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# Plistophora tahoensis sp. n (MICROSPORIDA, NOSEMATIDAE) IN THE BODY WALL OF THE PIUTE SCULPIN (Cottus beldingii) FROM LAKE TAHOE, CALIFORNIA-NEVADA

ROBERT C. SUMMERFELT [1] and VERLYN W. EBERT [2]

**Abstract**

A new microsporidian parasite from the muscular tissue of the body wall of the Piute sculpin from Lake Tahoe is described. The name *Plistophora tahoensis* is suggested in recognition of the habitat of the host and the uniqueness of the fauna of the lake. Fresh spores were ovoidal and measured 6.05 ± 0.14 by 3.03 ± 0.11 microns for spore length and width, respectively. Fixed and stained spores were somewhat smaller. The polar filament measured 140.9 ± 3.8 microns which was 23.28 times the mean spore length. Cysts, which measured 0.4 to 0.9 mm long, were present in the abdominal body wall between the points of insertion of the pelvic fins. The macroscopic cysts were composed of several smaller cysts derived from infected muscle bundles. Cysts displaced the muscles and weakened the body wall. Uninucleate, binucleate, and multinucleate schizogonic stages were recognized. Sporogony resulted in the development of a sporonts measuring 15-24 microns which produced 20 to 48 spores.

## Introduction

In the course of studying the life history of the Piute sculpin *(Cottus beldingii)* from Lake Tahoe, California-Nevada (Ebert and Summerfelt, 1969), we observed spindle-shaped cysts imbedded in the abdominal wall in 55 of 851 preserved specimens. These cysts were found to contain large numbers of spores of a protozoan parasite of the genus *Plistophora*. The object of this report is to present a description of a new parasite, *Plistophora tahoensis* sp. n.

The Microsporida of the genus *Plistophora* parasitize coelenterates, moluscs, arthropods, fishes and amphibians but they are most frequently described in fishes and insects. Species of *Plistophora* may parasitize fishes of economic importance (Kabat, 1959), affect the marketability (Nigrelli, 1946), and fecundity of the host (Summerfelt, 1964), or cause serious epizootics of hatchery (Wales and Wolf, 1955), or wild fishes (Putz et al., 1965). *Plistophora* have been known in fish hosts since *P. typicalis* Gurley was described in 1893. Although members of this genus parasitizing fish are world wide in distribution, only three of 20 species occurring in fish, *P. ovariae* Summerfelt, 1964, *P. salmonae* and *P. cepedianae* Putz et al., 1965, have been described from fresh waters of North America. The fact that so few species have been reported for North America is probably due to lack of attention and not due to their absence (Putz, 1964).

## Materials and Methods

Fish were obtained principally from preserved specimens collected by California Fish and Game Biologists between September, 1963 and September, 1964 as part of their study of the ecology of the nongame species of Lake Tahoe.[3]

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These fish were fixed in 10% formalin and later preserved in 40% isopropyl alcohol. Fresh spores for use in extruding the polar filament were obtained from specimens collected by sled trawl in May, 1964 from water 2 to 10 feet deep at the south end of the lake and air-mailed frozen to our laboratory.

Although the spore is of small size and it is difficult to make an accurate description of its internal structure by light microscopy, the spore, nevertheless, has remained since Kudo's (1924) monograph to the recent comprehensive treatise of microsporidia of insects by Weiser (1961) as the most important taxonomic feature. Thus, spore morphology has been given strong emphasis.

Biometrical data on both fresh and fixed spores were made at a magnification of 1000X with a calibrated ocular micrometer. Photomicrography and morphometric study of all but stained smears and tissue sections of spores was made by use of the paraffin oil method (POM) of Vávra (1964). Staining was with Heidenhain's iron hematoxylin or Giemsa; hematoxylin - stained sections were also counter-stained with alcoholic eosin. The polar filament was caused to extrude from water suspensions of spores, mixed with a drop of ordinary drawing India ink, by mechanical pressure. After air-drying, the ink smear preparations revealed the opaque spores and extruded polar filaments against a black background.

## Results and Discussions

### *Relation to Host*

Externally, infestation was detected by observing for the presence of spindle-shaped or ovocylindrical, white-colored cysts (figures 1 and 5) which show through the light tan-colored skin of preserved specimens. The cysts are less conspicuous on fresh specimens than the preserved because the light colored abdomen of fresh fish obliterates the contrast between body wall and cyst. Cysts were always found within an area between the base of the point of insertion of the pelvic fins and along the ventral, lateral margin of the abdomen within one cm from the base of the pelvic fins. Figure 4 illustrates a transverse section of the body wall of the host containing two cysts. The long axis of the cysts, like the abdominal musculature, were always oriented in a line parallel to the long axis of the body of the host. Large cysts force the visceral peritoneum, which contains numerous melanophores, to protrude into the abdominal cavity (Figures 1 and 6).

A second type of cyst was observed on one specimen (Figure 2). Cysts on this specimen were spherical protrusions of the dermis, covered by a thin epidermis. These cysts contained spores (Figures 3a and b) of the protozoan *Myxobolus* sp. (Class Myxosporea, Order Myxosporida). The cysts are illustrated here to point out the difference in their morphology and position compared with cysts of *P. ovariae*. The spherical cysts of *Myxobolus* sp. were principally located in the venter of the head, the branchiostegal rays and the base of the pectoral fins. In contrast, the splindle-shaped cysts of *P. tahoensis* were in the muscle tissue; they occurred principally in the body wall between the base of the pectoral fins posterior to the base of the pelvic fins.

Mature cysts of *P. tahoensis* are surrounded by a tough connective tissue sheath which permits the cyst to be easily separated from the musculature of the body wall (Figure 5). The cysts ranged from 2.0 to 6.5 mm in length, and 1.9 to 2.5 mm in diameter. The cysts were centrally located in the body wall (Figures 4 and 8), or excentrically located causing a protrusion inward in the direction of the body cavity (Figure 6), or toward body surface. Transverse and longitudinal sections through the cyst and body wall revealed that each macroscopic cyst was composed of groups of three to five smaller cysts (Figure 7). Each of the latter was surrounded by a fibrous connective tissue (Figures 7 and 8). The cysts correspond to destroyed bundles of muscle fibers of various sizes. The connective tissue sheat corresponds to the interstitial connective tissue, the perimysium. Life stages were

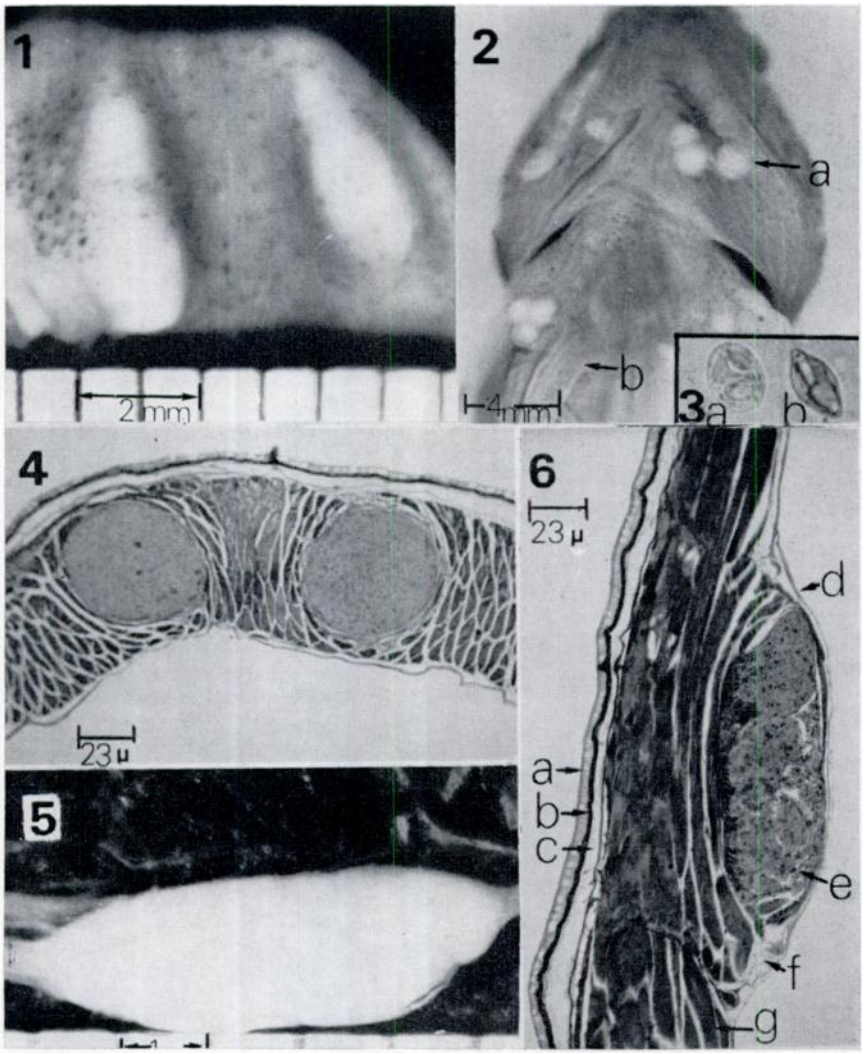


FIGURE 1. *Section of ventral body wall of* Cottus beldingii, *viewed from the inner side, containing two cysts produced by the parasite,* Plistophora tahoensis. *The black spots are melanophores in the visceral peritoneum.*

FIGURE 2. *Ventral view of head and pectoral fins (b) of the Piute sculpin with spherical dermal cysts of* Myxobolus *sp. shown for comparison with spindle-shaped cysts of* P. tahoensis.

FIGURE 3. *Spore of* Myxobolus *sp.: a. front view showing two polar capsules, sporoplasm and vacuole; b. side view showing sutural plane.*

FIGURE 4. *Transverse section of body wall showing two large cysts, like those in Figure 1, which represents fusion of many muscle blocks.*

FIGURE 5. *A cyst removed from host tissue showing the fibrous connective tissue sheath.*

FIGURE 6. *Transverse section through the body wall of a Piute sculpin: a. epidermis with mucous producing cells; b. compact fibrous layers of the dermis; c. thick spongy subcutis composed of reticulated connective tissue cells; d. visceral peritoneum; e. cyst containing numerous sporonts with spores of* P. tahoensis; *f. connective tissue; g. uninfected muscle blocks.*

limited to the cyst. Generally, the most abundant stages in cysts were sporonts, which in turn contained sporoblasts or 20-48 spores (Figures 20 and 21).

Histological detail of a transverse section of the body wall shows an epidermis with vacuoated mucous producing cells (Figure 6a), a thinner layer of compact dermis (Figure 6b), a thick layer of spongy subcutis or subdermis composed of reticulated connective tissue cells (Figure 6c), a large cyst containing numerous sporonts (Figure 6e), the cyst surrounded by intermuscular, fibrous connective tissue (Figure 6f), bundles of muscle (longitudinal section) displaced by the presence of the cyst (Figure 6g) and on the inner side, a thin layer of visceral peritoneum (Figure 6d).

The specific site of infection was muscular tissue (Figures 9 and 10) of the body wall between the points of insertion of the pelvic fins. The parasite invades the muscle fibers resulting in disintegration of the sarcoplasm and myofibrils (Figures 9 and 10). The surrounding cyst produces muscle displacement and weakens the body wall (Figures 4, 6, 7 and 8). Advanced microsporidiosis was characterized by disintegration of the integrity of the muscle fibers and presence of numerous large spore-filled sporonts. The entire cyst was surrounded by a dense intercellular substance of fibroblasts, collagen fibers, and fibroelastic connective tissue (Figure 8). Other organs of the host appear normal and spores were not found in squash preparations of liver, kidney, ovary, or testis.

The parasite occurred within an incidence of 6.5% of 851 preserved fish examined. There was no apparent relationship between frequency of occurrence of parasites in sculpin to depth of capture of the host. Parasite infected fish were captured at depths of 100, 200, 300 and 400 feet and in all months of the year except July. There was a higher frequency of occurrence in fish collected in the last four months of the year, i.e., September through December, than any other similar interval.

The average length of infected sculpin was 2.78 and 2.67 for females and males, respectively. All size classes were represented and there was no relationship to size or age. Cysts were found in 34 females, 19 males and 2 individuals too immature to determine the sex. Of 799 sculpin for which sex was determined, 382 were females and 417 males, i.e., the sex ratio was 1.00 females: 1.09 males is not statistically different from a hypotheses of a 1:1 sex ratio. A chi-square test of the difference in observed frequency of infected males to females was significant ($P<.05$). There is no obvious reason for this difference which may be due to factors influencing exposure to spores.

### *Morphology of the Spore*

The form of fresh and formalin-fixed spores was ovoidal in outline (Figures 11 and 12). The spore case or spore membrane of fresh or formalin-fixed spores, as observed in simple saline-suspensions or POM, were highly refractile and the spore membrane appeared quite thick, 25-30% of the width of the spore. Although spore projections have been revealed in some microsporidia in dry, smear preparations of water suspensions of spores mixed with India ink (Vávra, 1963), the spore margin of *P. tahoensis* is smooth. The shape of stained spores differed from fresh spores in a tendency toward a pyriform shape resulting from dehydration (Figure 13).

A length-frequency analysis of fresh spores produced a bimodal curve; one mode measuring approximately 5.2 mic-

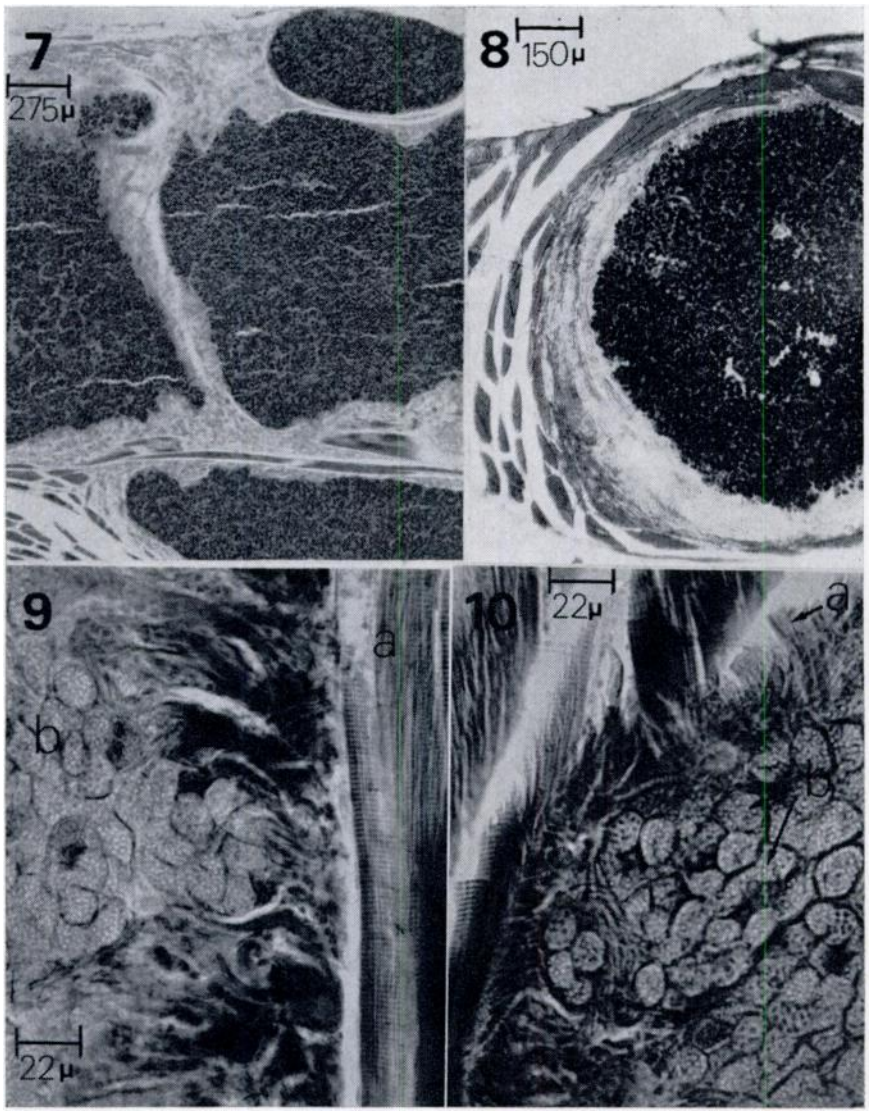


**FIGURE 7.** *Section showing multiple cysts surrounded by myosepta of connective tissue, and small bundles of uninfected muscle.*

**FIGURE 8.** *Advanced cyst containing sporonts and mature spores walled off from the surrounding muscle tissue by a dense band of fibroeleastic connective tissue.*

**FIGURE 9.** *Deposits of sporonts, with numerous spores containing highly refractive spores (b), showing uninfected striated myofibrils (a) and disorganized myofibrils and nuclei, and interstitial connective tissue.*

**FIGURE 10.** *Eroded myofibrils (a), and deposits of sporonts within primary muscle bundles.*

rons and another measuring 6.0 microns. However, this difference is not of the magnitude found in dimorphic form like *P. longifilis* Schuberg, 1910, which has macrospores 12 microns long and microspores 3 microns long (Kudo, 1924). The difference in the present species is not considered of sufficient magnitude to classify it as dimorhphic spore formation.

Inside the spore membrane or shell of fresh or formalin fixed spores two conspicuous vacuoles or clear spaces were visible (Figures 11 and 12), one on each side of the sporoplasm which forms a girdle-like ring located just anterior to the center of the spore. The anterior vacuole was 43.7 ± 2.9 of the spore length and contains the polaroplast (Lom and Vávra, 1961); the posterior vacuole, the larger of the two, which contains the coiled polar filament, was 57.0 ± 3.5% of spore length. The polar filament is attached on the internal corner of the spore (shown in the spore in the upper right corner of Figure 11). The spot represents what is called McMannus positive cap (Lom and Vávra, 1961) or polar mass (Kudo and Daniels, 1963). The polar filament passes on a diagonal through the anterior vacuole and sporoplasm, the thin line diagonally across the anterior vacuole in Figure 11a, to the posterior vacuole, where three or four coils were frequently seen; three coils are visible in the upper spore in Figure 12. The spore width is slightly larger than half the length of the spore; 52% in fresh spores (Table 1). In polar view (Figure 14) the spore is nearly spherical and the polar filament is shown extending from the polar mass located to the right center across to the upper right. The dark crescent in the center of the polar view is interpreted to represent the nucleus.

The appearance of fixed and stained spores, especially in smear preparation varied considerably from slide to slide and also depending on whether hematoxylin or Giemsa stain was used (Table 1). The mean length of unstained, formalin-fixed spores was significantly different ($P<.05$) from the mean length of stained spores; hematoxylin spores were not different from Giemsa stained spores. The width of the spore was not readily affected by fixation, although fixation and staining with the associated dehydration reduced the width of the spore. Identical findings were reported by Summerfelt and Warner (1969) for spores of *P. ovariae*. The ratios computed for vacuole length to spore length were not affected by shrinkage because the differences observed were apparently random (Table 1). Uniformity in these ratios in fresh and fixed spores suggest that the ratios may be of taxonmic value to compare and contrast spores of different species. The spore membrane of fresh (Figure 11c), or unstained fixed spores (Figure 12) is refractory, and appears thicker than in fixed and stained spores (Figure 13).

A single nucleus was present in a girdle-like ring of the sporoplasm of stained spores (Figure 13a) which lies near the middle of the spore. This position for the sporoplasm, and the general ovoidal shape of the spore places *P. tahoensis* in Kudo's type 2 category, differentiated from the type 1 which has a large anterior vacuole occupying the anterior two-thirds of the spore, and in which the sporoplasm is located in the posterior end of the spore (Kudo and Daniels, 1963). Spores of *Plistophora macrospora* (muscles of fish host, *Cobitis barbatula)* and *P. elegans* (ovary of hybrid fish, *Abramis brama* × *Leuciscus rutilus)* are of type 1 and quite distinct from *P. tahoensis* which is quite similar to the mature spore of *P. longifilis* (testis of fish, *Barbus fluviatilis).* A chromatinic mass of uncertain nature was visible in the posterior vacuole of the most stained spores (Figure 13b).

The filament was not present in a polar capsule but extended longitudinally from the anterior end, where it appeared to be attached to the polar mass, to lie coiled beneath the spore membrane in the posterior vacuole. Four or five coils of the filament were visible in the posterior vacuole of fresh or fixed spores. It was not visible in the posterior vacuole of fixed and stained spores. The ejected polar filament and spore appeared refractile in transmitted light against a dark dried ink background (Figures 15 and 16). The ejected polar filament was frequently detached from the spore or if

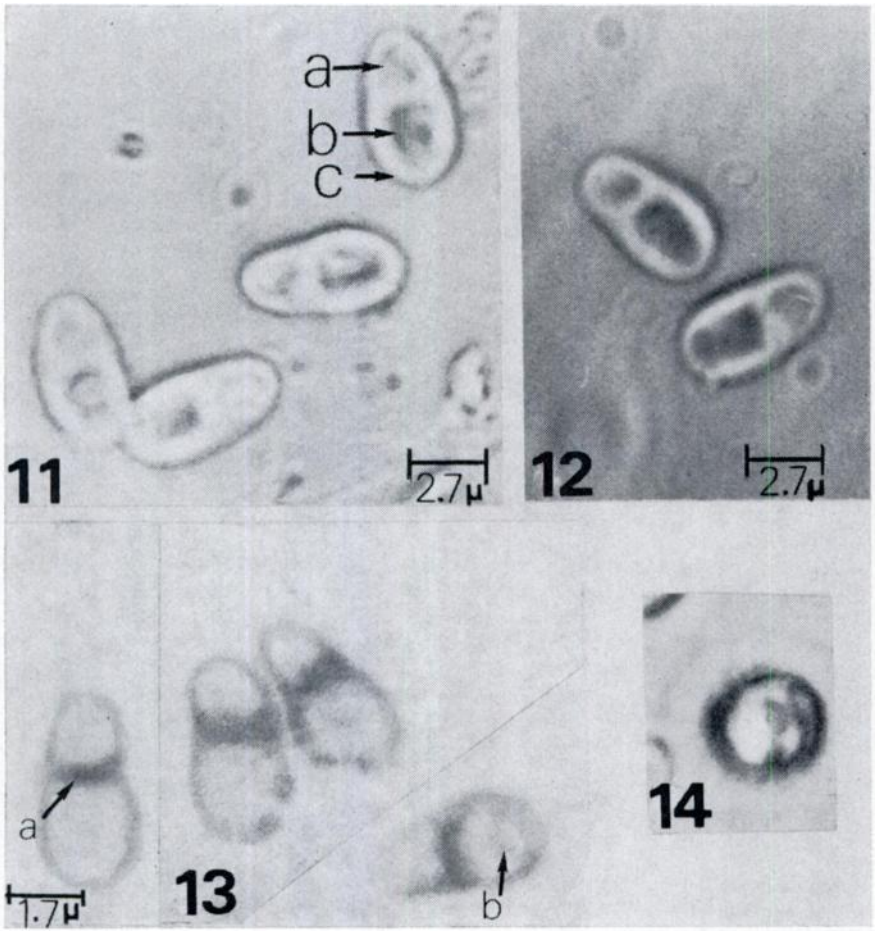


FIGURE 11. *Fresh spores in paraffin oil mount (POM) having polar filament in anterior vacuole (a), posterior vacuole (a), and thick, highly refractive spore membrane (c).*

FIGURE 12. *Formalin-fixed spores in POM showing in the anterior spore and four turns of the coiled filament.*

FIGURE 13. *Formalin-fixed, hematoxylin stained spores showing girdle-like sporoplasm and a single nucleus.*

FIGURE 14. *Polar view of unstained but formalin-fixed spore in water suspension showing nucleus and coiled filament.*

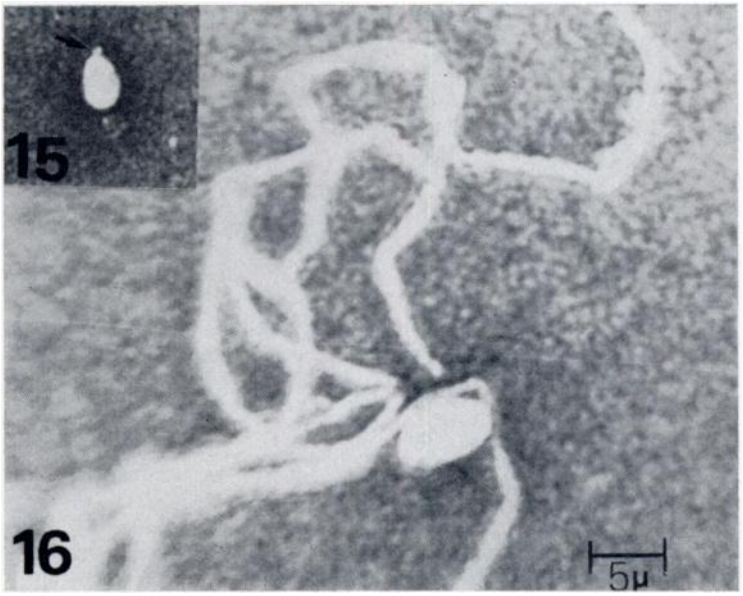


FIGURE 15. *Spore with ejected and detached polar filament showing a small knob on the anterior tip of the spore.*

FIGURE 16. *Ejected polar filament of fresh spores on dried India ink background.*

TABLE 1. *Morphometric comparison of fresh and fixed spores of* Plistophora tahoensis.

| | Mean ± standard error of mean (microns) | | | |
|---|---|---|---|---|
| | Fresh spores [1] | Formalin fixed spores [2] | | |
| | | Unstained | Giemsa | Hematoxylin |
| Length | 6.05 ± .14 | 5.52 ± .05 | 4.94 ± .15 | 4.63 ± .04 |
| Width | 3.03 ± .11 | 2.93 ± .03 | 2.40 ± .05 | 2.61 ± .08 |
| Anterior vacuole | 2.66 ± .08 | 2.43 ± .05 | 2.23 ± .04 | 1.94 ± .06 |
| Posterior | 3.44 ± .05 | 2.78 ± .04 | 2.75 ± .02 | 2.70 ± .06 |
| Spore width ÷ spore length | 0.52 ± .03 | .53 ± .01 | .49 ± .03 | .57 ± .04 |
| Anter. vac. ÷ spore length | 0.44 ± ..03 | .45 ± .04 | .45 ± .03 | .40 ± .07 |
| Post. vac. ÷ spore length | 0.57 ± .04 | .57 ± .03 | .59 ± .02 | .58 ± .03 |
| Polar filament [3] | 140.9 ± 3.8 | — | — | — |
| Polar fil. ÷ spore length | 23.3 | — | — | — |

[1] Measurements of 50 spores.
[2] Measurements of 25 spores.
[3] Measurements of 25 polar filaments.

attached, it was arranged in a haphazard manner around the spore. Spores with an ejected polar filament were swollen in appearance (Figures 15 and 16) when compared to an intact fresh spore (Figures 11 and 12). Also, the spore with an ejected polar filament as shown in Figure 15 frequently had a slight projection or raised area on the anterior end which may represent the point where the polar filament was broken from the spore. The breakage of the polar filament near the point of attachment to the spore may suggest escape of the sporoplasm from the spore rather than ejection through the polar filament as reported by Lom and Vávra (1961). The mean length of 25 ejected polar filaments measured 140.9 ± 3.8 microns which was 23.3 times the mean spore length (Table 1).

### *Developmental Stages*

Detritus consistently made up a high percentage of the total volume of food in sculpin tomachs (Ebert and Summerfelt, 1969). The benthoplagic feeding habits of sculpin would encourage consumption of spores with their food. Assuming then, transmission *per os* would be the most likely mode of transmission, fish would ingest spores which are released following death of the previous host. In the gut, the explosive ejection of the polar filament may inject the sporoplasm into the host's cells through the polar filament. However, if the sporoplasm escapes through an aperature made by the breakage of the polar filament from the spore, the parasite, an amoebula, must actively make its way to the gut epithelium. In either case, the parasite must find its way from the gut epithelium to the musculature of the body wall. This probably occurs via the blood stream. Polar filament extrusion was observed in *P. tahoensis,* emergence of the sporoplasm was not.

The trophozoite (vegetative stage) was not observed. The next life stage, the schizont, was found to dominate the cyst in certain specimens (Figure 17). Large numbers of developing schizonts were arranged in grape-like clusters (Figure 19) and these in turn were arranged in cysts divided by connective tissue septa. The earliest schizont measured 4 to 6 $\mu$ in diameter with a large compact nucleus. Schizonts with 2, 3, 4 and 8 nuclei were seen (Figures 17, 18 and 19). The largest schizonts measured 10 $\mu$. Schizont stages were easily distinguished from sporonts on the basis of their smaller size and indistinct limiting membrane. The thin membrane surrounding the multinucleate schizonts probably facilitates release of mature merozoites. The nature of the host cell was not conspicuous at this stage.

Eventually some merozoites undergo sporogony and formed an early sporont stage (Figure 20). The host cell was well defined, and the sporont membrane was often distinct, due to shrinkage (Figure 21b and c). The sporont nucleus divides several times to form numerous sporoblasts, each of which gives rise to a spore (Figure 21). The mature spores were surrounded by a sporont membrane, and an outer host cell membrane (Figure 21). About 20 to 48 spores were produced from each sporont as a result of sporogamy (Figure 21). Mature sporonts measured, means of measurements of 25 sporonts from tissue sections, 23.4 microns long by 15.3 microns wide and 15.3 microns in diameter.

### *Taxonomic Position*

The species described in the present paper is classified in the family Nosematidae because of the ovoid shape of the spore and because the spore length (6.05 ± 0.14) is less than 4 times the breadth of the spore (3.03 ± 0.11). The parasite is classified in the genus *Plistophora* because each sporont forms a variable number and more than 16 spores, 20 to 48 being the most common in the present species, and because the sporont has a distinct and persistent membrane.

It is assumed that species of *Plistophora* in hosts other than fish need not be considered to establish the validity of the present form as a new species.

The type species for the genus was described from two species of cottid fishes (Family Cottidae) by Gurley (1893), subsequently, according to our tabulations, approximately 51 additional

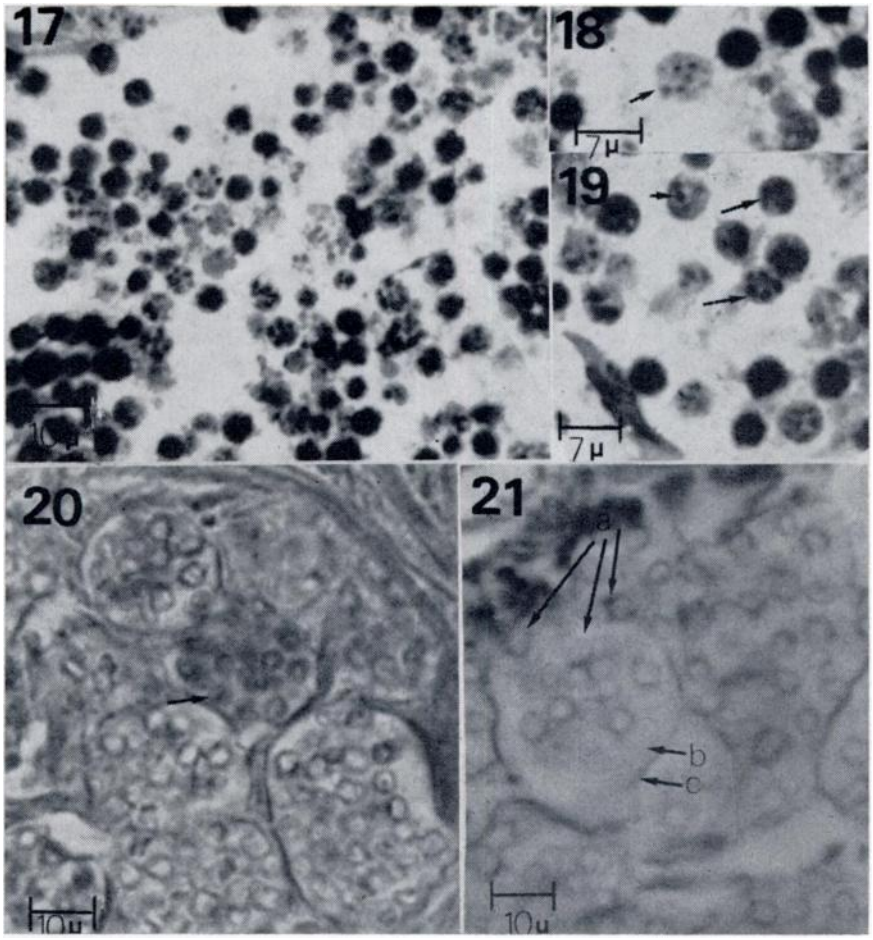


**FIGURE 17.** *Schizogonic stages showing numerous schizonts with one to eight nuclei.*

**FIGURES 18 and 19.** *Schizonts with 1 to 6 nuclei.*

**FIGURE 20.** *Early sporogonic stage with numerous spherical sporoblasts, each with a punctate nucleus (arrow).*

**FIGURE 21.** *Mature sporont, with numerous mature spores (a), and showing, due to dehydration, the fine sporont membrane (b) and the thicker membrane of an infected muscle bundle.*

species have been described of which 20 are known to us to have been described in fish hosts. Of the 16 species of the genus *Plistophora* listed in the first monographic work on the group by Kudo (1924), ten were described in fish; whereas, of the additional species subsequently described ten have been described from fish hosts: *P. ehrenbaumi* Reichenow, 1929; *P. hyphessobryconis* Schaperclaus, 1941; *P. macrozoarcidis* Nigrelli, 1946; *P. oolytica* Weiser, 1948; *P. gadi* Polyansky, 1955; *P. ovariae* Summerfelt, 1964; *P. salmonae* and *P. cepedianae,* Putz et al., 1965; *P. dalli* Zhukov, 1962; *P. peponoides* Schulman, 1962.

Measurements of spore length and width of *P. tahoensis* (Table 1) compared to spore measurements of microsporoidia found in fish places the present species intermediate in size between two of the smaller species, *P. acerinae* and *P. typicalis,* measuring approximately 3 × 2 $\mu$, and the largest, *P. longifilis,* a dimorphic species, with macrospores 12 × 6 $\mu$. Four species of *Plistophora* which occur in fish hosts have spore measurements within 2 microns of the mean length and width of the present species; *P. macrozarcidis, P. sciaenae, P. salmonae* and *P. cepedianae. P. macrozoarcidis* is the most similar in size (length 3.5 to 5.5, width 2.5 to 3.0), and also occurs in muscle tissue, but the host is the marine fish, *Macrozoarces americanus* (Nigrelli, 1946). Spores of *P. sciaenae* are slightly smaller in size and differs in that it was described from the connective tissue of the ovary of *Sciaena australis* in Australia. *P. salmonae,* synonymous to *Plistophora* sp., described by Wales and Wolfe (1955), is the only previous species described from a California fish, but it differs from *P. tahoensis* because it parasitizes gill filaments of the host rather than muscular tissue. The cyst of *P. cepedianae* is located in the visceral cavity. Although the Microsporida are not species specific, species occurring in more than one host are organ specific (Kudo, 1924).

The present species can be distinguished from other species of *Plistophora* in fishes on the basis of a combination of spore size and shape, length of polar filament, number of spores produced by the sporont, dimensions of the sporont, habitat and geographical location of the host and site of infection within the host.

It is proposed, therefore, that the present form be considered a new species designated *P. tahoensis* in recognition of Lake Tahoe. This lake ranks as the tenth deepest lake in the world and has a number of endemic invertebrate fauna (Frantz and Cordone, 1966).

## Acknowledgments

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