so much so that it is difficult to free the spores from it even long after the pansporoblasts have been removed from the host tissue. The octospores have a polar filament consisting of a broad basal portion abruptly constricting near the middle to form a narrow distal portion, and a lamellated polaroplast (fig. 38).

Little is known about the host-parasite relationships of these species, only that they cause death of their hosts. The genus is named in honor of Harold C. Chapman, who has contributed much to our knowledge of Microsporida. The type species is *C. cirritus*.

Chapmanium cirritus sp. n.

Thelohania sp. Chapman et al., 1969, Proc. N.J. Mosq. Exterm. Assoc. 56: 204.

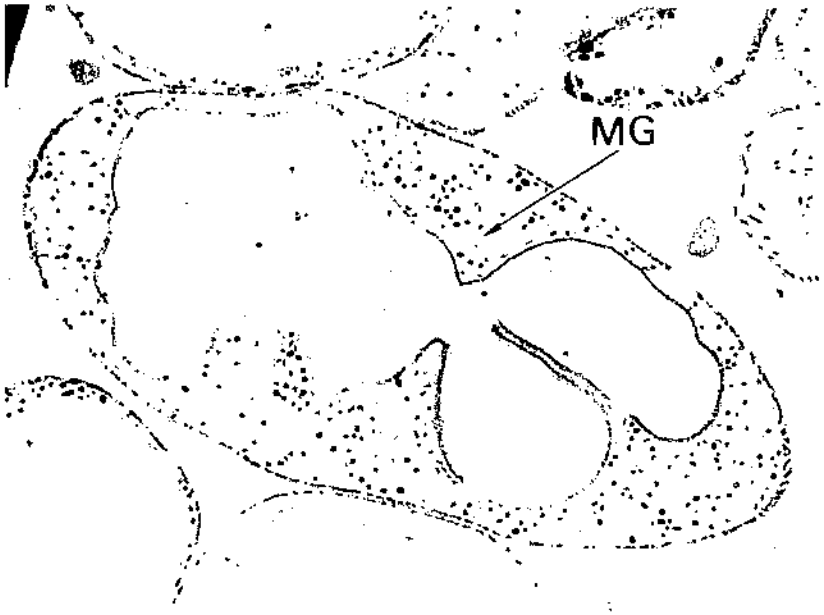
Spore size.—Living octospores measure 2.92–3.80 × 1.33–1.91 μm.

Host.—*Corethrella brakeleyi* (Coquillett), a phantom midge.

Infection site.—Adipose tissue of larvae.

Holotype.—Near Lake Charles, La., U.S.A., Feb. 22, 1974, USNM No. 24387 (Chapman and Glenn).

Paratype.—USNM No. 24388 (Chapman and Glenn).



PN 4188

FIG. 39.—Dividing sporont in *C. cirritus* pansporoblast. × 7,500. MG, metabolic granules.

The pansporoblastic membrane is persistent, so much so that it is difficult to free the spores from it even when much pressure is applied to the glass coverslip in fresh smear preparations. The pansporoblast is fusiform, somewhat resembling a pod (fig. 37).

The octospore has a thin exospore, a polar filament that abruptly constricts near its middle to form 6½ coils inside the spore (4½ coils formed by the broad basal portion), and a lamellated polaroplast (fig. 38). The sporont of the pansporoblast divides by endogenous budding (fig. 39). The fusiform pansporoblast contains many dense filaments that attach both to the surface of the spores and to the inner surface of the pansporoblastic membrane (fig. 40). The latter attachment probably explains why the spores are extremely difficult to free from the pansporoblast.

The Latin name *cirritus* means "having many threads" and refers to the numerous filaments in the pansporoblast.

Chapmanium macrocystis (Gurley, 1893) comb. n.

Pleistophora macrocystis Gurley, 1893, Bull. U.S. Fish Comm. for 1891, 11: 410; Garman, 1894, Rep. U.S. Fish Comm. 20: 196; Thelohan, 1895, Bull. Sci. Fr. Belg. 26: 362; Labbé, 1899, Sporozoa, *in* Das Tierreich (O. Butschli, ed.), p. 112; Auerbach, 1910, Die Cnidosporidien, p. 196; Kudo, 1924c, Ill. Biol. Monogr. 9(2-3): 136; Weiser, 1947, Ve. Moravské Přír. Spol. 18: 48; Sprague, 1965, J. Protozool. 12: 66; Sprague,

1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sprague and Couch, 1971, J. Protozool. 18: 530.

Sarcosporida Garbini, 1891, Rend. R. Accad. Lincei 7: 151, 152, and figs.

Sarcosporidae Henneguy and Thelohan, 1892a, C.R. Soc. Biol. 4: 586.

Spore size.—Measurements not given by Gurley.

Host.—*Palaemonetes varians* (Leach), a shrimp, collected from the Mincio near Verona, Italy.

Infection site.—Musculature.

The pansporoblasts are elongate and fusiform. Gurley reports the spores as having striated outer surfaces.

This species has not been studied since it was originally described by Gurley; therefore, nothing is known concerning its ultrastructure. Kudo (1924c) considered it a questionable species, and since Gurley's description is incomplete, we tentatively place it here because of the characteristic fusiform shape of the pansporoblast.

Chapmanium nepae (Lipa, 1966) comb. n.

Thelohania nepae Lipa, 1966, J. Invertebr. Pathol. 8: 163.

PN-4489

FIGURE 40.—Sporoblasts in *C. carritus* pansporoblast. × 6,750. TF, threadlike filaments.

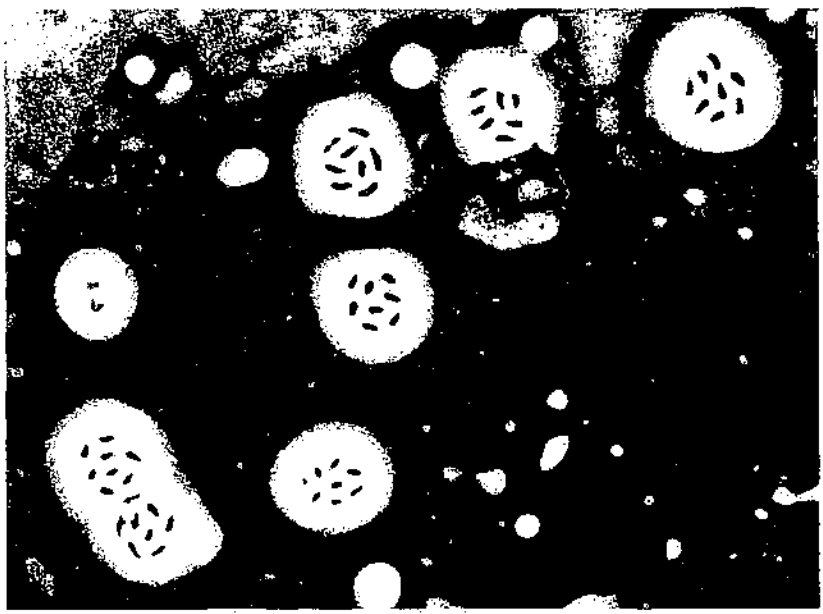
Spore size.—Preserved octospores measure 2.0–3.0 × 1.4–1.8 μm.
Host.—*Nepa cinerea* Linnaeus, a hemipteran, collected in Poland.
Infection site.—Adipose tissue of the adult insect.

We tentatively place this species in the genus *Chapmanium* because it has fusiform pansporoblasts, pending information concerning its ultrastructure. Weiser (1961) described another species, *Thelohania veliae*, from an aquatic hemipteran, *Velia currens* Fabricius, the authorship of which he credited to Poisson (1928). The date is incorrect, since he gave the name to a part of the sporogony of *Nosema veliae*, which was described by Poisson in 1929. Therefore, we consider the name *Thelohania veliae* Weiser, 1961, to be a synonym of *Nosema veliae* Poisson, 1929.

CRYPTOSPORINA gen. n.

Female hosts have not been examined; therefore, we do not know whether or not these microsporidia have a second sporogonic sequence resulting in transovarial infection of progeny and free spores.

The pansporoblasts are oval and contain small, pyriform octospores (fig. 41). These pansporoblasts also contain many dark, amber-colored, crystalliform particles that obscure the spores from



PN 4190

FIG. 41.—Fresh octospores of *Cryptosporina brachyfila* sp. n. × 2,000.

view in fresh preparations. Because of this, the pansporoblasts superficially resemble the sporangia of certain fungi. Once the pansporoblasts dry, they lose their color and refractiveness, making the spores visible. The pansporoblastic membrane is very persistent, remaining intact after the pansporoblasts have been removed from the body of the host. The octospore has a short polar filament of uniform diameter and a granular, structured polaroplast consisting of only a few lamellae near the polar cap (fig. 43). The spores have a somewhat wrinkled outer surface, the vacuole is not visible in fresh spores, and the spores do not have a mucous envelope.

Little is known concerning host-parasite relationships, but from the extent of the pathology observed in the hosts, we assume that these parasites eventually cause death. The name *Cryptosporina* means "hidden small spores." The type species, by monotypy, is *C. brachyfila*.

Cryptosporina brachyfila sp. n.

Spore size. Bouin's-fixed octospores average 1.86×0.71 μm, with a size range of 1.80–1.91×0.53–0.80 μm.

Host. *Piona* sp., a water mite.

Infection site. Adipose tissue.

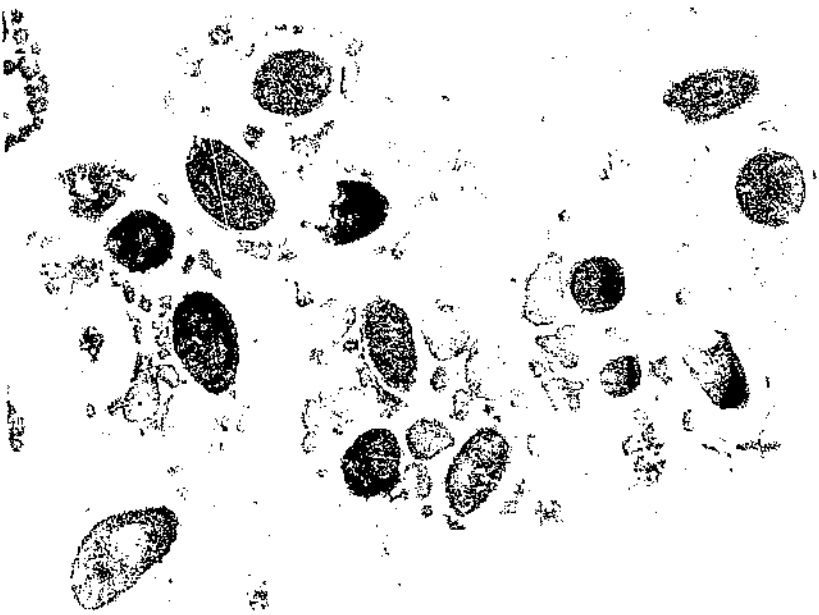
Holotype. West swamp in the Payne's Prairie on State Road 121 near Gainesville, Fla., U.S.A., April 1972. USNM No. 24389 (Federici and Hazard).

Paratype. USNM No. 24390 (Federici and Hazard).

The octospores are not visible through the pansporoblastic membrane until they have been dried or fixed and stained, and then the spores are not readily visible (fig. 41). Both fresh and fixed spores are pyriform, and they are not covered by a mucous envelope. No early sporogenic stages were observed as most of these stages had completed sporulation at the time the host was examined.

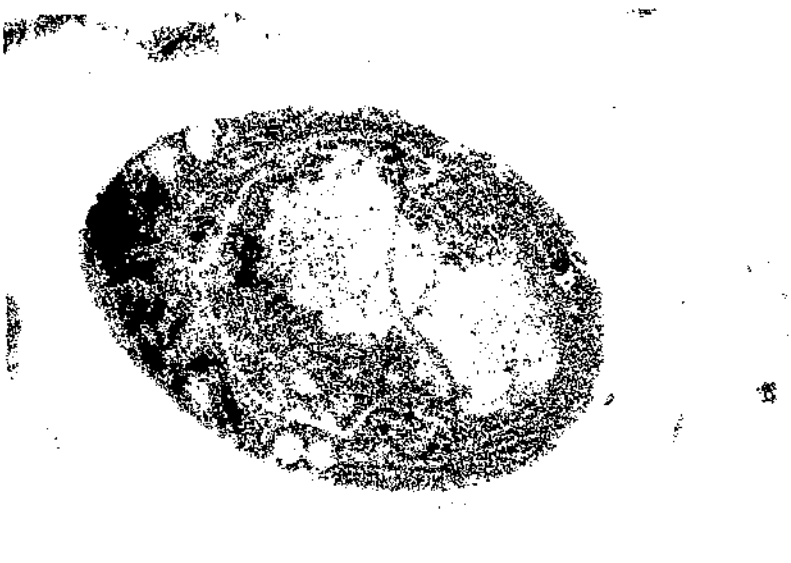
In electron photomicrographs the pansporoblastic membrane is seen to enclose eight uninucleate thin-walled spores and large crystalliform particles (fig. 41), the latter rendering the octonucleate sporonts dense and opaque. The outer surface of the spore wall is rugose. The polar filament is short, making no more than 3 coils in the posterior end of the spore, and is of uniform diameter throughout its length (fig. 43). The polaroplast is mostly granular in structure except near the polar cap, where it is lamellated.

Only octospores have been observed. Females, possibly carrying the infection via the ovaries, were not examined. The species name *brachyfila* means "short thread," referred to the short polar filament.



PN 4491

FIG. 42. Pansporoblasts of *C. brachyfila* containing large crystalliform particles and octospores. × 7,500.



PN 4492

FIG. 43. Ultrastructure of *C. brachyfila* octospore. × 39,000.

HYALINOCYSTA gen. n.

We do not know whether or not these species are dimorphic since we have not examined adult female hosts for a second sporogonic sequence possibly producing free spores in adult females and infections in their progeny via the ovaries.

The pansporoblasts are oval and contain pyriform octospores (fig. 44). The pansporoblastic membrane is thickened and somewhat persistent, holding the octospores together for a short time after the pansporoblasts have been removed from the body of the host. The pansporoblasts containing mature spores are completely void of crystalliform particles, granules, and microtubules; therefore, the octospores lie on a clear background making them sharply visible in both fresh and stained smears (figs. 45 and 46). Fresh spores are thinly covered by a mucous envelope difficult to resolve in dilute india ink. The polar filament has a broad basal portion abruptly constricting near its middle to form a narrow distal portion, and the wall of the spore is smooth and without surface structure (fig. 47).

These microsporidia are known only from mosquitoes and cause death of their larval hosts. The name *Hyalinocysta* means "clear bag," referring to the granular and microtubule-free pansporoblasts. The type is *H. chapmani* by monotypy.

Hyalinocysta chapmani sp. n.

Spore size.—Preserved octospores measure 4.34–4.76×2.38–2.80 μm, the average being 4.41×2.62 μm.

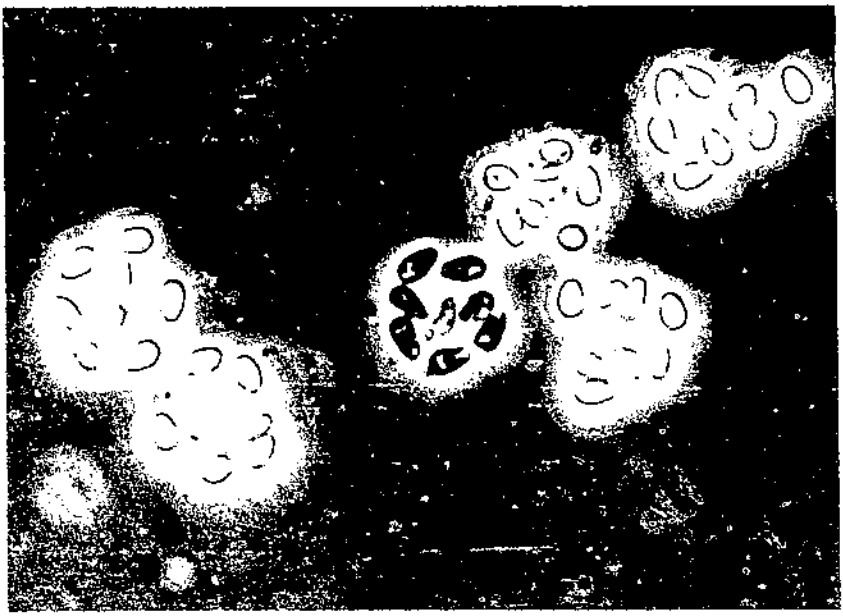
Host.—*Culiseta melanura* Coquillett, a mosquito.

Holotype.—Near Kinder, La., U.S.A., Dec. 27, 1971, USNM No. 24391 (Chapman).

Paratypes.—USNM No. 24392; World Health Organization Reference Center, Ohio State University, Accession No. 1667 (Chapman).

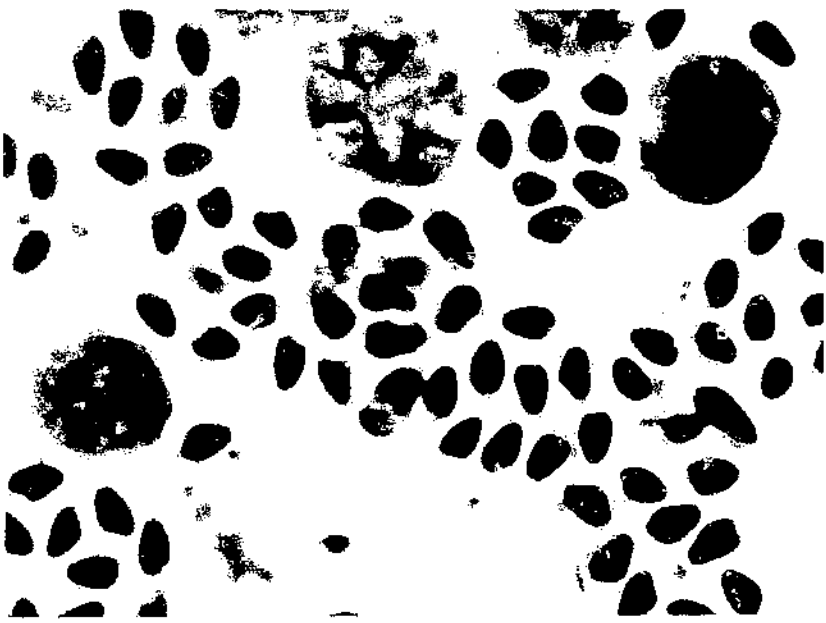
Living and preserved octospores are pyriform (fig. 44), have a large prominent vacuole, and are covered by a mucous envelope. Pansporoblasts containing mature octospores are void of granules, tubules, or crystalliform particles, providing for easy observation of spores in both fresh and preserved preparations (fig 45). Some granules can be seen, however, in stained preparations of pansporoblasts containing young sporonts.

Pansporoblasts, especially those containing mature spores, are void of metabolic products (fig. 46). The polar filament of octospores consists of 7 or 8 coils, 3½ being formed by the much narrower distal portion (fig. 47). Both the endospore and exospore are thin, the latter having a relatively smooth outer surface in mature spores. The



PN-4493

FIGURE 44.—Fresh octospores of *Hyalinocysta chapmani* sp. n. × 1,100.



PN 4494

FIGURE 45.—Heidenhain's-hematoxylin-stained octospores of *H. chapmani*. × 2,000.

PN 4195

FIGURE 16.—Pansporoblast of *H. chapmani* free of metabolic products. × 12,000.

PN 4196

FIGURE 17.—Ultrastructure of *H. chapmani* octospore. × 32,750.

polaroplast is composed of tightly compressed lamellae in the anterior half and widely separated lamellae forming cell-like areas in the posterior half.

This species is named in honor of Harold C. Chapman, who found it in Louisiana. We have seen one other species which, when described, will be placed in this genus.

INODOSPORUS Overstreet and Weidner, 1974

Inodosporus Overstreet and Weidner, 1974, Z. Parasitenkd. 44: 171

These microsporidia are known to have only one sporogonic sequence in their hosts, producing octospores.

The pansporoblasts are oval and contain pyriform octospores. The pansporoblastic membrane is very persistent, remaining intact around the spores long after they have been removed from the body of the host. The octospores have four or five long appendages, have an otherwise thin exposure, a polar filament of nearly uniform diameter, and a distinct lamellated polaroplast.

Although we have not seen species of this genus and know nothing of their host-parasite relationships, we are led to believe they cause death of their hosts. The type species is *I. sprague*i Overstreet and Weidner, 1974, by original designation.

Inodosporus octosporus (Henneguy, 1892) Overstreet and Weidner, 1974

Pleistophora octospora Henneguy, *in* Henneguy and Thelohan, 1892b, Ann. Microgr. 4: 639; Henneguy, *in* Thelohan, 1892, Bull. Soc. Philomath. Paris 4: 174; Gurley, 1893, Bull. U.S. Fish Comm. for 1891, 11: 410; Gurley, 1894, Rep. U.S. Fish. Comm. 26: 197; Thelohan, 1895, Bull. Sci. Fr. Belg. 26: 361; Pfeiffer, 1895, Die Protozoen als Krankheitserreger, p. 72; Leger and Hagenmuller, 1897, C. R. Assoc. Fr. Av. Sci. 26: 553; Labbe, 1899, Sporozoa, *in* Das Tierreich (O. Butschli, ed.), p. 112; Stempell, 1909, Arch. Protistenkd. 16: 340; Pixell-Goodrich, 1920, Arch. Zool. Exp. Gen., Notes Rev. 59: 17, 19; Kudo, 1924c, Ill. Biol. Monogr. 9(2-3): 134; Weiser, 1947, Pr. Moravské Přír. Spol. 18: 27; Poisson, 1953, *in* Traite de zoologie (P. P. Grasse, ed.), p. 1063; Sprague, 1965, J. Protozool. 12: 66; Codreanu, 1966, Proc. Int. Congr. Parasitol., 1st, 1964: 602; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 424; Sprague and Couch, 1971, J. Protozool. 18: 530.

Inodosporus octospora (Henneguy, 1892) Overstreet and Weidner, 1974, Z. Parasitenkd. 44: 173

Spore size.—Octospores measure 2-3 μm (Henneguy and Thélohan 1892b); 3-4 μm (Gurley 1894); 2.8 × 1.9 μm (Codreanu 1966).

Host.—*Palaemon adspersus* Rathke (=*Palaemon rectirostris* Zaddach) and *P. serratus* (Pennant), a shrimp, collected in France and England (Henneguy and Thélohan 1892b; Gurley 1894; and Pixell-

Goodrich 1920); and *Palaemon elegans* Rathke, a shrimp, collected from the Black Sea in Romania (Codreanu 1966).

Infection site.—Musculature.

Nothing is known of the ultrastructure of this species. Pixell-Goodrich (1920), examining octospores from *Palaemon serratus*, discovered that the spores have three posterior tails. Codreanu (1966) reported the same number of tails on the spores taken from another host species, *Palaemon elegans*. We wonder how many acicular appendages are on spores of the microsporidium from *P. adspersus* and, more importantly, how much difference there may be in the ultrastructure of the spores from these three forms since Overstreet and Weidner (1974) place taxonomic importance on the number and arrangement of appendages.

Inodosporus spraguei Overstreet and Weidner, 1974

Inodosporus spraguei Overstreet and Weidner, 1974, Z. Parasitenkd. 44: 171.

Spore size.—Living octospores measure 2.0–3.7× 1.7–2.5 μm and average 2.9×2.0 μm.

Host.—*Palaemonetes pugio* Holthius, the grass shrimp, collected in Mississippi, U.S.A., and *P. kadiakensis* Rathbun, collected in Texas, U.S.A.

Infection site.—Abdominal musculature.

Electron photomicrographs (Overstreet and Weidner 1974) show the spores as having a thin and externally smooth exospore (except in the areas where the appendages are formed) and a polar filament of nearly uniform thickness from base to apex. The appendages, three at the posterior end and one, usually branched, at the anterior end, are extensions of the exospore.

PARATHELOHANIA Codreanu, 1966

Parathelohania Codreanu, 1966, Proc. Int. Congr. Parasitol., 1st, 1964: 602.

These microsporidia have two developmental sequences in their hosts. One sporogonic sequence, producing octospores, is found in one-half of larval progeny and produces patent infections only in male larvae or in both male and female larvae, depending on the species. The other sporogonic sequence, producing free spores, is found in female larvae not having patent infections of the first sequence. In this sequence the sporonts form plasmodia (having 8 to 40 nuclei) in oenocytes that sporulate in adult females to form spores not enclosed in a pansporoblastic membrane. This sequence produces infections in the progeny of these females via the ovaries.

The fresh octospore of many species has a characteristic internal bottle-shaped structure when viewed through the phase-contrast microscope (figs. 51, 59, and 63). Also, the fresh octospore is not covered by a mucous envelope, nor does the fresh or preserved spore usually have a visible vacuole. Octospores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin have constricted posterior ends (figs. 48, 52, 58, 64, and 68). This constriction, as well as the deeply ridged surface structure, can be discerned in scanning electron photomicrographs (figs. 49, 53, and 66). The octospore has a thick exospore, a polar filament usually abruptly constricting near the middle to form a thin distal portion, and a prominent lamellated polaroplast (fig. 50).

The pansporoblasts are oval and contain oval octospores. The pansporoblastic membrane remains intact only for a short time after the pansporoblasts are removed from the body of the host. The sporonts secrete metabolic products that form granules, these often clumping to form large dense masses (fig. 55). Microtubules are not usually formed during sporulation.

Fresh free spores are elongate oval to cylindrical and have a large dark vacuole. They appear to have a smooth, delicate spore wall, two nuclei, and a polar filament of uniform diameter from base to apex (fig. 56).

PN 1097

FIG. 48.—Heidenhain's-hematoxylin-stained octospores of *Parathelohania africana* Hazard and Anthony. × 2,000.

PN 4498

FIGURE 49.—Surface ultrastructure of *P. africana* octospores, × 7,500.

PN 4199

FIGURE 50.—Internal ultrastructure of *P. africana* octospore, × 30,750.

The sporonts producing free spores lie dormant as uninucleate stages in the hemolymph of young female larvae invading oenocytes of the last stadium, where they undergo many nuclear divisions and become large plasmodia (fig. 57). Sporonts producing free spores do not have a pansporoblastic membrane and do not secrete metabolic products. Sporulation of these large plasmodia usually begins shortly after the first blood feeding, producing numerous free spores in the ovaries or oenocytes of adult females.

This genus represents a large number of species found primarily in anopheline mosquitoes. However, two species have been found in the mosquito genera *Aedeomyia* and *Aedes*. All apparently are transmitted to their hosts via the ovaries of infected females. The type species is *P. legeri* (Hesse) as originally designated by Codreanu (1966).

Parathelohania africana Hazard and Anthony, 1974

Parathelohania africana Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 30.

Duboscqia legeri Fantham et al. (in part), 1941, Parasitol. 33: 202. New synonymy.

Spore size.—Living octospores from male larvae measure 3.7×2.3 μm; living free spores from adult females measure 4.8×2.0 μm.

Host.—*Anopheles gambiae* Giles, a mosquito, collected in Nigeria and Zululand, Africa.

Infection site.—Adipose tissue of larvae; oenocytes and ovaries of adult females.

Holotype.—Kaduna, Nigeria, September 1970, USNM No. 24393 (Hazard).

Paratypes.—USNM No. 24394; World Health Organization Reference Center, Ohio State University, Accession No. 1661 (Hazard).

Scanning electron photomicrographs show the surface of octospores to be wrinkled and not as noticeably constricted at their posterior ends as are the octospores of other species (fig. 49). Transmission electron photomicrographs show the polar filament to have 5 to 6 coils inside the spore, usually 2 of these coils formed by the broad basal end (fig. 50). Nothing is known of the ultrastructure of free spores.

Patently infected male larvae succumb in the last instar. The free spores develop in blood cells surrounding the ovarioles, are elongate and slightly bent or curved, and narrow to nearly a point at one end.

Parathelohania anomala (Sen, 1941) Hazard and Anthony, 1974

Thelohania anomala Sen, 1941, J. Malar. Inst. India 1: 258; Thomson, 1960, J. Insect Pathol. 2: 356.

Perezia legeri Kudo (in part), 1929, Arch. Protistenkd. 67: 2; Sen (in part), 1941, J. Malar. Inst. India 1: 258; Tour et al. (in part), 1971, Ann. Parasitol. Hum. Comp. 46: 208. New synonymy.

Parathelohania anomala (Sen, 1941); Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 18.

Spore size.—Living free spores (?) from larvae measure 5.1–6.1 × 2.0–2.1 μm.

Host.—*Anopheles ramsayi* Covell, a mosquito, collected in India.

Infection site.—Adipose tissue of larvae.

Nothing is known of the ultrastructure of this species. We tentatively place it in this genus, as did Hazard and Anthony (1974). We do this believing that the sporonts and oblong spores described by Sen (1941) represent stages and free spores in females, which occasionally prematurely develop in the oenocytes among the adipose cells in female larvae. Sen's description is vague; therefore, we place it here with uncertainty.

Parathelohania anophelis (Kudo, 1924) Hazard and Anthony, 1974

Nosema anophelis Kudo, 1924c, Ill. Biol. Monogr. 9(2-3): 110; Weiser, 1947, Pr. Moravske Prir. Spol. 18: 37.

Perezia illinoisensis Kudo, 1922, J. Parasitol. 8: 74.

Perezia legeri Kudo (in part), 1924a, Arch. Protistenkd. 49: 147; Kudo (in part), 1924c, Ill. Biol. Monogr. 9(2-3): 143; Kudo (in part), 1925a, Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. I. Orig. 96: 431; Chapman et al. (in part), 1966, J. Invertebr. Pathol. 8: 453; Weiser, 1966, Nemoci hmyzu, p. 459; Hazard and Weiser, 1968, J. Protozool. 15: 818.

Nosema stegomyiae Fox and Weiser (in part), 1959, J. Parasitol. 45: 21; Thomson (in part), 1960, J. Insect Pathol. 2: 352; Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 108; Weiser (in part), 1966, Nemoci hmyzu, p. 455.

Parathelohania anophelis (Kudo, 1924); Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 10.

Spore size.—Living octospores in larvae measure 4.0–5.5×2.5–3.6 μm (Hazard and Weiser 1968), 5.18±0.04×3.16±0.02 μm (Chapman et al. 1966); living free spores measure 4.7–5.8×2.3–3.2 μm (Kudo 1925a), 4.5×2.2 μm (Hazard and Weiser 1968).

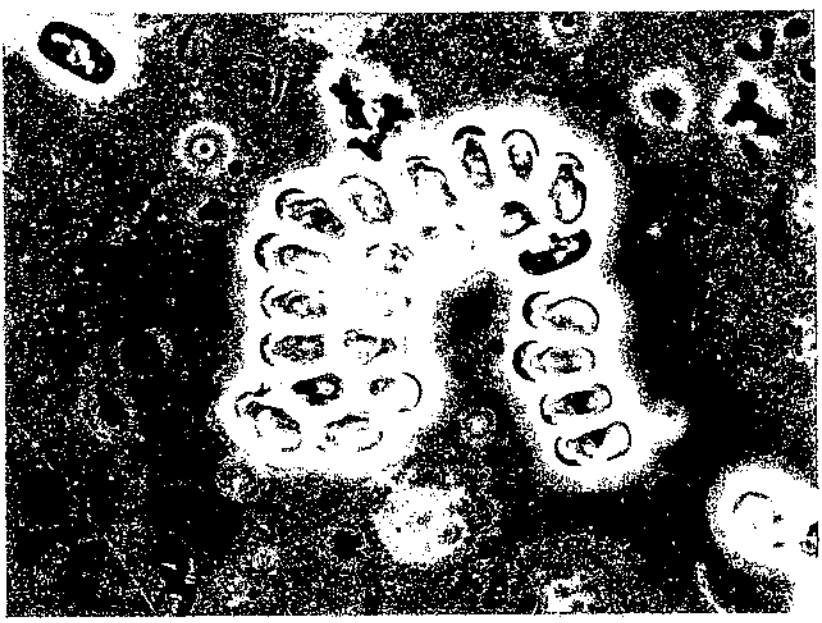
Host.—*Anopheles quadrimaculatus* Say, a mosquito, collected in Florida, Georgia, and Louisiana, U.S.A.

Infection site.—Hemocytes and adipose tissue of male larvae and the oenocytes and ovaries of females.

The living octospore has a bottle-shaped internal structure visible in phase-contrast microscopy (fig. 51). The vacuole is not visible in living octospores. The free spores, however, have a distinct dark vacuole, viewed under the phase-contrast microscope. Free spores are somewhat pyriform and are found in the ovaries.

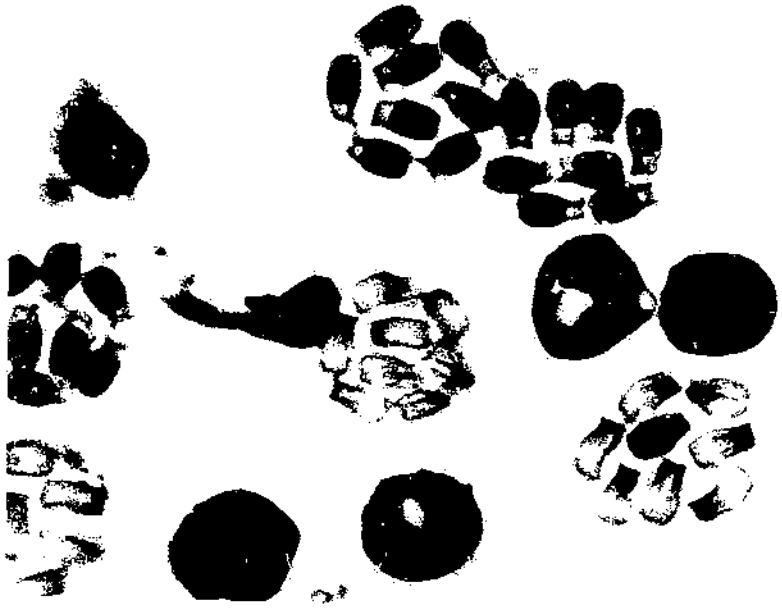
The octospore has four strong ridges on its constricted posterior end formed by the thick exospore of the spore wall (fig. 53). The polar filament makes 8 coils inside the spore, the first 3½ of these formed by the broad basal portion (fig. 54). The sporonts secrete granules that clump to form large dense masses (fig. 55). The ultrastructure of the free spore is much different from that of the octospore found in larvae. The free spore has a very thin and smooth exospore, a large empty vacuole, a short polar filament of uniform diameter, and a small indistinct polaroplast (fig. 56). This is the only species of *Parathelohania* in which the ultrastructure of the unbound spore has been studied.

All male larvae become patently infected in the last instar and succumb. All female larvae survive to become adults, which transmit the disease to their progeny via the ovaries. Uninucleate sporonts in female larvae lie dormant in the hemolymph until the last larval molt, at which time they invade oenocytes that have migrated to the ventral diverticula. Here they become closely appressed to it at the time the adult emerges from the pupal skin. Shortly after the



PN 4500

FIGURE 51 Fresh octospores of *Parathelohania anophelis* (Kudo). × 2,000.



PN 1501

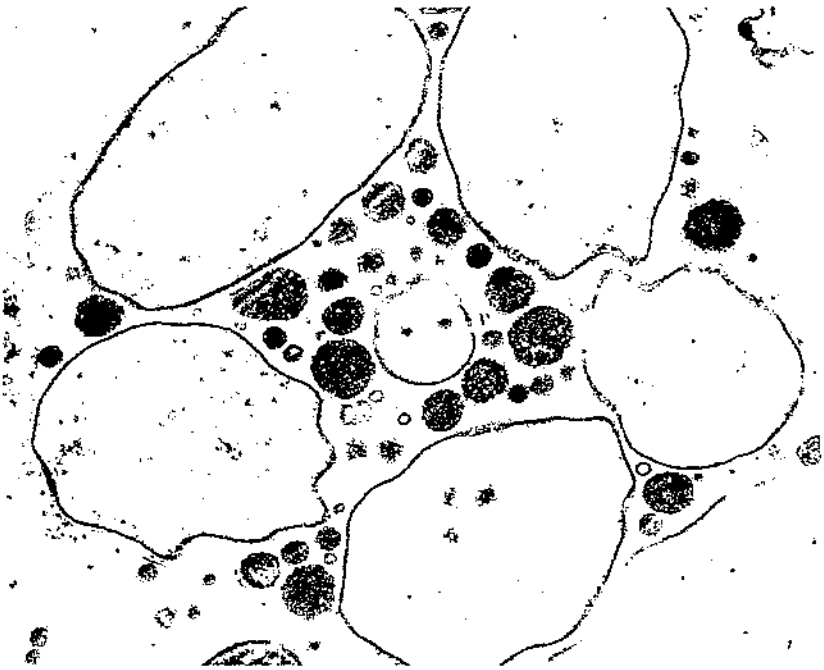
FIGURE 52.—Heidenhain's-hematoxylin-stained octospores of *P. anophelis*. × 2,000.

PN 4502

FIGURE 53.—Surface ultrastructure of *P. anophelis* octospores. × 9,600.

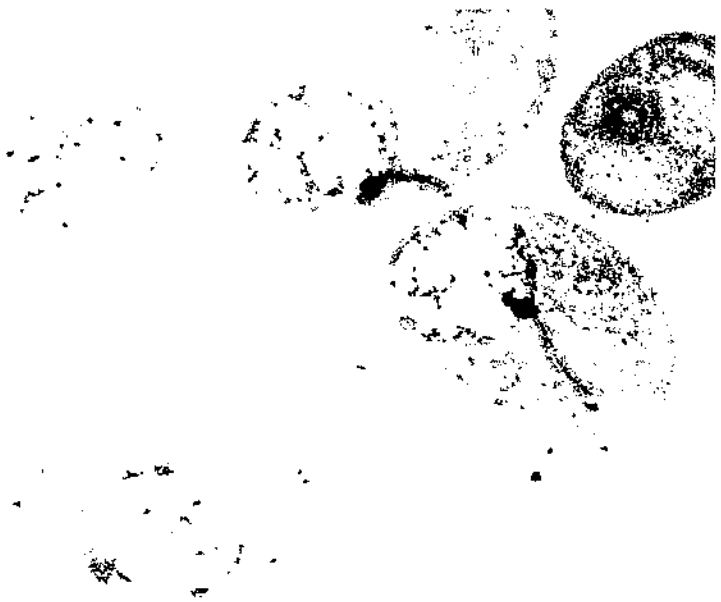
PX 1561

FIG. 54. Internal ultrastructure of *P. aroides* octospore. × 22,500



PX 1561

FIG. 55. Pansporoblast of *P. aroides*, showing sporoblasts and metabolic products. × 10,800



PN 1505

FIGURE 56.—Ultrastructure of *P. anophelis* free spores. × 12,150.

PN 4506

FIGURE 57.—Giemsa-stained sporonts of *P. anophelis* that give rise to free spores. × 2,000

female takes her first blood feeding, the multinucleate sporonts divide by cytoplasmic division, producing many binucleate sporonts (fig. 57), and the oenocytes break free of the ventral diverticula and migrate to the ovaries. These binucleate sporonts become sporoblasts and eventually free spores sometime during the transformation of ovarioles to eggs.

Parathelohania barra (Pillai, 1968) comb. n.

Thelohania barra Pillai, 1968, Z. Angew. Entomol. 62: 395.

Spore size.—Living octospores measure 3.77±0.34×2.63±0.28 μm; macrospores measure 5.73–7.80×3.64–5.09 μm.

Host.—*Aedes australis* (Erichson), a mosquito, collected in New Zealand.

Infection site.—Adipose tissue of larvae.

This species is polymorphic, having spores that vary greatly in size and shape, some being very large. It is the first *Parathelohania* species reported from a mosquito in a genus other than *Anopheles*. Nothing is known of the spore ultrastructure in larvae or of the sporogonic stages in adult female hosts.

Parathelohania chagrasensis sp. n.

Spore size.—Living octospores measure 3.07–3.92×2.12–2.44 μm.

Host.—*Aedeomyia squamipennis* (Lynch Arribálzaga), a mosquito.

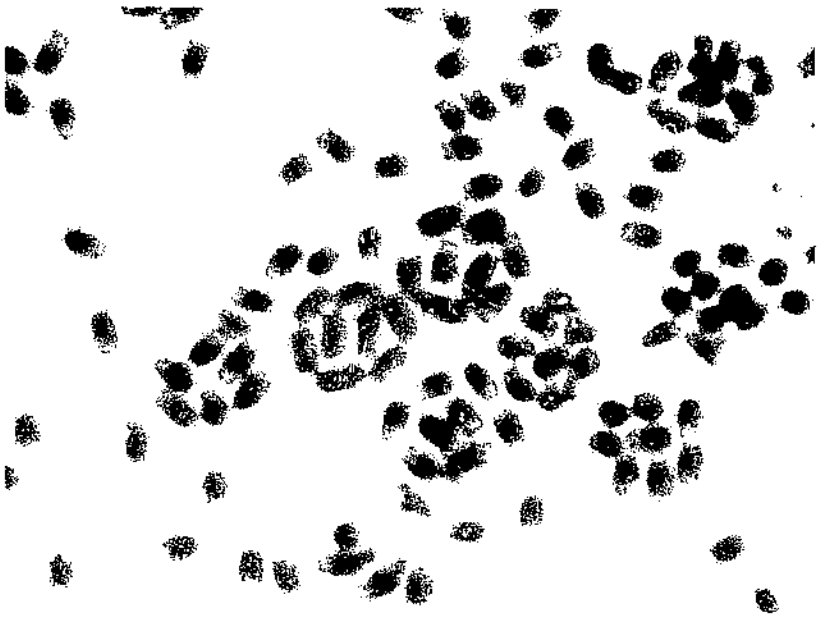
Infection site.—Adipose tissue of larvae.

Holotype.—Chagras River, Canal Zone, Mar. 6, 1973, USNM No. 24395 (Anthony, Hazard, and Keenan).

Paratypes.—USNM No. 24396; World Health Organization Reference Center, Ohio State University, Accession No. 1660 (Anthony, Hazard, and Keenan).

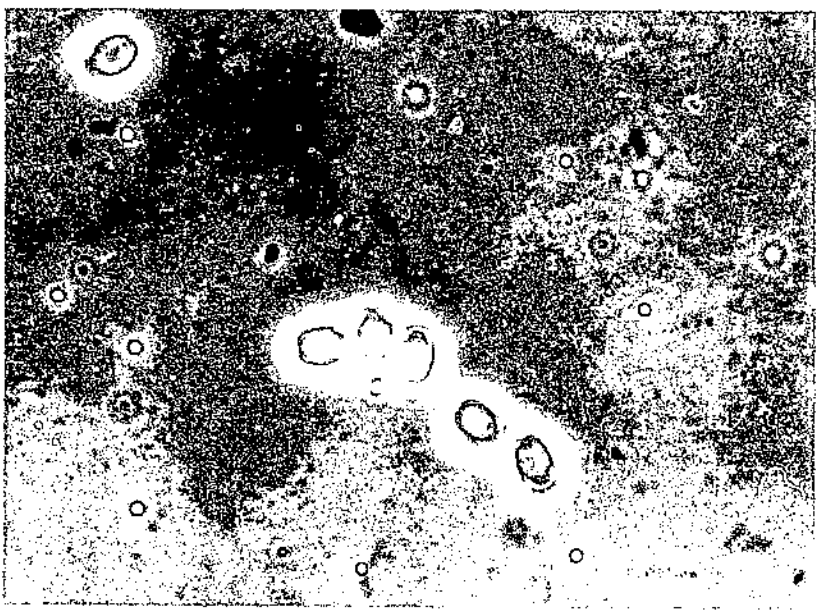
Octospores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin are similar in size and shape to the stained octospores of *P. africana* (fig. 58), and fresh octospores have a similar bottle-shaped internal structure (fig. 59).

The sporonts secrete granules that clump to form large dense masses within the pansporoblastic membrane (fig. 60). Tubular structures are also formed. These metabolic products disappear for the most part during sporulation, but remnants of the tubular structures remain even after the spores mature. The constricted posterior end of the octospore is short and looks similar to the spore of *P. africana*. Its polar filament is also similar to that of *P. africana*, hav-



PN 4507

FIGURE 58.—Heidenhain's-hematoxylin-stained octospores of *Parathelohania chagrasensis* sp. n. × 2,000.

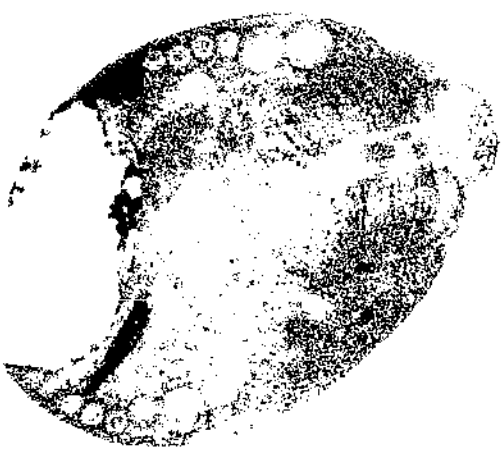


PN 4508

FIGURE 59.—Fresh octospores of *P. chagrasensis*. × 2,000.

PN 1509

FIGURE 60.—Dividing sporont and metabolic products in *P. chagrasensis* pansporoblast. × 12,000.



PN 1510

FIGURE 61.—Ultrastructure of *P. chagrasensis* octospore. × 33,300.

ing 6 coils, the first 2 formed by the broad basal portion (fig. 61). *P. chagrasensis* differs, however, by being more strongly constricted and having more prominent ridges at the posterior end.

Nothing is known of the sporonts and free spores in adult females or if disease is transmitted via the ovaries. All patently infected larvae die in the last stadium. The sex of the larvae having octospores was not determined. Nothing more is known about the host-parasite relationship of this species. We name this species *chagrasensis* after the Chagras River in the Canal Zone, where its host was collected.

Parathelohania illinoisensis (Kudo, 1921) Hazard and Anthony, 1974

Perezia illinoisensis Kudo, 1921b, J. Morphol. 35: 167.

Parathelohania illinoisensis (Kudo 1921) Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 16.

Perezia legeri: Kudo (in part), 1924a, Arch. Protistenkd. 49: 148; Kudo (in part), 1924c, Ill. Biol. Monogr. 9(2-3): 143; Kudo (in part), 1925a, Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. I. Orig. 96: 131; Missiroli (in part), 1929, Rev. Malariol. 8: 395; Fantham et al. (in part), 1941, Parasitology 33: 202; Weiser (in part), 1947, Pr. Moravske Prir. Spol. 18: 38; Poisson (in part), 1953, *in* Traité de zoologie, P. P. Grassé, ed., p. 1063; Thomson (in part), 1960, J. Insect Pathol. 2: 359; Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 113; Kudo, 1962, J. Insect Pathol. 4: 453; Chapman et al. (in part), 1966, J. Invertebr. Pathol. 8: 453; Anderson, 1968, J. Invertebr. Pathol. 11: 442; Tour et al. (in part), 1971, Ann. Parasitol. Hum. Comp. 46: 208.

Parathelohania legeri: Summers, 1974a, J. Invertebr. Pathol. 23: 402.

Spore size.—Living octorpores measure 4.8–6.0×3.0–4.0 μm (Kudo 1921b), 4.94±0.05×3.18±0.02 μm (Chapman et al. 1966); Giemsa-stained octospores measure 4.3±0.07×2.9±0.07 μm (Anderson 1968).

Host.—*Anopheles punctipennis* (Say), a mosquito, collected in Connecticut, Illinois, and Louisiana, U.S.A., and Quebec, Canada.

Infection site.—Hemocytes and adipose tissue of male larvae; oenocytes and ovaries of females.

The octospores of *P. illinoisensis* (fig. 62) are morphologically similar in size and shape to those of *P. anophelis* but differ in the number of coils of the polar filament, there being 6 coils in *P. illinoisensis* (2 formed by the broad basal portion) and 8 coils in *P. anophelis* (3½ formed by the broad basal portion).

We do not know whether or not sporulation of octospores occurs in both larval sexes or only in male larvae as it does in *P. anophelis*. Also, we do not know anything concerning a sporogonic sequence in surviving females.

PS 1511

FIGURE 62.—Ultrastructure of octospore of *Parathelohania illinoisensis* (Kudo). × 22,500

Simmers (1947a) has reported finding *Parathelohania legeri* (=*P. illinoisensis*) in a mosquito predator, *Psoraphora ciliata* (Fabricius). We hesitate to list it as a host of *P. illinoisensis* until a diagnostic comparison of the ultrastructure of the microsporidia in these two mosquitoes has been given, since he based his identification only on spore size.

Parathelohania indica (Kudo, 1929) Hazard and Anthony, 1974

Thelohania indica Kudo, 1929, Arch. Protistenkd. 67: 3; Sen, 1941, J. Malar. Inst. India 4: 258; Weiser, 1947, Pr. Moravské Přír. Spol. 18: 38; Thomson, 1960, J. Insect Pathol. 2: 359; Weiser, 1966, Nemoci hmyzu, p. 460; Tour et al. (in part), 1971, Ann. Parasitol. Hum. Comp. 46: 208.

Thelohania obesa Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 111.

Parathelohania indica (Kudo, 1929) Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 18.

Spore size.—Preserved octospores measure 4.0–5.2×2.4–2.8 μm.

Host.—*Anopheles hyrcanus* (Pallas), a mosquito, collected in India.

Infection site.—Adipose tissue of larvae.

Kudo (1929) gave little information concerning the structure and shape of the octospores of this species. Since all of the material he examined had been either fixed in "Carnoy-Lebrum" solution for paraffin sectioning or prepared as dried smears stained with Giemsa, it was not possible for him to see the characteristic structure of the spores. The senior author of this paper had an opportunity to examine many species of microsporidia-infected anopheline larvae collected in India some years ago, all of which were somewhat similar in structure to the previously listed species. We tentatively place this species in this genus, as did Hazard and Anthony (1974), based on our knowledge of the prevalence of *Parathelohania* in anopheline mosquitoes in India.

Parathelohania legeri (Hesse, 1904) Codreanu, 1966

Thelohania legeri Hesse, 1904a, C.R. Soc. Biol. 57: 570; Hesse, 1904b, C.R. Soc. Biol. 57: 571; Auerbach, 1910, Die Cnidosporidien, p. 196; Kudo (in part), 1924a, Arch. Protistenkd. 49: 147, Kudo (in part), 1924c, Ill. Biol. Monogr. 9(2-3): 143; Kudo (in part), 1925a, Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. I. Orig. 96: 431, Kudo (in part), 1929, Arch. Protistenkd. 67: 3; Missiroli, 1929, Rev. Malariol. 8: 395, Fantham et al. (in part), 1941, Parasitology 33: 202; Sen (in part), 1941, J. Malar. Inst. India 4: 258, Weiser (in part), 1947, Pr. Moravske Přír. Spol. 18. 38; Poisson (in part), 1953, *in* Traite de zoologie (P. P. Grasse, ed.), p. 1063; Thomson (in part), 1960, J. Insect Pathol. 2. 359, Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 113, Weiser, 1963b, *in* Insect pathology (E. A. Steinhaus, ed.), vol. 2: 318; Weiser (in part), 1966, Nemoci hmyzu, p. 458, Weiser, 1969, An atlas of insect diseases, p. 245; Kudo (in part), 1971, Protozoology, p. 816; Tour et al. (in part), 1971, Ann. Parasitol. Hum. Comp. 46: 208.

Parathelohania legeri (Hesse, 1904) Codreanu, 1966, Proc. Int. Congr. Parasitol., 1st, 1964: 602, Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 8.

Toxoglugea missiroli Weiser, 1961, Monogr. Angew. Entomol. 17: 117; Weiser, 1963c, Bull. W.H.O. 28: 125.

Spore size.—Living octospores measure 6–8×3.4 μm (Hesse 1904a and 1904b), 4.8–6.0×3.4 μm (Weiser 1961); macrospores measure 12×5 μm (Hesse 1904a and 1904b); free spores in adult females measure 3.0–4.0×1.5–2.0 μm (Missiroli 1929).

Host.—*Anopheles maculipennis* Meigen, a mosquito, found in Czechoslovakia, France, and Italy.

Infection site.—Adipose tissue of male larvae; oenocytes and ovaries of adult females.

According to the photomicrographs of Heidenhain's-hematoxylin-stained octospores of Weiser (1961) this species has spores similar to those of *P. obesa*, but they have a more constricted posterior end and are proportionately longer than their width. The free spores in adult females are elongate and curved or bent and develop in blood cells in the body cavities. Since this species is the type for the genus, its

ultrastructure should be studied so it can be compared to that of other species. Nothing is known of the ultrastructure of the sporonts or spores from either sporulation sequence in this species. Larvae having frank infections apparently succumb to disease in the last instar.

Kudo (1929) lists six other *Anopheles* species from India—*Anopheles barbirostris* Van der Wulp, *A. annularis* Van der Wulp, *A. hyrcanus* (Pallas), *A. ramsayi* Covell, *A. subpictus* Grassi, and *A. varuna*, Iyengar (= *A. funestus* of Kudo)—as hosts of *T. legeri*. Also, Tour et al. (1971) added an additional host, *Anopheles labranchiae atroparvus* Van Thiel, from France. Before identification of these microsporidia can be determined, their ultrastructure must be studied.

Parathelohania obesa (Kudo, 1924) Hazard and Anthony, 1974

Thelohania obesa Kudo, 1924c, Ill. Biol. Monogr. 9(2–3): 161; Kudo, 1925a, Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. I. Orig. 96: 432; Missiroli, 1929, Rev. Malariol. 8: 396; Weiser, 1947, Pr. Moravske Prir. Spol. 18: 38; Thomson, 1960, J. Insect Pathol. 2: 360; Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 111; Wills and Beaudoin, 1965, J. Invertebr. Pathol. 7: 12; Weiser, 1966, Nemoci hmyzu: p. 160; Hazard and Weiser, 1968, J. Protozool. 15: 820; Fulton et al., 1974, Mosq. News 34: 89.

Parthelohania legeri Chapman et al., 1966, J. Invertebr. Pathol. 8: 453; Tour et al. (in part), 1971, Ann. Parasitol. Hum. Comp. 46: 208.

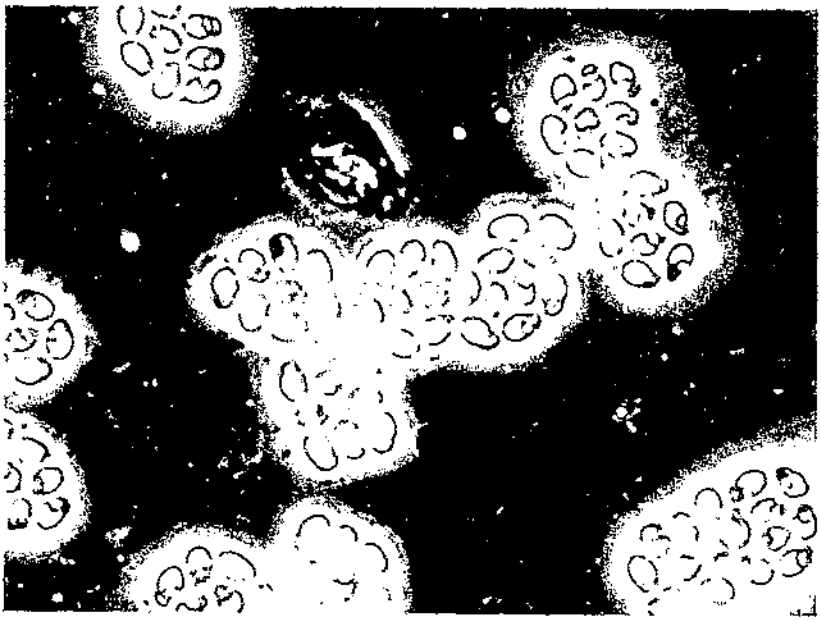
Parathelohania obesa (Kudo, 1924) Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 11.

Spore size.—Living octospores measure 3.5–5.7 × 2.8–4.2 μm (Hazard and Weiser 1968); preserved octospores measure 4.0–4.5 × 3.0–3.5 μm (Kudo 1924c); living free spores measure 7.9 × 3.5 μm (Hazard and Weiser 1968).

Host.—*Anopheles crucians* Wiedemann and *Anopheles quadrimaculatus* Say, mosquitoes, collected in Florida, Georgia, Louisiana, and Pennsylvania, U.S.A.

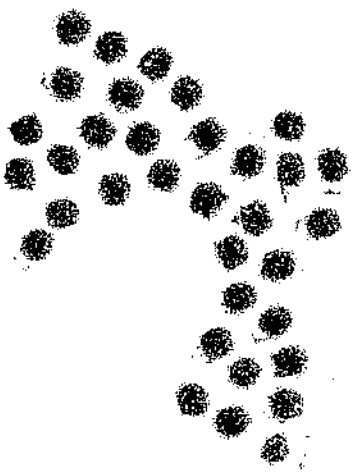
Infection site.—Hemocytes and adipose tissue of male and female larvae; oenocytes and ovaries of adult females.

The octospore is short and oval, being only a little longer than it is wide (figs. 63 and 64). Unlike many other species, no internal bottle-shaped structure is seen in phase-contrast microscopy of living octospores in fresh smears. Instead, only a dark, thin, elliptical line is seen in the posterior end of the spore. Sporonts producing these octospores contain densely staining granules easily seen in Giemsa-stained smears and Heidenhain's-hematoxylin-stained wet smears. Free spores are broad and cylindrical and have a large vac-



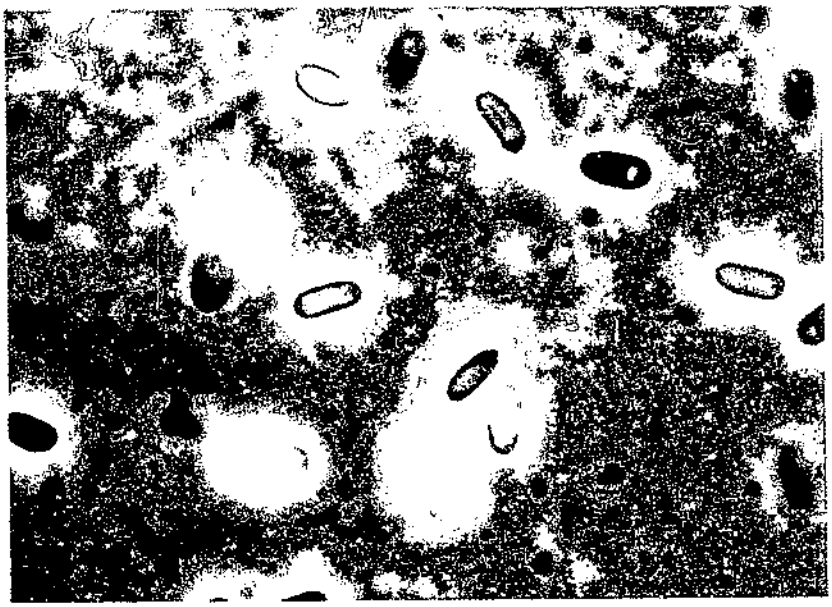
PN 1512

FIGURE 63.—Fresh octospores of *Paranthelohania obesa* (Kudo). × 1,100.



PN 1513

FIGURE 64.—Heidenhain's-hematoxylin-stained octospores of *P. obesa*. × 2,000.



PN 1511

FIG. 65.—Fresh spores of *P. obesa*. × 1,380.

PN 1515

FIG. 66.—Surface ultrastructure of *P. obesa* octospores. × 15,600.

uole (fig. 65). These sporulate in oenocytes in the hemocoel, causing the blood cells to become greatly hypertrophied. Each sporont produces six to eight free spores.

The surface ultrastructure of the octospore is similar to *P. anopheliis*. However, the heavily ridged constricted posterior end is much shorter in *P. obesa* (fig. 66). The polar filament forms 7 coils in the spore, 3 of which are formed by the broad basal portion (fig. 67). Nothing is known of the ultrastructure of free spores or of the sporonts from which they develop.

Octospores sporulate in about one-half of male and female larval progeny, causing their death in the last instar. This microsporidium is carried via the ovaries to female progeny for only one generation, surviving female progeny being free of the infection. This species differs from others by its short and broad octospores, by the number of the coils of the polar filament and number of coils formed by the broad basal portion, and by the unusually large size of the free spores in adult females.

PN 4516

FIG. 67.—Internal ultrastructure of *P. obesa* octospore. × 24,400.

Parathelohania obscura (Kudo, 1929) Hazard and Anthony, 1974

Perezia obscura Kudo, 1929, Arch. Protistenkd. 67: 4; Weiser, 1947, Pr. Moravske Prir. Spol. 18: 30; Thomson, 1960, J. Insect Pathol. 2: 360; Weiser, 1966, Nemoc. hmyzu, p. 460.

Perezia obscura Weiser (in part), 1961, Monogr. Angew. Entomol. 17: 111.

Perezia legeri Sen (in part), 1941, J. Malar. Inst. India 4: 258. New synonymy.

Parathelohania obscura (Kudo, 1929), Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 19.

Spore size. Preserved octospores measure 4.5–5.0×3.0–3.5 μm.

Host. *Anopheles varuna* Iyengar (= *A. funestus* of Kudo, 1929), a mosquito, collected in India.

Infection site. Probably adipose tissue of larvae, but not reported by Kudo.

This species may be similar to *P. octolagenella*, according to the description of the spores by Kudo. We tentatively place this species in *Parathelohania* for the same reasons we indicated for *P. indica*.

Parathelohania octolagenella Hazard and Anthony, 1974

Parathelohania octolagenella Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 22.

Spore size. Living octospores measure 6.0×2.6 μm; living free spores measure 5.5×1.5 μm.

Host. *Anopheles pretoriensis* (Theobald), a mosquito.

Infection site. Hemocytes and adipose tissue of male larvae; oenocytes and ovaries of adult females.

Holotype. Kaduna, Nigeria, September 1970, USNM No. 24397 (Hazard).

Paratypes. USNM No. 24398; World Health Organization Reference Center, Ohio State University, Accession No. 1658 (Hazard).

The octospores of this species are elongate and have a long attenuated posterior end (fig. 68). The mature free spores in the adult female are narrowly cylindrical, narrower on the anterior end, curved (some so drastically that they are U-shaped), and without a visible vacuole.

The octospores are heavily ridged, some of the ridges running the full length of the spore (fig. 69). The constricted posterior end is often attenuated to a blunt point (Hazard and Anthony 1974). The polar filament is long, making 8 coils inside the spore, the first 2½ formed by the broad basal portion (fig. 70). Nothing is known of the

PN 4[illegible]

[illegible]. Heterogeneous ornamented stained ectospores of *Parathelohania* [illegible] Hazard and Anthony. × 2,000.

PN 4[illegible]

[illegible]. Surface ultrastructure of *Plistophora hyphessobryconis* ectospores. × 7,500.

ultrastructures of sporonts and sporoblasts in larvae or of sporonts, sporoblasts, and free spores in adult females.

The octospores sporulate in male larvae, all of which succumb to the infection in the last instar. The octospores of this species are easily distinguished by the ridges that run their full length and by the long attenuated posterior end.

PN 1549

FIG. 70. Internal ultrastructure of *P. octolagenella* octospore. × 21,000.

Parathelohania periculosa (Kellen and Wills, 1962) Hazard and Anthony, 1974

Perezia periculosa Kellen and Wills, 1962a, J. Insect Pathol. 4: 54; Weiser, 1968, Neptun Innov. p. 460.

Parathelohania periculosa Kellen and Wills 1962; Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 19.

Nosema periculosa Kellen et al. 1967, J. Invertebr. Pathol. 9: 20.

Perezia sp. Hazard and Weiser, 1968, J. Protozool. 15: 817.

Spore size.—Living octospores measure 4.71±0.06×2.62±0.02 μm, and preserved octospores measure 3.81±0.06×0.04 μm (Kellen and Wills 1962a); living free spores measure 5.54±0.20×1.73±0.07 μm, and preserved free spores measure 5.78±0.18×1.48±0.10 μm (Kellen et al. 1967).

Host.—*Anopheles franciscanus* McCracken, a mosquito, collected in California, U.S.A.

Infection site.—Hemocytes and adipose tissue of male larvae; oenocytes and ovaries of females.

Frank infections are found only in male larvae, where the octospores are seen as whitish masses on the ventral side of the abdomen (described as "dough belly" by Kudo). Patently infected larvae usually die in the last instar. The stained octospores of this species are similar in shape and structure to those of *P. anophelis*, but the free spores in adult females differ; those of *P. anophelis* are oval to oblong and sporulate in the ovaries, and those of *P. periculosa* are elongate to cylindrical and sporulate in oenocytes in the hemocoel.

Other Hosts of Undescribed *Parathelohania* Species

Anopheles albimanus Wiedemann
Parathelohania sp. Hazard and Oldacre, hoc loco
Anopheles annularis Van der Wulp
Thelohania legeri. Kudo, 1929
Anopheles barbirostris Van der Wulp
Thelohania legeri: Kudo, 1929
Anopheles bradleyi King
Thelohania sp. Chapman et al., 1966; Chapman et al., 1967
Anopheles claviger (Meigen) [= *A. bifurcatus* of Kudo]
Thelohania legeri: Kudo, 1924a; Weiser, 1947, 1961
Anopheles funestus Giles
Parathelohania sp. Hazard and Oldacre, hoc loco
Anopheles labranchiae atroparvus Van Thiel
Thelohania legeri: Tour et al., 1971
Anopheles nili (Theobald)
Parathelohania sp. Hazard and Oldacre, hoc loco
Anopheles pharoensis Theobald
Parathelohania sp. Hazard and Oldacre, hoc loco
Anopheles pseudopunctipennis pseudopunctipennis Theobald
Thelohania legeri: Carney-Pacheco, 1968
Anopheles subpictus Grassi
Thelohania legeri: Kudo, 1929
Anopheles triannulatus (Neiva and Pinto)
Parathelohania sp. Hazard and Oldacre, hoc loco
Anopheles vagus Donitz
Thelohania legeri: Sen, 1941
Anopheles walkeri Theobald
Thelohania sp. Laird, 1961

PEGMATHECA gen. n.

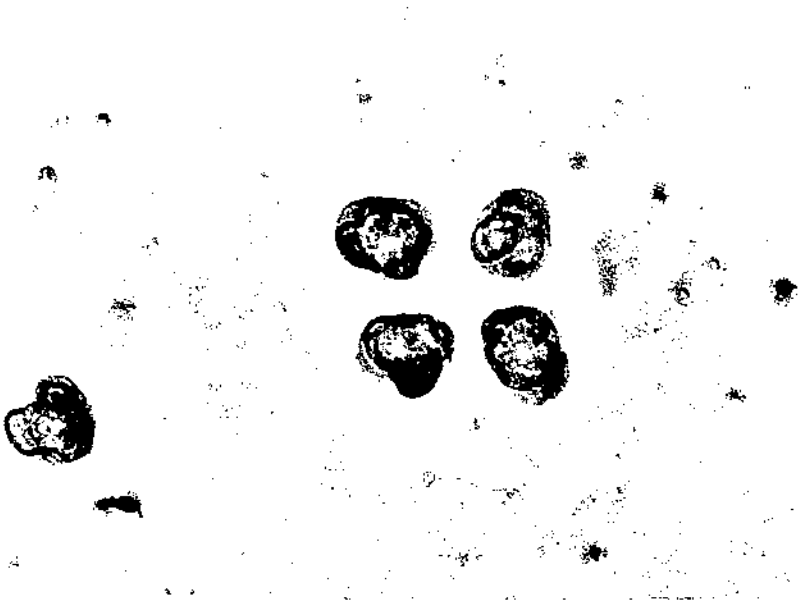
Only one sporogonic sequence, producing octospores in larval hosts, is known in these microsporidia, but adult females have not been examined to determine whether or not there is a transovarial sequence.

The pansporoblast is oval, has a subpersistent pansporoblastic membrane, and contains oval octospores with equally rounded ends (fig. 71). The octospore retains its shape when preserved and stained with Heidenhain's hematoxylin and has a short polar filament of uniform diameter. This genus is unique in that multinucleate meronts give rise to several pansporoblasts (4 to 16) that remain attached by thin strands of protoplasm after division until sometime after sporulation (figs. 72 and 73). The young sporonts secrete small granules that are retained by the pansporoblastic membrane until sporulation (fig. 74), at which time most are apparently utilized by the sporoblasts in the formation of the spore wall. Microtubules are not formed in sporulating pansporoblasts. Electron photomicrographs show the octospore to have a thin and relatively smooth exospore and a polar filament of uniform diameter (fig. 75).

This genus may contain numerous species because they appear to be common parasites of blackflies. We name the genus *Pegmatheca*, meaning "fastened cases" and referring to the many connected pansporoblasts developing from single meronts. We describe only one species, *Pegmatheca simulii*, which is the type by monotypy.

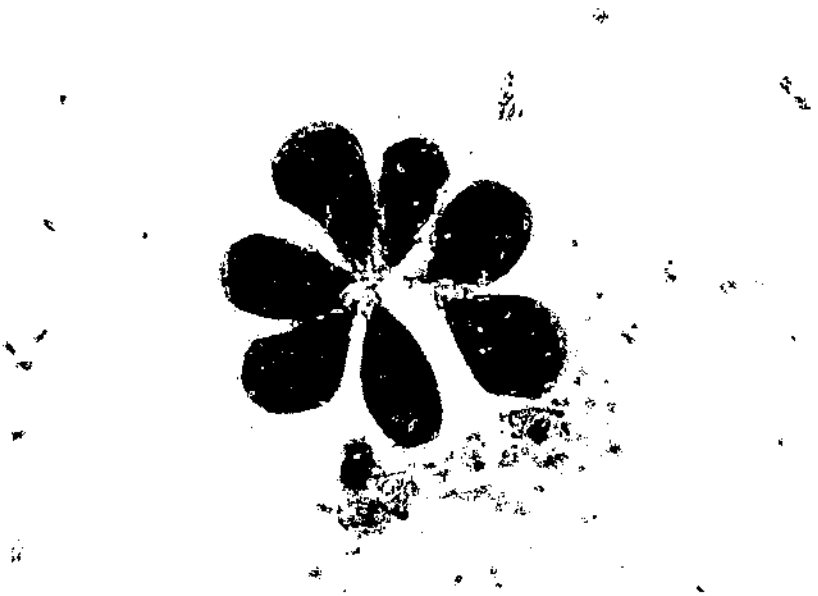
PN 4549

FIGURE 71.—Heidenhain's hematoxylin-stained octospores (melanized) of *Pegmatheca simulii* sp. n. × 2,000



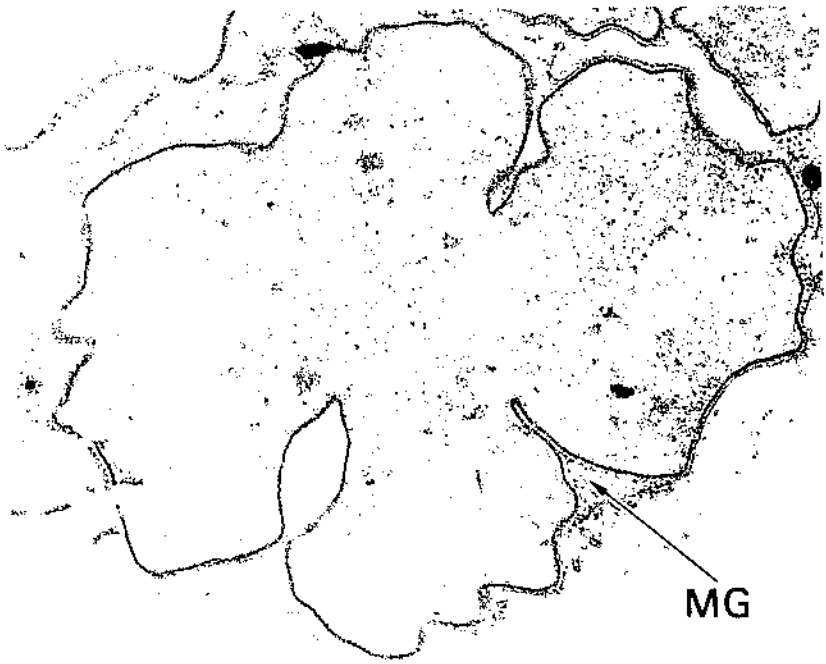
PN-4521

FIGURE 72.—Fresh smear showing group of connected pansporoblasts of *P. simulii*. × 1,100.



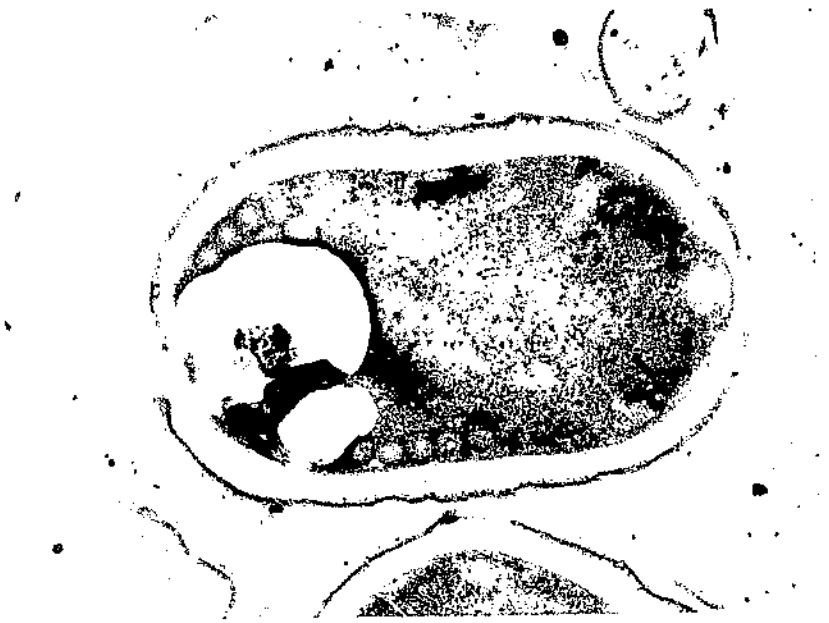
PN-4522

FIGURE 73.—Heidenhain's-hematoxylin-stained group of connecting pansporoblasts of *P. simulii*. × 2,000.



PN 4523

FIGURE 74.—Dividing sporont in *P. simulii* pansporoblast. × 12,000. MG, metabolic granules.



PN 4524

FIGURE 75.—Ultrastructure of *P. simulii* octospore. × 22,500.

Pegmatheca simulii sp. n.

Spore size.—Living octospores measure 3.04–3.72×1.92–2.48 μm.

Host.—*Simulium tuberosum* (Lindström), a blackfly.

Infection site.—Adipose tissue of larvae.

Holotype.—Hatchet Creek on Waldo Road near Gainesville, Fla., U.S.A., Mar. 9, 1971, USNM No. 24402 (Hazard and Swain).

Paratypes.—USNM No. 24403; World Health Organization Reference Center, Accession No. 1662 (Hazard and Swain).

The living octospore is oval, has no observable vacuole, and has equally rounded ends, the ends of some appearing to be triangularly pointed in fresh preparations. The spores have not been examined in dilute india ink for mucous envelopes. Octospores fixed in aqueous Bouin's fluid and stained with Heidenhain's hematoxylin are oval, but never appear to have pointed ends. Mature spores are nearly impervious to this stain and many appear to be melanized (fig. 71). The pansporoblastic membrane retains the octospores for a short time after removal from the body of the host.

The sporonts secrete moderately sized, uniform granules within the pansporoblastic membrane (fig. 74). These granules remain in the pansporoblast long after the sporoblasts are formed, but they are reduced to very fine, irregularly spaced granules during sporulation. The octospores have a thin exospore, a moderately thick endospore, a small, tightly compressed, lamellate polaroplast, and a short, thick polar filament of uniform diameter (fig. 75).

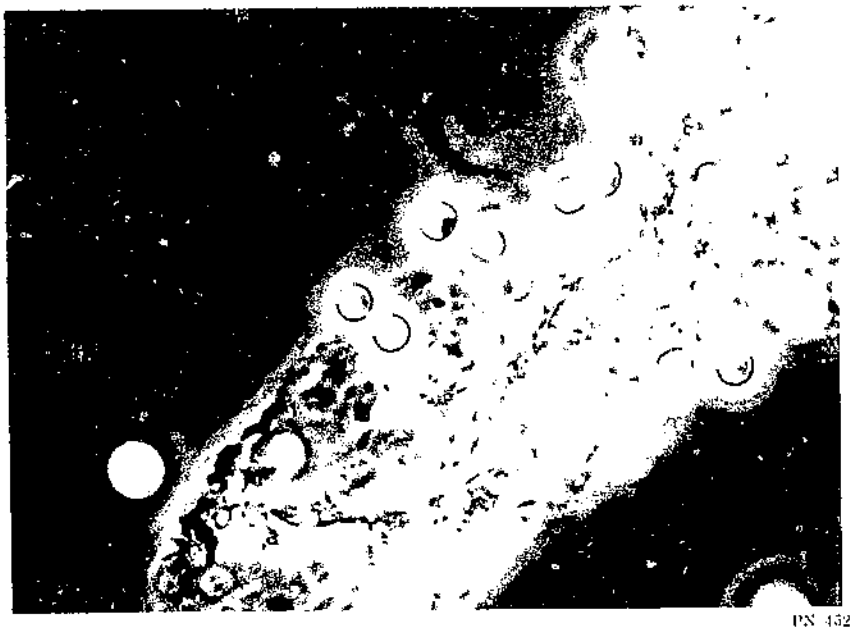
We name this species after the genus of its host.

PILOSPORELLA gen. n.

The adult female hosts have not been examined; therefore, we know nothing about probable transovarial transmission of infection or production of free spores.

The pansporoblasts are oval and contain subspherical or spherical octospores (figs. 76 and 79). The pansporoblastic membrane is fragile and bursts immediately when removed from the body of the host. The octospore has a thin exospore, a relatively thick endospore, a somewhat short polar filament of uniform diameter from base to apex, and a polaroplast made up of indistinct and widely separated lamellae (figs. 77 and 80). The octospore wall is smooth, and the octospores are without a mucous envelope. Young pansporoblasts contain numerous granules (fig. 81), which are replaced by microtubules (fig. 78) when octospores are formed.

Only two species are known, both from mosquitoes. The name *Prilosporella* means "small, ball-like spores," referring to the shape of the octospores. We designate *P. fishi* as the type species.

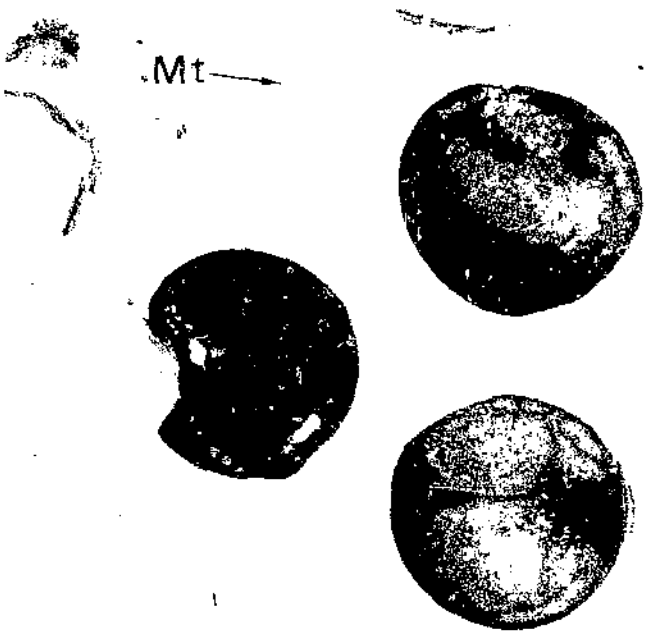


PN 4525

FIGURE 76.—Fresh octospores of *Pilosporella chapmani* sp. n. × 2,000.

PN 4526

FIGURE 77.—Ultrastructure of *P. chapmani* octospore. × 45,400.



PN 1527

FIGURE 78.—Pansporoblast of *P. chapmani* containing octospores. × 17,100. Mt, microtubules.

Pilosporella chapmani sp. n.

Spore size.—Living octospores measure 2.80 μm.

Host.—*Aedes triseriatus* (Say), a mosquito.

Infection site.—Small isolated areas in the adipose tissue, often in the head and sixth abdominal segment of larvae.

Holotype.—In old rubber tires near Lake Charles, La., U.S.A., July 1, 1972, USNM No. 24399 (Chapman and Glenn).

Fresh and preserved octospores are spherical (fig. 76).

The octospore has a short polar filament making about 4 coils (fig. 77). Mature pansporoblasts contain a few granules and many microtubules (fig. 78), but only the granules are seen in young pansporoblasts.

We suspect that this species is not lethal to its host, at least not larval hosts, because the infection never appears to be extensive in immature mosquitoes. We name it *P. chapmani* in honor of Harold C. Chapman, who found it in mosquitoes breeding in old rubber tires in Louisiana, U.S.A.

Pilosporella fishi sp. n.

Spore size.—Living octospores measure 2.33–3.13 μm, the average being 2.92 μm.

Host.—*Wyeomyia vanduzeei* Dyar and Knab, a mosquito.

Infection site.—Adipose tissue of larvae.

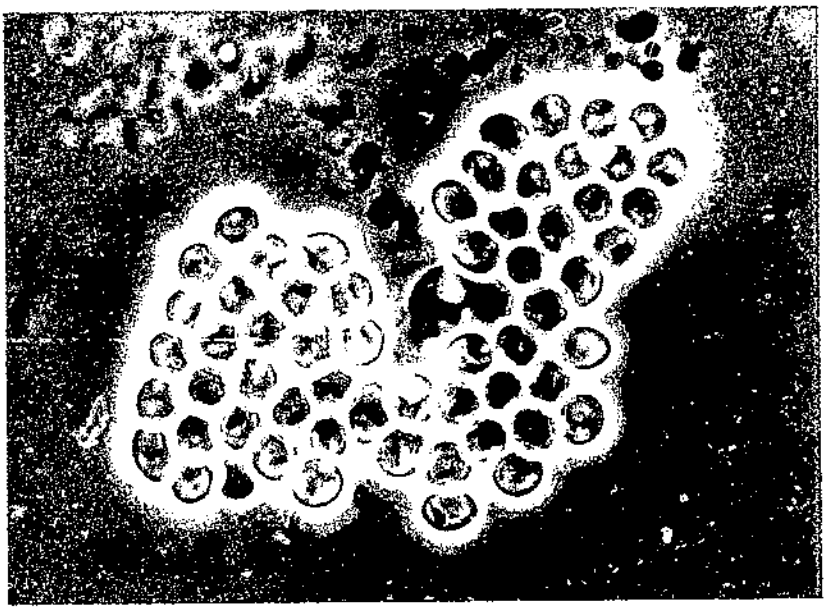
Holotype.—In bromeliads near Vero Beach, Fla., U.S.A., Mar. 21, 1973, USNM No. 24400 (Fish).

Paratypes.—USNM No. 24401; World Health Organization Reference Center, Ohio State University, Accession No. 1659 (Fish).

The octospores are subspherical and have a small, indistinct vacuole (fig. 79).

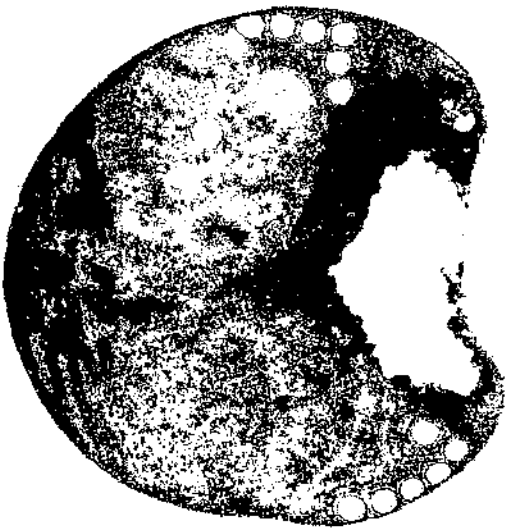
The octospore differs from that of *P. chapmani* by having a thicker endospore and a longer (6 coils) and narrower polar filament (fig. 80). The young pansporoblasts contain many granules (fig. 81), most of which are replaced by microtubules during sporulation. Microtubules appear to be fewer in the mature pansporoblasts of this species than in *P. chapmani*.

P. fishi causes more extensive pathologies in its host than does *P chapmani* in *A. triseriatus*, usually involving a greater part of the larval fat body and often causing death in late larval instars. Some infected individuals, however, survive to become adults when the infection occurs in small isolated areas of the host body. A spore



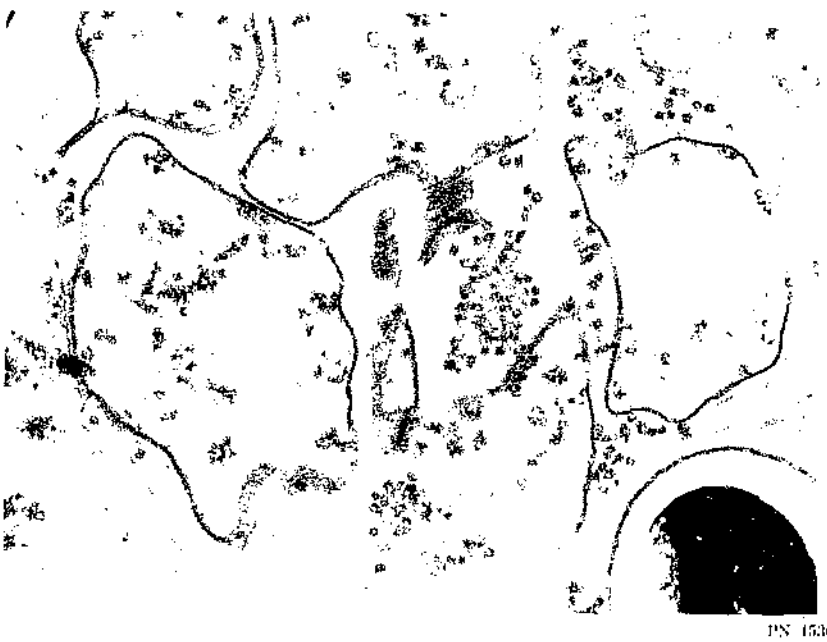
PN-4528

FIGURE 79.—Fresh octospores of *Pilosporella fishi* sp. n. × 2,000.



PN 1529

FIGURE 80.—Ultrastructure of *P. fishi* octospore. × 32,500.



PN 1530

FIGURE 81.—Pansporoblast of *P. fishi* containing sporoblasts and metabolic granules. × 12,000.

having a different shape has been seen mixed with the subspherical octospores in one larva. These spores are pyriform, somewhat elongate, and not enclosed in pansporoblastic membranes. They measure 3.18–5.14 × 1.38–1.64 μm. We do not know whether or not these spores represent dimorphism or another species. We name it after Durland Fish, a graduate student at the University of Florida, who found it in *W. vanduzeei* breeding in bromeliads near Vero Beach, Fla.

SYSTENOSTREMA gen. n.

We know of only one developmental sequence in these microsporidia, and it produces octospores.

The pansporoblasts are subspherical and contain small, oval to pyriform octospores (fig. 82). The pansporoblastic membrane is subpersistent, remaining intact only for a short time after the pansporoblasts have been removed from the body of the host. The octospore has a thin exospore, a long polar filament abruptly constricting near its middle, and an indistinct polaroplast (fig. 85). The poor resolution of the latter may be due to improper spore fixation. The surface of the spore wall is covered with fine ridges, making it somewhat wrinkled. Dividing sporonts secrete granules (fig. 83) of uniform size within the pansporoblastic membrane, which are mostly replaced by microtubules during sporulation (fig. 84).

We name this genus *Systenostrema*, meaning "thread tapering to a point" and relating to the polar filament which abruptly constricts to a narrow distal portion. Only one species, *S. tabani*, represents the genus and is the type by monotypy.

Systenostrema tabani sp. n.

Spore size.—Living octospores average 3.32 × 2.08 μm, the range being 3.13–3.45 × 1.91–2.17 μm.

Host.—*Tabanus lineola* Fabricius, a horse fly.

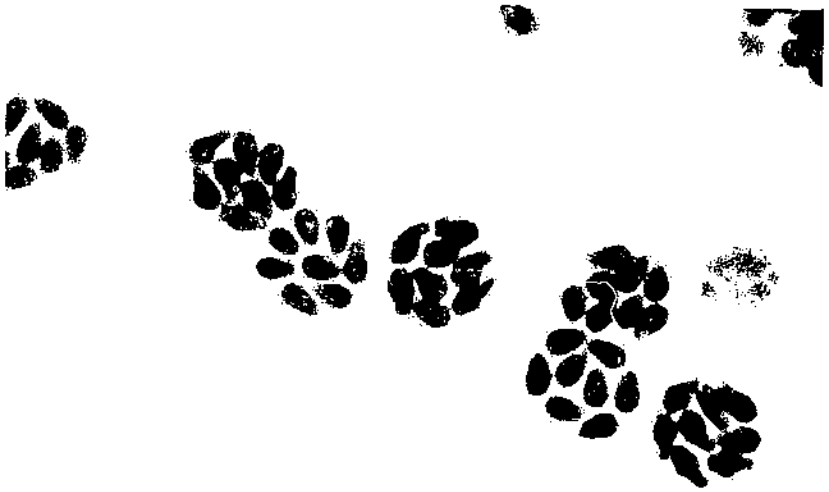
Infection site.—Adipose tissue of larvae.

Holotype.—Orange Lake, Fla., U.S.A., Apr. 2, 1973, USNM No. 24404 (Knell).

Paratypes.—USNM No. 24405; World Health Organization Reference Center, Ohio State University, Accession No. 1666 (Knell).

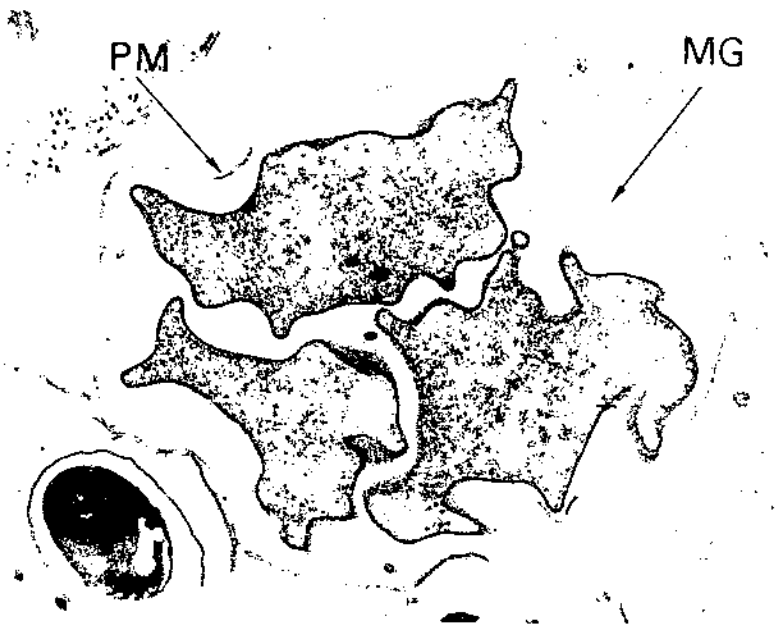
Fresh and preserved octospores are oval to pyriform and are without a visible vacuole (fig. 82).

Young sporonts secrete granules of uniform size (fig. 83), and these are replaced by microtubules in pansporoblasts during sporulation (fig. 84). The octospores have a thin rugose layer, a somewhat long and abruptly constricting polar filament, and an ill-defined



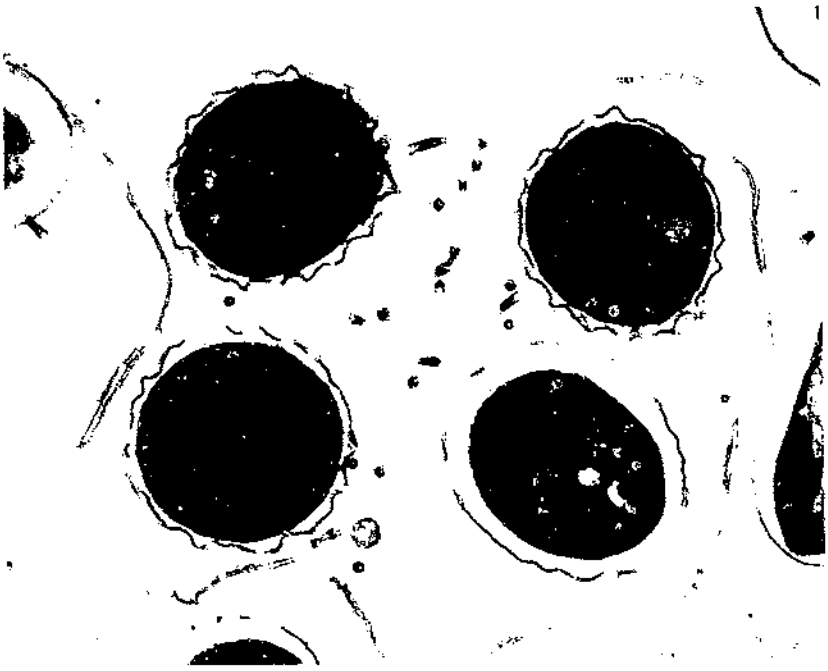
PN 1531

FIGURE 82.—Heidenhain's-hematoxylin-stained octospores of *Systenostroma tabani* sp. n. × 2,000.



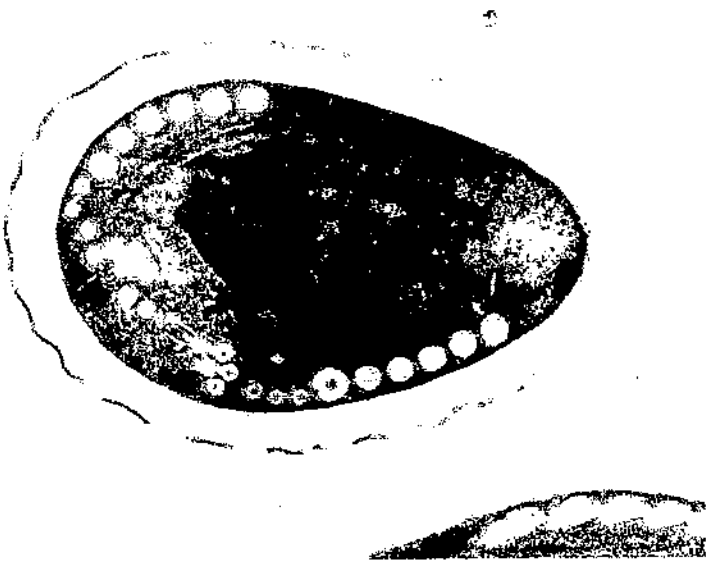
PN 1532

FIGURE 83.—Dividing sporont of *S. tabani* pansporoblast. × 12,000. MG, metabolic granules; PM, pansporoblastic membrane.



PN 1533

FIG. 84.—Pansporoblast of *S. tabani* containing spores and microtubules. × 17,100



PN 1534

FIG. 85.—Ultrastructure of *S. tabani* octospore. × 30,750.

polaroplast (fig. 85). The polar filament makes 14 to 16 coils within the spore, the first 6 coils being formed by the broad basal portion.

This microsporidium causes death of larval hosts. Little else is known about its host-parasite relationship. We name this species after the genus of its host.

THELOHANIA Henneguy, 1892

Thelohania Henneguy, *in* Henneguy and Thelohan, 1892b, Ann. Microgr. 4: 639.

As far as we know, the species of this genus have only one developmental sequence, which produces octospores.

The pansporoblasts are subspherical and contain small, oval or pyriform octospores. The pansporoblastic membrane may be persistent, or it may burst shortly after the pansporoblast is dissected from the body of the host. The octospore has a thin exospore, a long polar filament of uniform diameter, and a distinct polaroplast composed of compressed lamellae. The wall of the spore is without surface structure.

Nothing is known about the ultrastructure of the type species. We can only describe the ultrastructure of a microsporidium found in *Astacus pallipes* Lereboullet (= *Austropotamobius pallipes* of Vey and Vago) reported to be *Thelohania contejeani* Henneguy by Vey and Vago (1973). The sporonts of this microsporidium secrete small dense granules that are held by the pansporoblastic membrane until sporulation, when they are replaced by small tubules (Vey and Vago 1973). The octospore is uninucleate and has a thin exospore, a polar filament of nearly uniform diameter (or only narrowing gradually toward its apical end), and a polaroplast composed of tightly compressed lamellae. The outer surface of the exospore has no noticeable surface structure in the electron photomicrographs of Maurand and Vey (1973).

We suspect that this genus is represented by several species found in decapod crustaceans, but we cannot be certain until we have studied their ultrastructure and the ultrastructure of the type species, *Thelohania giardi* Henneguy.

Thelohania contejeani Henneguy, 1892

Thelohania contejeani Henneguy, *in* Henneguy and Thélohan, 1892b, Ann. Microgr. 4: 639; Henneguy *in* Thelohan, 1892, Bull. Soc. Philomath. Paris 4: 174; Dubois, 1893, C.R. Soc. Biol. 5: 158; Gurley, 1893, Bull. U.S. Fish Comm. for 1891, 11: 410; Gurley, 1894, Rep. U.S. Fish. Comm. 26: 196; Thelohan, 1895, Bull. Sci. Fr. Belg. 26: 362; Pfeiffer, 1895, Die Protozoen als Krankheitserreger, p. 72; Léger and Hagenmuller, 1897, C.R. Assoc. Fr. Av. Sci. 26: 553; Labbé, 1899, Sporozoa, *in* Das Tierreich (O. Bütschli, ed.), p. 112; Auerbach, 1910, Die Cnidosporidien, p. 196; Kudo, 1924c, Ill. Biol. Monogr. 9(2-3): 135; Schereschewsky, 1926, Zool. Anz. 65: 71; Dollfus, 1935, B.C.I. Soc. Centr. Aquicc. 42(10-12): 119; Weiser, 1947, Pr. Moravské Přír.-Spol. 18:

27. Poisson, 1953, *in* Traite de zoologie (P. P. Grasse, ed.), p. 1063; Schaperclaus, 1954. Fischkrankheiten, p. 377; Sprague, 1965, J. Protozool. 12: 66; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sumari and Westman, 1970, Ann. Zool. Fenn. 7: 193; Voronin, 1971, Parazitologiya 5: 186; Sprague and Couch, 1971, J. Protozool. 18: 530; Vey et al., 1971, C. R. Acad. Agric. Fr. 57: 1540; Maurand and Vey, 1973, Ann. Parasitol. Hum. Comp. 48: 411; Vey and Vago, 1973, *in* Freshwater crayfish (S. Abrahamsson, ed.), p. 166.

Thelohania sp. Vey and Vago, 1972, Ann. Hydrobiol. 3: 61.

Sporozoaire Henneguy and Thelohan, 1892a, C. R. Soc. Biol. 4: 585.

Spore size.—Octospores measure 2.0–3.0 μm (Henneguy and Thelohan 1892b; Thelohan 1895); 3.0–4.0 μm (Gurley 1894); 1.5–2.5 × 1.25–1.5 μm (Dollfus 1935); 4.6 × 2.2 μm (Sumari and Westman 1970); 3.3–3.8 × 2.0–2.4 μm, living spores (Voronin 1971); 3.4 × 2.3 μm (Vey and Vago 1972); 3.5–4.0 × 2.0 μm, living spores (Maurand and Vey 1973).

Host.—*Astacus fluviatilis* Fabricius (=*Astacus astacus*) (Henneguy and Thélohan 1892b; Gurley 1894; Sumari and Westman 1970; Voronin 1971); *Astacus pallipes* Lereboullet (=*Austropotamobius* (*Atlantoastacus*) *pallipes*) (Dollfus 1935; Schäperclaus 1954; Vey et al. 1971; Vey and Vago 1972; Maurand and Vey 1973; Vey and Vago 1973), crayfish, collected in Finland, France, Germany, and U.S.S.R.

Infection site.—Musculature (Henneguy and Thélohan 1892b); musculature, heart, brain, connective tissue surrounding the gut, and the envelopment of the ovary (Vey and Vago 1973).

The octospores are ovoid (Henneguy and Thélohan 1892b; Thélohan 1895) or slightly pyriform (Maurand and Vey 1973). Since they are not always enclosed by a membrane, according to Henneguy and Thelohan (1892b) and Gurley (1894), the pansporoblastic membrane must not persist.

Vey and Vago (1972 and 1973) and Maurand and Vey (1973) have reported on the ultrastructure of a microsporidium, which they identified as *Thelohania contejeani*, in *Astacus pallipes*. Whether or not this microsporidium is identical to that of Henneguy in *Astacus fluviatilis* remains to be determined through electron microscopy studies of infected type hosts. The electron photomicrographs of Maurand and Vey (1973) show that the spore has a thin, externally smooth exospore, a gradually narrowing polar filament, and a large, tightly compressed polaroplast. Electron photomicrographs of Vey and Vago (1973) show granules in young pansporoblasts, which are replaced by short tubules when spores are formed.

This species is tentatively held in the genus *Thelohania* pending electron microscopy studies of the type species, *Thelohania giardi* Henneguy.

Thelohania duorara Iversen and Manning, 1959

Thelohania duorara Iversen and Manning, 1959, Trans. Am. Fish. Soc. 88: 130; Iversen and Van Meter, 1961, Bull. Mar. Sci. Gulf Carib. 14: 549; Hutton, 1964, Proc. Am. Microsc. Soc. 83: 440; Sprague, 1965, J. Protozool. 12: 66; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sprague and Couch, 1971, J. Protozool. 18: 530.

Thelohania sp. Jones, 1958, Assoc. Southeast. Biol. Bull. 5: 10.

Thelohania sp. Kruse, 1959, Tulane Stud. Zool. 7: 128.

Spore size.—Living octospores measure 5.4 × 3.6 μm.

Host.—*Penaeus duorarum* Burkenroad (Iversen and Manning 1959); *P. brasiliensis* Latreille (Iversen and Van Meter 1964); *P. aztecus* Ives (Kruse 1959; Hutton 1964), a shrimp, collected off the coast of Florida, U.S.A.

Infection site.—Musculature (Iversen and Manning 1959); heart, gonads, brain, and musculature (Iversen and Van Meter 1964).

Nothing is known of the ultrastructure of this species, it is held in this genus pending electron microscopy studies of this species and the type species, *Thelohania giardi* Henneguy.

Thelohania giardi Henneguy, 1892

Thelohania giardi Henneguy, *in* Henneguy and Thelohan, 1892b, Ann. Microgr. 4: 639; Henneguy, *in* Thelohan, 1892, Bull. Soc. Philomath. Paris 4: 174; Gurley, 1893, Bull. U.S. Fish Comm. for 1891, 11: 410; Gurley, 1894, Rep. U.S. Fish. Comm. 20: 201; Thelohan, 1895, Bull. Sci. Fr. Belg. 26: 362; Pfeiffer, 1895, Die Protozoen als Krankheitserreger, p. 72; Leger and Hagenmuller, 1897, C.R. Assoc. Fr. Av. Sci. 26: 551; Labbé, 1899, Sporozoa, *in* Das Tierreich (O. Butschli, ed.), p. 112; Mercier, 1908, C.R. Acad. Sci. 146: 34; Mercier, 1909, Acad. R. Belg., Cl. Sci., Mem. 2(2): 30; Stempell, 1909, Arch. Protistenkd. 16: 340; Auerbach, 1910, Die Cnidosporidien, p. 196; Schaberg, 1910, Arb. Kais. Gesundh. 33: 406, 415, 417, 418; Kudo, 1924c, Ill. Biol. Monogr. 9(2-3): 130; Weiser, 1947, Pr. Moravske Přír. Spol. 18: 28; Poisson, 1953, *in* Traité de zoologie (P. P. Grasse, ed.), p. 1062; Sprague, 1965, J. Protozool. 12: 66; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 424; Sprague and Couch, 1971, J. Protozool. 18: 530; Overstreet and Weidner, 1974, Z. Parasitenkd. 44: 171; Hazard and Anthony, 1974, U.S. Dep. Agric. Tech. Bull. 1505: 3.

"Sporozoaire" Henneguy and Thelohan, 1892a, C.R. Soc. Biol. 4: 585.

Spore size.—Living octospores measure 5.0–6.0 μm in length (Henneguy and Thélohan 1892a) and 2.0–3.0 μm in diameter (Henneguy and Thélohan 1892b).

Host.—*Crangon crangon* Linnaeus (=*Crangon vulgaris* Fabricius), a shrimp, collected in France.

Infection site.—Musculature.

The living spore, very refringent under the microscope, is pyriform and, according to Gurley (1894), has longitudinal striations. Frequently, macrospores are formed in pansporoblasts and these have abnormalities—for example, constrictions near their

middle. These apparently result when nuclei fail to make the last division just prior to sporoblast formation. The pansporoblastic membrane is very thin, but it is easily seen in fixed and stained preparations.

Nothing is known about the ultrastructure of this species; therefore, the generic status of other species found in decapod crustaceans may possibly change after the ultrastructure of this species, the type, has been studied.

Thelohania maenadis Pérez, 1904

Thelohania maenadis Perez, 1904, C.R. Soc. Biol. 57: 214; Pérez, 1905a, C.R. Soc. Biol. 58: 148; Perez, 1905b, Soc. Sci. Arachon. Trav. Lab. 8: 16; Pérez, 1906, C.R. Soc. Biol. 60: 1091; Stempell, 1909, Arch. Protistenkd. 16: 341; Auerbach, 1910, Die Cnidosporidien, p. 120; Kudo, 1924c, Ill. Biol. Monogr. 9(2-3): 141; Weiser, 1947, Pr. Moravske Prir. Spol. 18: 27; Sprague, 1965, J. Protozool. 12: 66; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sprague and Couch, 1971, J. Protozool. 18: 530.

Spore size.—Octospores measure 5.0×4.0 μm.
Host.—*Carcinus maenas* Pennant, a crab, collected in France.
Infection site.—Musculature and ovaries.

The pansporoblastic membrane is persistent and remains intact in salt water for several weeks. Vivares and Tuzet (1974) reported an additional host of *T. maenadis*, *Carcinus mediterraneus* Czernavsky. The microsporidium in this host cannot be considered the same species until the microsporidia in both hosts have been examined by electron microscopy. Since nothing is known of the ultrastructure of *T. maenadis* or the type species of this genus, we tentatively hold it in *Thelohania*.

Thelohania paguri Pérez, 1927

Thelohania paguri Pérez, 1927, Bull. Soc. Zool. Fr. 52: 99; Sprague, 1965, J. Protozool. 12: 66; Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sprague and Couch, 1971, J. Protozool. 18: 530.

Spore size.—Octospores measure 4.7×2.9 μm.
Host.—*Eupagurus bernhardus* Linnaeus, the hermit crab, collected in France.
Infection site.—Abdominal space between the viscera.

Nothing is known of the ultrastructure of this species. It is held in this genus pending electron microscopy studies of it and of the type species, *Thelohania giardi* Henneguy.

Thelohania petrolisthis Sprague, 1970

Thelohania petrolisthis Sprague, 1970, Am. Fish. Soc. Spec. Publ. 5: 425; Sprague and Couch, 1971, J. Protozool. 18: 530.

Thelohania sp. Sprague, 1950a, Occas. Pap. Mar. Lab. La. State Univ. 5: 5;

Sprague, 1954, U.S. Fish Wildl. Serv. Fish. Bull. 55: 251; Sprague, 1965, J. Protozool. 12: 66.

Spore size.—Octospores measure approximately 3.0×2.0 μm.

Host.—*Petrolisthis armatus* (Gibbs), a crab, collected in Louisiana, U.S.A.

Infection site.—Musculature.

The pansporoblastic membrane is persistent, remaining intact in salt water for several weeks. Nothing is known of the ultrastructure of this species; it is held in this genus pending electron microscopy studies of it and of the type species, *Thelohania giardi* Henneguy.

Doubtful *Thelohania* Species

Additional species have been placed in the genus *Thelohania* that are not accompanied by clear definitions concerning the enclosure of spores in a pansporoblastic membrane. Others obviously do not even represent species of the family. The former are listed here pending verification of their ultrastructure through electron microscopy for proper taxonomic placement, and the latter, indicated by an asterisk (*), are listed for reference.

**Thelohania acuta* (Moniez, 1887) Schröder, 1914
**Thelohania apodemi* Doby et al., 1963
Thelohania argyresthiae Issi and Lipa, 1968
Thelohania asterias Weiser, 1963a
**Thelohania baetica* Kudo, 1923
Thelohania barbata Weiser, 1969 (nomen nudum)
Thelohania baueri Voronin, 1974
Thelohania brasiliensis Kudo, 1924c (nomen nudum)
Thelohania breindli Weiser, 1946b
Thelohania cambari Sprague, 1950b
**Thelohania cepede* Hesse, 1905
**Thelohania chaetogastris* Schröder, 1909
**Thelohania cheimatobiae* Kreig, 1956
**Thelohania chironomi* Jirovec, 1940
**Thelohania cladocera* (Pfeiffer, 1895) Jirovec, 1936
Thelohania columbaczense Weiser, 1960
**Thelohania corethrae* Schuberg and Rodriguez, 1915
Thelohania cyclopis Weiser, 1945
Thelohania dasychirae Issi and Lipa, 1968
**Thelohania diazomx* Kramer, 1965
**Thelohania disparis* Timofejeva, 1956
**Thelohania ephestiae* Mattes, 1928

**Thelohania eriogastri* Weiser, 1957b
**Thelohania fibrata* (Strickland, 1913) Debaisieux and Gastaldi, 1919
Thelohania grassii Missiroli, 1929
Thelohania heredeteria Bulnheim, 1971
**Thelohania hessei* Weiser, 1961
**Thelohania hyphantriae* Weiser, 1953
**Thelohania janus* Hesse, 1903
**Thelohania mesnili* Paillot, 1924
**Thelohania minor* Georgévitch, 1954
**Thelohania mutabilis* Kudo, 1923
Thelohania nana Kellen and Lindegren, 1969
**Thelohania ochridensis* Georgévitch, 1952
**Thelohania ovicola* (Auerbach, 1910) Kudo, 1924c
**Thelohania pinguis* Hesse, 1903
Thelohania plectrocnemiae Weiser, 1946a
**Thelohania pristiphorae* Smirnoff, 1966
**Thelohania pyriformis* Kudo, 1924c
Thelohania reniformis Kudo and Hetherington, 1922
**Thelohania rhithrogenae* Weiser, 1946a
**Thelohania similis* Weiser, 1957a
**Thelohania tabani* Gingrich, 1965
**Thelohania thomsoni* Kramer, 1961
**Thelohania tipulae* Weissenberg, 1926
**Thelohania vandeli* Poisson, 1924
**Thelohania vanessae* Chorine, 1930
**Thelohania varians* (Léger, 1897) Debaisieux, 1919
Thelohania veliae Weiser, 1961 (=*T. veliae* Poisson, 1929 of Weiser in 1961)
**Thelohania virgula* (Moniez, 1887) Kudo, 1921a
**Thelohania weiseri* Günther, 1960
Thelohania wurmi Weiser, 1946a

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