

## ***Microsporidium rhabdophilia* n. sp. from rodlet cells of salmonid fishes**

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**Abstract.** A microsporidian parasite, *Microsporidium rhabdophilia* n. sp. is described from rodlet cells of salmonid fishes. Chinook salmon, *Oncorhynchus tshawytscha*, coho salmon, *Oncorhynchus kisutch*, steelhead rainbow trout, *Salmo gairdnerii gairdnerii*, and various strains of domesticated rainbow trout were found infected. In living wet mount phase-contrast observations of various tissues, 16 mature spores could be seen infecting the nucleus of rodlet cells. The number of spores infecting each nucleus generally numbered 16 and spores were not found in any cell or organelle other than the nucleus of the rodlet cell. All observations were limited to California hatchery-reared fish.

### **Introduction**

Routine phase-contrast examinations of wet mount preparations of various tissues and organs of fish often reveal structures thought by some to be of a parasitic nature and by others to be glandular cells of fish epithelium. Thélohan (1892) originally described these cells as protozoan parasites of fishes. Plehn (1906) described them as glandular cells and reported them in the endothelial lining of the circulatory system. Subsequently, Plehn (1924) referred to them as glandular rod cells and reported them not only from the heart and circulatory system, but also from the lymphoid tissue of the liver and epithelial tissues of the intestine, uriniferous tubules and ureters.

The nature and function of the rodlet cell remains controversial. Bannister (1966), Iwai (1968), Mourier (1970) and Mayberry, Marchiondo, Ubelaker & Kazić (1979) considered it to be a parasite. Flood, Nigrelli & Gennaro (1975) and Leino (1974), studying the cell's ultrastructure, favoured either a physiological or secretory interpretation. Bullock (1963) described the intestinal histology of salmonid fish and on the basis of the occurrence of rodlet cells in many species of fish and in a variety of organs of these fish, suggested that they are normal components of fish tissues.

The purpose of this report is to describe a new microsporidian parasite, *Microsporidium rhabdophilia* and its unique affinity for the nucleus of the rodlet cell. Host cell specificity within the Microspora has long been recognized. Kudo (1924) called attention to the microsporidian characteristic of host cell specificity and stated that there are numerous cases of microsporidia invading only a specific cell of the host

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animal. Observations of this parasite suggest an even greater degree of specificity, invading only the nucleus of the rodlet cell. Uninfected rodlet cells from intestinal epithelium of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), are illustrated for reference (Figs 1 & 2). The typical crescent-shaped character of the rodlet cell nucleus shown here is significantly altered when infected by *M. rhabdophilia*, becoming, in most cases, imperfectly spherical in character.

## Materials and methods

Materials used in this study were collected from hatchery-reared fish from the Mad River and Mojave River installations of the State of California Resources Agency, Department of Fish and Game. Three additional state hatcheries, located on the American, Feather and Mokelumne rivers, have been found infected.

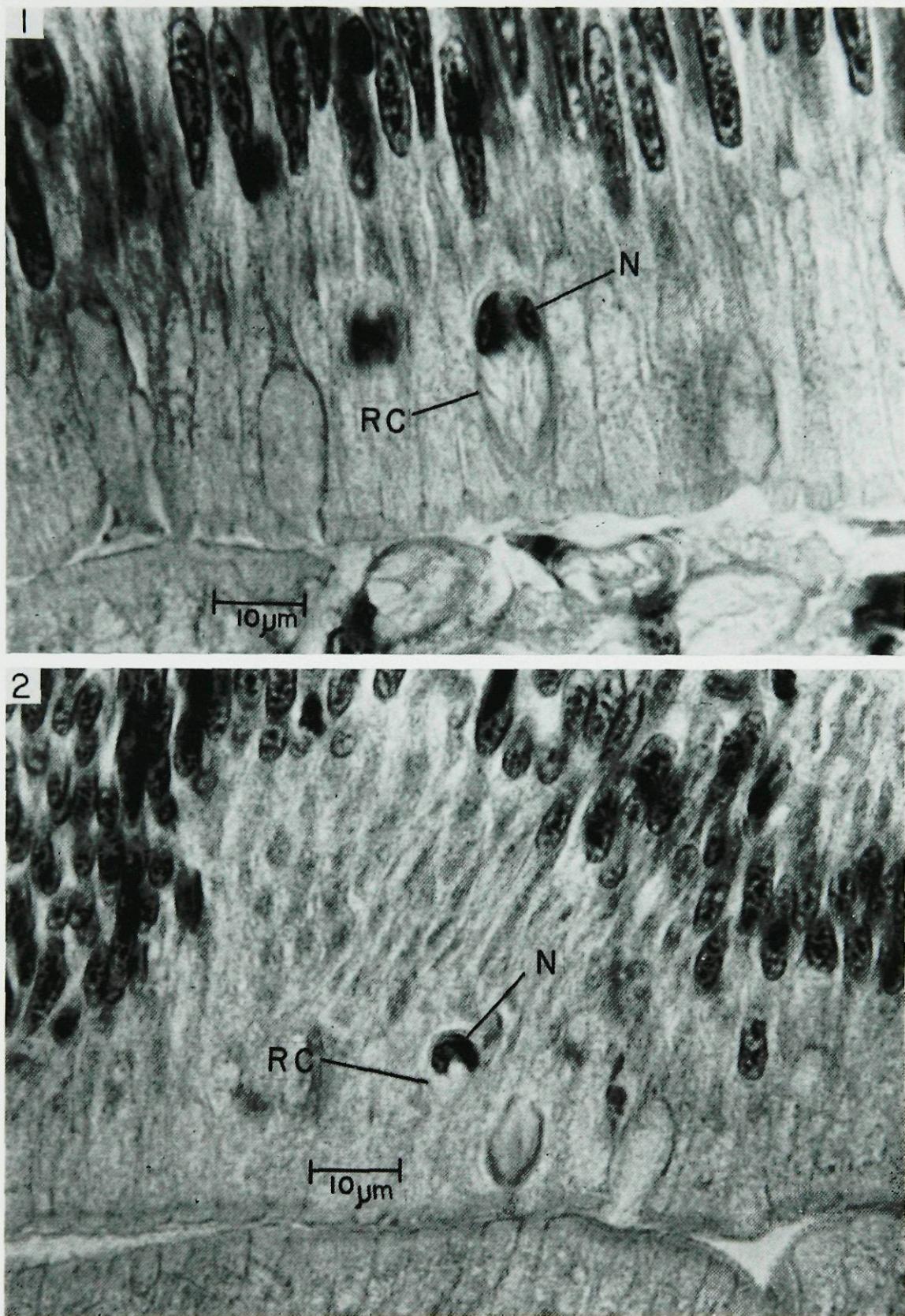
Observations of living tissue (wet mount preparations made by coverslip scrapings of intestine, gill and skin) were made with a Leitz, Dialux phase-contrast microscope. Phase-contrast photomicrography was done with a Nikon, microflex automatic camera.

Sectioned material (intestine) was fixed in Bouin's solution, embedded in paraffin wax and stained with Delafield's haematoxylin and eosin (H&E). Photomicrography was done with a Zeiss photomicroscope equipped with apochromatic brightfield objectives.

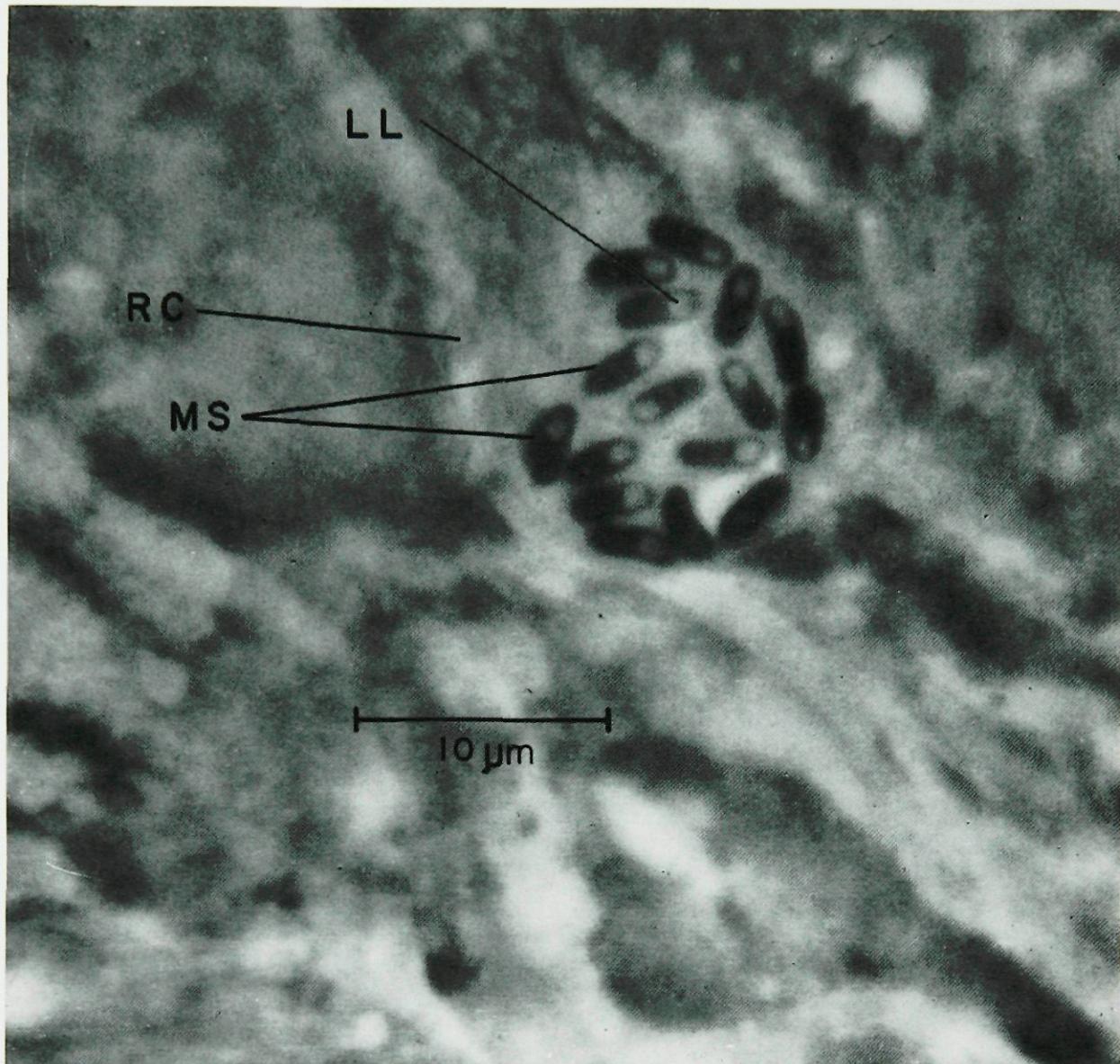
Biometrical data on fresh spores were obtained at  $\times 900$  with a calibrated ocular micrometer using phase-contrast equipment. Sectioned spores were measured at  $\times 1250$  with a brightfield  $\times 100$  apochromatic oil immersion objective and an ocular micrometer.

## Observations

In December 1972, during a routine examination of fish from the Mad River Hatchery, rodlet cells from the intestinal epithelium of a 20-cm yearling chinook salmon, *O. tshawytscha*, were found to be parasitized by an intranuclear microsporidian (Fig. 3). This parasite was recognized because of the general similarity to the species *Pleistophora salmonae* (Putz, Hoffman and Dunbar, 1965), a gill parasite seen in some California salmonid fish (Fig. 4). Subsequent observations of *M. rhabdophilia* were made in March 1973 in rodlet cells from intestinal scrapings of 6–8-cm juvenile steelhead trout, *Salmo gairdnerii gairdnerii* Richardson, and again in August 1973 in rodlet cells from gill scrapings of 15–20-cm yearling chinook and coho salmon, *O. tshawytscha* and *O. kisutch* (Walbaum). On one occasion this parasite was found as a mixed infection with *P. salmonae* on the gills of a chinook salmon from the Mad River Hatchery. Figure 4 shows a group of what is presumed to be a liberated pansporoblast containing 16 fully developed subcylindrical spores of *M. rhabdophilia* which contrast with the much larger spores of *P. salmonae*. Since 1976, *M. rhabdophilia* has



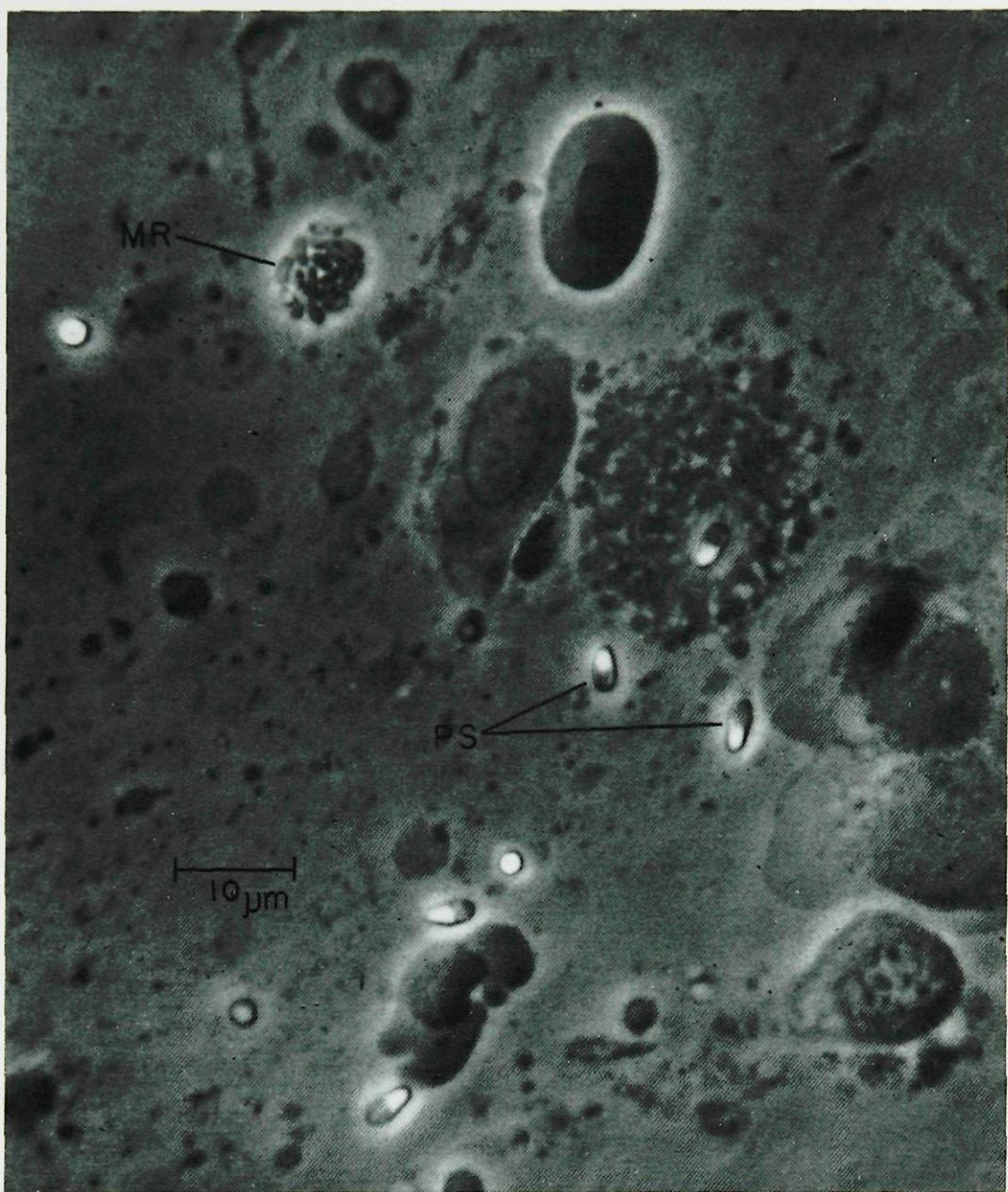
**Figures 1 and 2.** Haematoxylin and eosin sections of intestinal epithelium of chinook salmon illustrating shape and position of the uninfected nucleus of the rodlet cell. Figure 1. Longitudinal section through rodlet cell and nucleus. Figure 2. Cross section through rodlet cell and nucleus; RC, rodlet cell; N, nucleus (uninfected).



**Figure 3.** Intestinal scraping of yearling chinook salmon showing disintegrating rodlet cell and 16 distinct spores; RC, rodlet cell; MS, microsporidian spore; LL, longitudinal line (phase contrast).

been observed in various rainbow trout stocks at several California hatcheries. At the Mojave River Hatchery, rodlet cells from intestinal epithelium, gills and skin from various stocks of rainbow trout are frequently infected. Occasionally, heavy infections occur in the intestinal epithelium and skin (Melvin Willis, personal communication, California Department of Fish and Game). Intestinal inflammation and above-normal hatchery mortalities have been observed with a high prevalence of infection, but it is not known whether the inflammation is due to the presence of large numbers of microsporidia.

The source of this parasite in California hatcheries is unknown, but it is most likely from fish populations in major rivers serving as a water supply to many California hatcheries. The American River, Feather River and Mokelumne River hatcheries are located below impoundments on major river systems and receive their water from reservoirs above. The Mad River Hatchery water supply is from wells located in river gravel and is known to contain common fish pathogens. At Mojave River



**Figure 4.** Individual microsporidian spores of *Pleistophora salmonae* from gill scraping of chinook salmon, photographed as a mixed infection with spores of *Microsporidium rhabdophilia* contained within what is presumed to be the unseen pansporoblastic membrane (phase contrast). PS, spores of *Pleistophora salmonae*; MR, spores of *Microsporidium rhabdophilia*.

the water is pumped from an underground river that rarely carries a surface flow. There are frequent exchanges of fish here, however, and it is highly possible *M. rhabdophilia* was introduced into this hatchery from another area.

#### Description

**Type host.** Chinook salmon, *O. tshawytscha*.

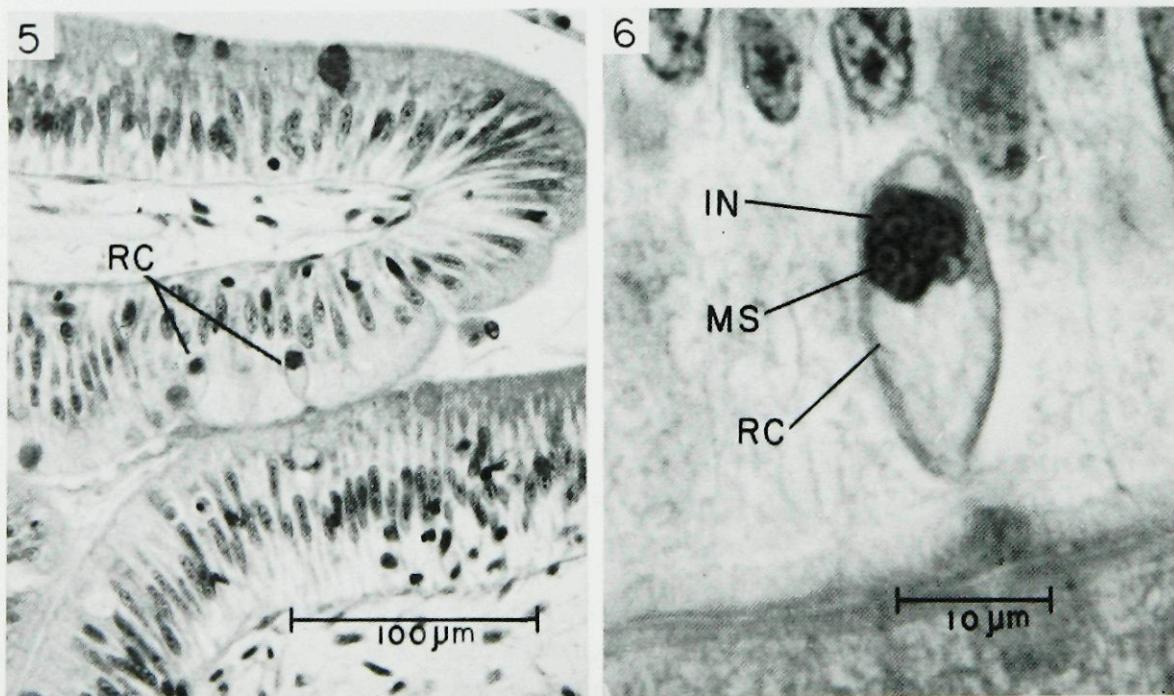
**Site of infection.** Nucleus of rodlet cells in skin, gill and intestine (Figs 3-6).

**Type locality.** Mad River Fish Hatchery, Arcata, California, U.S.A.

**Type slides.** U.S. National Museum, Smithsonian Institution. Holotype, USNM 30071; Paratype, USNM 30072.

**Spore.** In fresh smears the vacuolated, slightly curved subcylindrical spores measure 2·90  $\mu\text{m}$  (2·25–3·47  $\mu\text{m}$ ) long by 1·05  $\mu\text{m}$  (0·82–1·22  $\mu\text{m}$ ) wide. The vacuolated portion occupies approximately one-third of the spore length (Fig. 3). Repeated observations of living spores and subsequent fixation of identical material have shown that the fixation process significantly alters spore morphology. In H&E sections, spores can be seen tightly packed within the nucleus of the rodlet cell (Figs 5 & 6) where they appear spherical and measure 1·48  $\mu\text{m}$  (1·35–1·58  $\mu\text{m}$ ) in diameter. A coiled polar filament was not seen, either in fresh or in sectioned material. In phase contrast  $\times 900$  oil immersion observation, a very fine longitudinal line can be seen within the vacuolated portion of the spore. This line, possibly the straight or basal portion of the polar filament, is unmistakably present when living spores are viewed under these conditions. Its fine character, however, makes it difficult to resolve photographically and for this reason is discernible in only one of the spores illustrated in Fig. 3.

**Vegetative stages.** In living wet mount preparations liberated spore clusters, possibly representing pansporoblasts, were observed. Spores within these clusters commonly numbered 16, but on a number of occasions, clusters were found with spores too numerous to count. In these instances it could not be determined whether sporogony resulted in the formation of more than 16 spores developing from a single sporont, or whether two or more sporonts in one cell each produced 16 spores that were being held intact by an unseen rodlet cell nuclear membrane. Based on hundreds of observations of infected rodlet cells it is this author's belief that the infrequent occurrence of more than 16 spores appearing in free spore clusters and in intact



**Figure 5.** Section illustrating the location of rodlet cells infected with *Microsporidium rhabdophilia* within the intestinal epithelium of chinook salmon; RC, rodlet cell (H&E).

**Figure 6.** Oil immersion photomicrograph of infected rodlet cell shown on right in Figure 5; IN, infected nucleus; MS, microsporidian spores; RC, rodlet cell (H&E).

Table 1. A comparison of three Microsporidia found in California fish

	<i>Microsporidium rhabdophilum</i>	<i>Pleistophora salmonae*</i>	<i>Pleistophora tahoensis†</i>
Host	Chinook salmon, coho salmon, rainbow trout, steelhead, rainbow trout	Kamloops rainbow trout, steelhead, rainbow trout, kokanee, sculpin	Paiute sculpin
Site of infection	Nucleus of rodlet cells from skin, intestine and gills	Cysts throughout tissue of gill filaments	Muscle tissue
Locality reported	American River, Feather River, Mad River, Mojave River, Mokelumne River State Fish Hatcheries	Widespread in hatchery and wild fish	Lake Tahoe
Morphometrics‡	Fresh § (mean ± mean deviation)	Fixed ¶ (mean ± mean deviation)	Fresh (mean ± mean deviation)
Spore:			Fixed (mean ± mean deviation)
length	2.90 ± 0.24	Spherical 1.48 ± 0.05	7.5 —
width	1.05 ± 0.05	2.4 —	— —
		Mean Range	Range
		—	4.2–5.3 1.7–2.8
		—	6.05 ± 0.14 3.03 ± 0.11
		—	4.63 ± 0.04 2.61 ± 0.03

\* Information from Wales &amp; Wolf (1955); Putz, Hoffman &amp; Dunbar (1965).

† Information from Summerfelt &amp; Ebert (1969).

‡ All measurements in µm.

§ Measurements of 38 spores.

¶ Measurements of 17 spores.

rodlet cells is most likely the result of a multiple infection, rather than in a variation in the number of spores being produced by a single sporont. In sectioned material spore clusters within the rodlet cell nucleus were irregular in shape and appeared to be modified, in some cases, by the shape of the rodlet cell nucleus (Fig. 6). Their maximum dimensions, in sectioned material, averaged 6.17  $\mu\text{m}$  and ranged between 5.67 and 7.52  $\mu\text{m}$ . In no case have other vegetative stages been recognized, either in wet mount preparations or in sections.

## Discussion

Rodlet cells are routinely observed in California, in a variety of domesticated and wild fish and from a variety of organs of these fish. Their common occurrence in various species and organs of fish, combined with their apparently benign character, leads this author to conclude that rodlet cells are normal components of fish tissues. Because *M. rhabdophilia* is found in what is believed to be a normal component of fish tissue, it is described as a parasite of fish.

Since the present species does not clearly show any of the known characters of presently described genera it is placed in a collective group, *Microsporidium*, according to Sprague (1977). This is an assemblage of identifiable species of which the generic positions are uncertain and treated as a generic group for taxonomic convenience. Most likely this species is a new genus but present data are too few for characterizing it. Species designation *M. rhabdophilia* is suggested because of the apparent unvarying affinity of this parasite for the nucleus of the rodlet cell.

At the present time only one other microsporidian is known to infect salmonids. It was originally reported in gills of rainbow trout by Wales & Wolf (1955) and later described as *Pleistophora salmonae* by Putz, Hoffman & Dunbar (1965).

Another microsporidian known to infect native fish in California, *P. tahoensis* is found in the muscle tissue of the Paiute sculpin from Lake Tahoe, California (Summerfelt & Ebert 1969). *M. rhabdophilia* can be distinguished from the two species of *Pleistophora* in California fish on the basis of spore size and shape and by this species unique affinity for the nucleus of the rodlet cell. A comparative analysis of these three species is given in Table 1.

## Acknowledgment

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