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Ultrastructure and Description of a New Species of *Telomyxa* (Microspora: Telomyxidae) from the Semiaquatic Beetle, *Ora texana* Champ. (Coleoptera: Helodidae)[1]

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ABSTRACT. A new species of the uncommon microsporidian genus *Telomyxa* (Microspora: Telomyxidae) has been found parasitizing the larval fat body of the semiaquatic beetle, *Ora texana*. In this species, the sporogonic sequence results in the formation of sporocysts measuring 7.7 × 6.5 μm that contain two crested uninucleate spores (averaging 5.7 × 2.2 μm). The spores are essentially oblong/ovate, tapering toward the anterior end and remaining bound together after sporogony by a persistent accessory membrane or sporocyst. The two spores in the sporocyst are produced by an unusual morphogenetic sequence in which, after one mitosis, the binucleate sporont elongates, forming two lobes that fold toward one another and cleave along a central plane, forming two parallel sporoblasts. The general ultrastructural features of this process are described, and diagnostic characters of this new species of *Telomyxa* are presented.

THE genus *Telomyxa* Léger & Hesse, 1910 is comprised of a small group of rare and inadequately studied microsporidia. The genus and the type species, *Telomyxa glugeiformis*, were originally described from naiads of the mayfly, *Ephemera vulgata* L. by Léger & Hesse (11). They placed the genus in a new family, Telomyxidae, believing that the sporocyst of the type species contained a single spore having two polar filaments. Subsequently, Léger & Hesse (12) placed the family in a new suborder, Dicnidea, and suggested that it represented a phylogenetic link to the myxosporidia. Fantham & Porter (5) described, without giving it a specific name, a *Telomyxa* from a calypterate muscoid fly, which Weiser (17) later named *Telomyxa muscarum*.

With the advent of electron microscopy, a more detailed understanding of the fine structure of the sporogonic stages and spores of *Telomyxa* was revealed by Codreanu & Vávra (3) in a study of a microsporidium that they found in naiads of *Ephemera danica* Müller. They determined that the sporocyst of this parasite, which they identified as *T. glugeiformis*, contained two spores obscured by dense metabolic granules and that each spore possessed a single polar filament. Actually, Codreanu (2) had earlier reported that the "pansporoblast" of *Telomyxa* contained two spores when he proposed the term "diplospores" for the paired spores and placed the genus in a new suborder, Polycytosporea. Codreanu & Vávra (3) suggested that the suborder Dicnidea should be rejected but allowed the family Telomyxidae to stand, due to the unique structure of the sporocyst and spores and their occurrence in pairs. More recently, Sprague (15) placed the family in the suborder Pansporoblastina and simultaneously transferred two previously described species, *Glugea campanellae* Kruger, 1956 (8), and *Perezia trichopterae* Weiser, 1946 (16), to the genus *Telomyxa*. Weiser (18) concurrently placed the family Telomyxidae in a new order, Pleistophoridida, in an alternate taxonomic revision when he designated *Telomyxa trichopterae* as the type for a new genus, *Issia*. Larsson (9) presented the ultrastructure of some of the sporulation stages of *T.*

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glugeiformis from *E. danica* collected in Sweden, but gave little new information concerning the fine structure of sporogonic stages, sporoblasts, and spores. Although these revisions leave the proper placement of the family Telomyxidae uncertain, the family and its genus *Telomyxa* are sound taxonomic groups. Later, Larsson (10) proposed a new genus for the family Telomyxidae. The type species, *Berwaldia singularis,* was found in a cladoceran, *Daphnia pulex.*

In the present paper, a fifth species of *Telomyxa,* isolated from the semiaquatic beetle, *Ora texana* Champ., is described and diagnostic characters, based on observations made with the light and electron microscope, are given. As we believe that the term sporophorous vesicle, proposed by Canning & Hazard (1) is somewhat ambiguous, we prefer to use the simpler term, sporocyst, proposed by Debaisieux (4), to identify the membrane-like sac enclosing sporogonic stages and spores.

MATERIALS AND METHODS[2]

Larvae of *Ora texana* Champ. were collected during the months of September through December, 1975–1983, from a semipermanent woodland pool located on S.W. 23rd Drive in Gainesville, Florida. The prevalence of infection increased with each successive generation, exceeding 60% near the end of the breeding season in October.

Light microscopy. Healthy and infected larvae were dissected in the laboratory and microscopic examination indicated that the adipose tissue was the site of infection. Dissected fat cells were used for preparing lacto-aceto-orcein-stained smears (6) and fixed for studies of the ultrastructure of the microsporidium. Fresh spores were photographed using a Zeiss Photomicroscope II equipped with phase contrast objectives.

Electron microscopy. Small pieces (1 mm^3) of infected fat body tissue were excised from larvae and fixed overnight in 4% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.4) at 8°C. Tissues were post-fixed in 1% (w/v) OsO_4, dehydrated in an ethanol series, and embedded in Epon-Araldite (13). Sections were stained with methanolic uranyl acetate, followed by lead citrate (14), and examined at an accelerating voltage of 50 kV on an Hitachi HS-8.

RESULTS

Light microscopy. Infected late instar larvae were sluggish in comparison to healthy larvae and could be recognized by their swollen and discolored abdomens which, in the advanced stages of disease, were noticeably white, particularly along the intersegmental membranes. The enlargement of the abdomen was due to the reproduction of the parasite and concurrent hypertrophy of the fat body, in which large numbers of spores accumulated in the cytoplasm of infected cells. The accumulations of spores imparted the white color to the fat body. The appearance of typical mature rigid sporocysts in fresh smears, each containing two spores, is illustrated in the light photomicrograph (Fig. 13).

Electron microscopy. The earliest stage of the parasite observed in ultra-thin sections were small, oval, uninucleated sporonts (Fig. 1), which were frequently grouped together and intermixed with more advanced stages. The cytoplasm of early sporonts contained a small number of vesicles, numerous dispersed ribosomes, and a few parallel arrays of rough endoplasmic reticulum (rER) as seen in Fig. 2. More advanced sporonts had numerous arrays of rER as well as a greater number of small vesicles. Electron-dense metabolic granules were observed in many vesicles and long, thin, dense plaques were formed external to the plasmalemma (Fig. 2). Prior to nuclear division, the amount of rER within the cytoplasm was noticeably greater and numerous vesicles of various sizes were apparent. Also, fewer dispersed ribosomes were observed (Fig. 3). Concomitantly, small vesicles containing dense granules appeared to be migrating from the nucleus, and the dense plaques formed external to the plasmalemma thickened. A fine granular matrix also began to accumulate on the surface of the dense plaque.

Nuclear division was apparently rapid since few stages undergoing this process were observed; however, cytokinesis and spore differentiation were more gradual and a variety of sequential stages were observed. Cytokinesis was initiated with a slight constriction of the cell midway between the nuclei of the binucleate sporont (Fig. 4). During this process, the cytoplasmic vesicles containing dense secretions appeared to migrate to the plane of cell division (Figs. 4, 5). The fine granular matrix also increased greatly and encompassed the entire sporont (Figs. 4–7). The two lobes of the binucleate sporont appeared to elongate as they grew and fold toward one another along a cleavage plane, forming two parallel sporoblasts joined at their posterior ends (Figs. 4–7). The migration of the vesicles containing dense granules to the edge of the cleavage furrow was apparent until cytokinesis was complete. As cytokinesis neared completion, the rER became concentrated near the nuclei at what becomes the anterior ends of the sporoblasts (Figs. 6, 7).

Sporogenesis began after cytokinesis was complete. As spore formation proceeded, the layer of fine granules external to the spore wall reorganized, forming the sporocyst and a network of tubular structures between the spore wall and the sporocyst (Fig. 8). In the early stages a prominent Golgi-like system developed near the posterior end of each sporoblast in the region where the polar filament was forming (Figs. 8, 9). As the polar filament formed, vesicles with dense granules continued to migrate to the posterior end of the sporoblast where they were apparently expelled from the cell, forming a dense crest on the developing spore wall (Figs. 8, 10). The anchoring disc of the polar filament was also differentiated at this time (Fig. 9). The polaroplast was one of the last spore structures formed and consisted of numerous tightly appressed lamellae. At this time the anchoring disc of the polar filament appeared as a bulbous structure which protruded into the thick endospore wall (Fig. 11). In the mature spore, the polar filament was of a nearly equal diameter along its entire length, narrowing only slightly toward the apex (Fig. 10).

[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee of warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be available.

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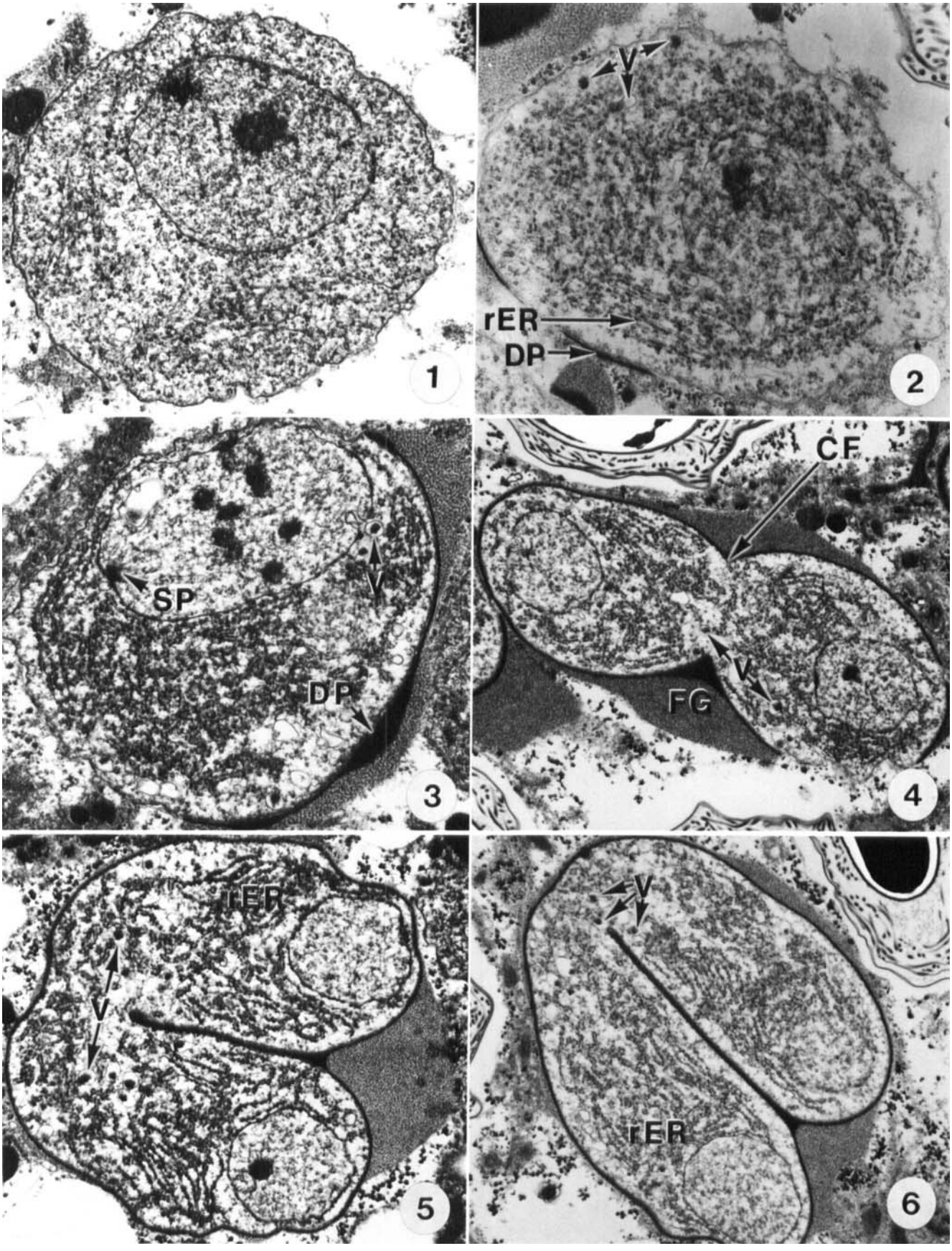
Figs. 1–6. Electron photomicrographs of sporonts of *Telomyxa orae* in larval fat body cells from the semiaquatic beetle, *Ora texana.* 1. Young uninucleate sporont prior to initiation of differentiation. ×13,700. 2. Sporont showing metabolic granules forming a dense plaque (DP) on unit membrane. Vesicles (V) and rough endoplasmic reticulum (rER). ×16,000. 3. Nucleus of a sporont just prior to division. Note the spindle plaque (SP), the thickened dense plaque (DP), and vesicle (V) containing dense granules that apparently have been liberated from the nucleus. ×10,800. 4. A bilobed sporont after mitosis in which cytokinesis has begun. Note the vesicles (V) containing metabolic granules in the vicinity of the cleavage furrow (CF), and the accumulation of fine granules (FG) outside the plasma membrane. ×7100. 5. Parallel lobes of the sporont which have folded toward one another. Note the apparent migration of vesicles (V) toward the edge of the cleavage fold. ×7900. 6. Bilobed sporont showing migrations of rER and vesicles containing metabolic granules to opposite ends of the developing spores. ×7300.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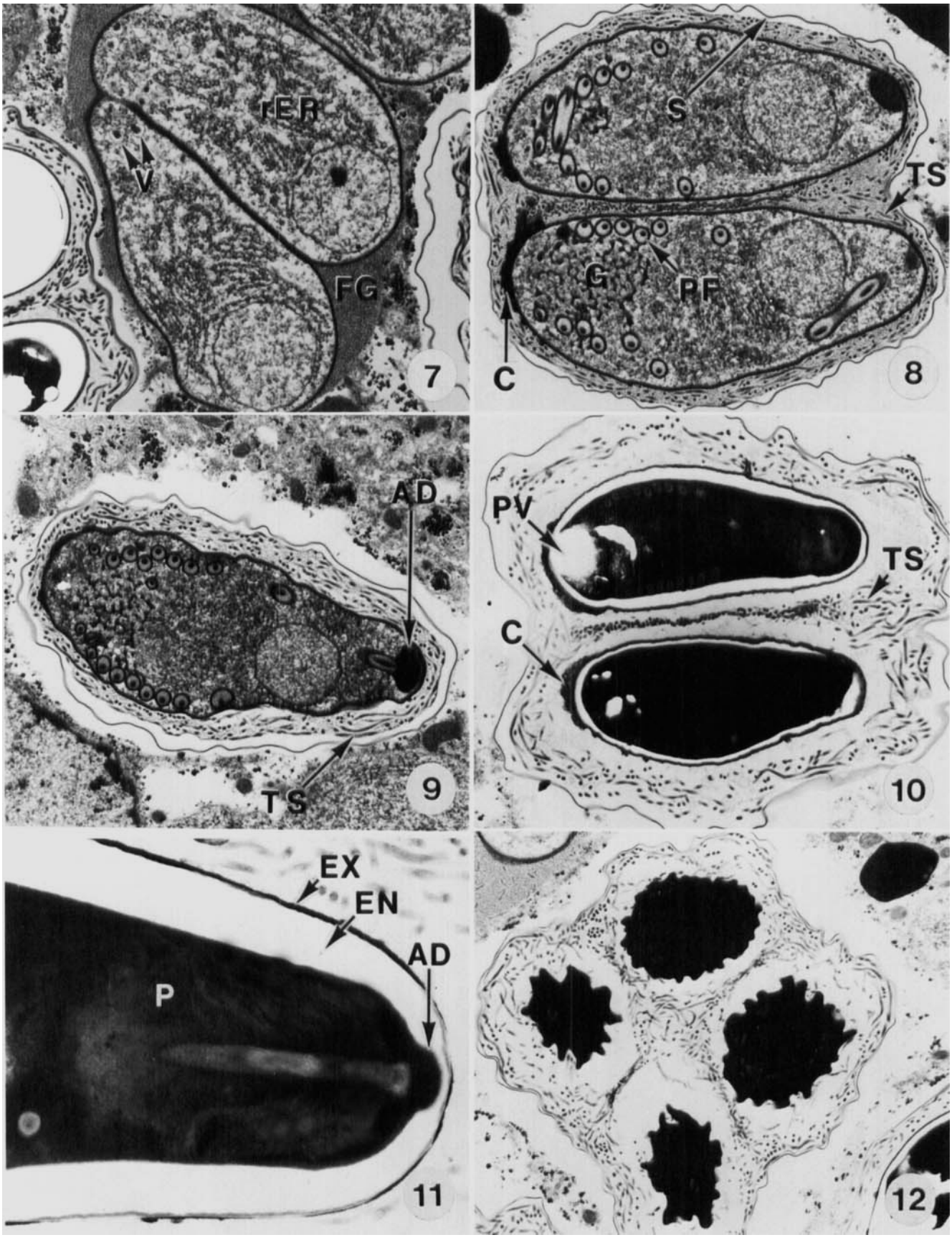
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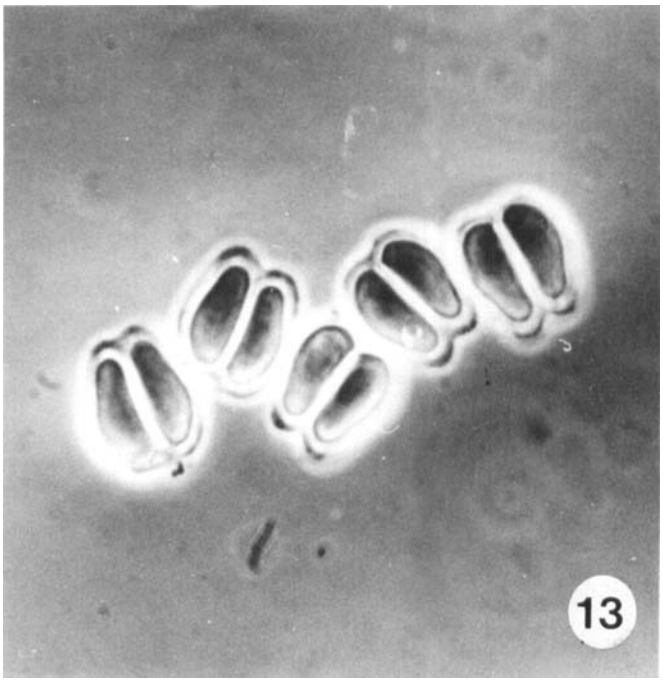

Fig. 13. Light photomicrograph of a fresh smear of sporocysts containing spores from adipose tissue of *Ora texana*. Note the characteristic shape of the sporocysts, each containing two spores, and a highly refractile sporocyst wall. Phase contrast, ×2000.

The sequence described above, which resulted in the formation of two spores in each sporocyst, was typical; however, occasionally, some sporocysts were observed containing three or four spores (Fig. 12). Such sporocysts, containing more than two spores, are apparently the results of an uncommon additional division of one or both nuclei in the binucleate sporont.

DISCUSSION

Although the earliest stages observed were uninucleate sporonts, we believe the microsporidium is a monomorphic species, since only one sporogonial sequence was found in larvae and since no diplokaryotic meronts (stages characteristic of polymorphic forms) were observed in female hosts (larvae or adults).

This *Telomyxa* species differs from *T. glugeiformis* Léger & Hesse of Codreanu & Vávra (3) and Larsson (9) by several characteristics: 1) the structure of metabolic granules which begin as very fine particles in early sporonts, 2) the formation of tubular structures at sporogony, 3) the presence of a posterior vacuole, 4) a crest on the posterior end of each spore. Larsson (9) published an electron micrograph of a sporogonic nucleus illustrating what he interpreted as "modified" synaptonemal complexes, but we believe the structures represent simple mitotic chromosomes. He suggested that the single division of the sporont is a meiosis. It is, however, difficult to understand how both reduction division and chromatid separation can take place in a single division process. Also, we did not observe synaptonemal complexes or meiotic chromosomes in electron photomicrographs or lacto-aceto-orcein-stained preparations of sporogonic stages. It should be noted here that the host for the microsporidium called *T. glugeiformis* by Codreanu & Vávra (3) and Larsson (9) came from the mayfly *E. danica*, not the type host, *E. vulgata* L. of Léger & Hesse. Therefore, the microsporidium in *E. danica* may possibly represent yet another species.

It is difficult to compare our species with *T. campanellae* Kruger and *T. muscarum* (Weiser) because the descriptions of these species (5, 8, 16) are incomplete, indicating mainly only the shape of spores as seen in the light microscope; however, the shape of the spores of these two species are unlike that of our microsporidium.

We find no reason to compare this new species with *Berwaldia singularis* Larsson (10) since the latter appears to be a misidentified species of *Tuzetia* (7). Larsson proposed this species as the type for a new genus of the family Telomyxidae based on the absence of diplokaryotic meronts, the number of merogonial sequences, and the occasional pairing of spores. Larsson's electron micrographs of secondary meronts and early sporonts are identical in structure. Primary meronts are shown only in light micrographs of stained smears and therefore can not be compared with electron micrographs of secondary meronts and young sporonts. Also, photographs presented by Larsson of stained smears illustrating a primary meront, give the appearance of a typical sporogonic rosette commonly observed in stained smears of species of *Tuzetia* (7). Stages illustrated in both light and electron micrographs suggest an advanced state of development where few if any merogonic stages remain; therefore, diplokaryotic stages would not be observed at this time. It is difficult to understand why the author placed this species in the Telomyxidae, a family having spores consistently paired, when only occasional spores are observed in pairs as indicated by Larsson's light micrographs of stained smears.

As mentioned earlier, there is some ambiguity in the use of the word vesicle in the term "sporophorous vesicle" pertaining to the membrane-like sac that contains sporogonic stages and spores of Microspora. Webster's International Dictionary defines vesicle as "a small bladder or blister, a body felt to resemble a bladder, a small thin-walled cavity; and a cyst, vacuole, or cell having the general form of a membranous cavity." Thus, the connotation of this definition is that the word vesicle refers to a walled bladder, cavity, or vacuole that contains little if any substance. On the other hand, Webster defines cyst, among other things, as "a capsule or rounded sheath formed about certain cells when going into a resting stage or becoming transformed into spores"; "the whole structure including the contents of the capsule"; and "one that many protozoans and other animals secrete about themselves as a prelude to a resting stage or a specialized reproductive phase". The term sporophorous vesicle becomes even more ambiguous when we take into consideration those microsporidia that are formed within a membrane-bound-

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Figs. 7–12. Electron photomicrographs of sporoblasts and spores of *Telomyxa orae*. **7.** A developing sporocyst containing two sporoblasts at the completion of cytokinesis. Note the vesicles (V) containing dense granules in the region of the extending cleavage furrow, and the fine metabolic granules (FG) around the sporoblasts. ×7300. **8.** Young sporoblasts in which the polar filament (PF) has begun to form in association with a Golgi apparatus (G). Note that the sporocyst (S) and tubular structures (TS) between the spore wall and the sporocyst have differentiated from the fine granules. Also note crest (C) formed by metabolic granules on spore wall. ×7500. **9.** A sporoblast in which the anchoring disc (AD) of the polar filament is apparent and tubular structures (TS) are prominent. ×7300. **10.** Sporocyst containing two mature spores illustrating tubular structures (TS), posterior vacuoles (PV), and crests (C) on exospore wall. ×89,000. **11.** Section through the anterior end of a mature spore illustrating the polaroplast (P), anchoring disc (AD), endospore (EN), and exospore (EX). ×28,000. **12.** An atypical sporocyst containing four rather than two spores. ×7300.

ed vacuole in the host cell cytoplasm such as in species of *Steinhausia* Sprague, Ormières & Manier (15). Therefore, we prefer the term sporocyst which refers to a membrane-like sac of parasite origin, in which sporogonic stages form spores.

Telomyxa orae sp. n.

Host: Ora texana Champ.

Site: Fat body cells.

Diagnosis: An asexual, monomorphic species. Meiosis not taking place during sporogony and stages of merogony not observed, but believed to be present in very young larvae. Sporogony simple, usually involving a single protracted division, giving rise to two spores enclosed in a rigid sporocyst. Metabolic secretions in the form of very fine granules are involved in synthesis of long tubular structures, which are observed surrounding uninucleate spores within the sporocyst late in sporogony. Spores oblong/ovate, slightly bent or curved, with a thick dense crest on the posterior ends. Sporocysts so rigid that the spores cannot be disrupted from sporocyst even when it is subjected to extreme pressure.

Average size of sporocysts 7.7 × 6.5 μm (fresh), spores measure 5.0–6.1 × 1.9–2.4 μm (fresh) averaging 5.7 × 2.2 μm. Polar filament of nearly uniform thickness throughout, only tapering slightly towards its distal end.

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Myxosporean Infections in Cultured Tilapias in Israel[1]

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ABSTRACT. Five new species of myxosporean parasite are described from cultured tilapias in Israel. These are: *Myxosoma sarigi, Myxosoma equatorialis, Myxobolus israelensis, Myxobolus agolus,* and *Myxobolus galilaeus.* The first four were found in hybrids of *Oreochromis aureus* × *Oreochromis niloticus* while *Myxobolus galilaeus* was found in *Sarotherodon galilaeus.* In addition, *M. sarigi, M. israelensis,* and *Myxobolus* sp. were also found in *S. galilaeus.* In the light of the present study, the taxonomy of myxosporean infections in tilapias is modified. Mature spores may localize in the melano-macrophage centers of the spleen and kidney where they may eventually be destroyed. No cases of mortality have so far been associated with these parasites.

WITH the emerging worldwide interest in the intensification of tilapia culture, it is surprising how little is known of their parasitic infections. This may not only be a reflection of their resistance to disease, but also (33) due to the fact that tilapias are often cultured in areas where diagnostic facilities are minimal or because prior to their cultivation, little interest was shown in these species.

Reports of myxosporean infections in wild tilapias are fairly sparse and have been limited to isolated areas in Africa, both in the west and the great lakes in the east (1, 5, 9, 25, 26). Myxosporeans that have been described are restricted to the genera *Myxosoma* and *Myxobolus* (Table I). (The taxonomic nomenclature of tilapias adopted by Trewavas [40], to genus level only, is used throughout this paper.) Reports from cultured tilapias are minimal (1, 26) and little information other than species and site distribution is known.

Tilapia culture in Israel has been an ongoing concern for about twenty years. At present, the most commonly cultured variety is the hybrid *Oreochromis aureus* (Steindachner) × *Oreochro-*

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