COMPARATIVE MORPHOLOGY AND HOST-PARASITE STUDIES OF *TRICHOPHYRA CLARKI* (N. SP.) ON CUTTHROAT TROUT (*SALMO CLARKI*)

by

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of

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in

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VITA

Richard Anderson Heckmann was born in Salt Lake City, Utah, on December 7, 1931, the son of William C. and Olivea A. Heckmann. He moved to Providence, Utah in 1932 where he attended elementary school. He graduated from South Cache High School in June 1950 and attended Utah State University from September 1950 to June 1954 receiving a Bachelor of Science degree with a major in Zoology in June 1954. He then entered the military service for two years and was given an honorable discharge with the rank of First Lieutenant November 1956. He entered graduate school at Utah State University January 1957 and received a Master of Science degree in Zoology June 1958. He started a doctoral program at the University of California (Davis) September 1958 but terminated studies to accept a position in the biology department at Contra Costa College January 1961 where he taught biology courses for six years. During the academic years 1958-59 and 1959-60 he received Pauley Research Fellowships to study protozoan parasites of sea urchins in Hawaii. During his tenure at Contra Costa College he received two National Science Foundation Fellowships for summer study at the University of Washington (Radiation Biology) and at the University of California (Berkeley) (Recent Advances in Protozoology). He entered Montana State University September 1967 to continue his doctoral requirements. He held a graduate teaching assistantship from 1967-68 and received a National Science Foundation Traineeship (Intermediate and Terminal year) in September 1968. He was married to the former Karen Lynn Olson of El Cerrito, California in 1963 and they have three daughters, Lisa, Nancy and Amy Elizabeth. He has accepted a teaching position at Fresno State College, Fresno, California.

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Dr. C. J. D. Brown directed the study and assisted in the preparation of this manuscript. Dr. Glenn Hoffman confirmed the identification of the protozoa. Drs. Gary Strobel, Akiyo Shigematsu, Robert Hull, Lyle Meyers, Datus Hammond, and Thomas Carol provided certain materials and laboratory facilities. The National Fish Hatchery, Ennis, Montana and the State Fish Hatchery, Big Timber provided experimental fish. The Fish Hatchery Development Center at Bozeman provided tanks for transporting fish. Excellent cooperation was received from the National Park Service and U. S. Sport Fisheries and Wildlife. Help in the field was given by Messrs Lee Mills, Jim Liebelt, Keith Johnson, George LaBar, Gary Lewis and Dr. Robert Olson. Mr. Richard Jacobson helped with Ouchterlony plate preparation. I wish to thank my wife, Karen, for her encouragement and patience during this study.

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ABSTRACT

The host-parasite relationship was studied for two new species of *Trichophyra* in Yellowstone Lake fishes. *T. clarki* and *T. catostomi*, parasitic in cutthroat trout *(Salmo clarki lewisi)* and longnose suckers *(Catostomus catostomus)* respectively, were differentiated by; mensural data, host specificity, morphology and electron microscopy. *T. clarki* is larger (80.7 versus 43.6 microns average length) and has more microtubules within the tentacles (85 and 111 versus 57 and 62 outer and inner rings) than *T. catostomi*. Auxiliary tentacles are present in 95% of *T. catostomi* and 35% of *T. clarki*. All cutthroat trout 14 cm in total length and 50% of the adult longnose suckers from Yellowstone Lake were infected with trichophyrans. No suctorians were found in fry or fingerlings. *Trichophyra* was also found in the gills of brown trout *(Salmo trutta)* and rainbow trout *(Salmo gairdneri)*. Light microscopy disclosed extensive pathology of gill epithelium in longnose suckers due to *T. catostomi* but no damage was observed for *T. clarki*. Electron microscopy shows damage to host gill cells by both parasites which probably is related to the respiratory activity of the host. Both parasites form attachment helices, which originate in the protozoan cell membrane, and function for maintenance of parasite position on the host cell. There was no uptake of ^{14}C, patent experimental infection or specific antibodies by *T. clarki*. A prepared trichophyran antigen generated antibody formation by injected fish. *T. catostomi* may use necrotic gill tissue for food and there may be use of mucous by *T. clarki*. Statistical differences were observed between hatchery and wild cutthroat trout for nonprotein nitrogen and oxyhemoglobin. There was no significant differences between experimental (infected) and control hatchery fish. There was sexual differences (40% male and 37% female) for hematocrits of wild trout.

INTRODUCTION

During the summer of 1968, 137 cutthroat trout *(Salmo clarki lewisi)* from Yellowstone Lake, Yellowstone National Park, Wyoming, were examined for parasites. The survey disclosed two new records for protozoa inhabiting the gills and blood of this species. The gill parasite was present in all fish examined while the blood protozoan was found in only one specimen. The following summer, the same gill protozoan was present in all adult cutthroat trout, in 12 of 20 longnose suckers *(Catostomus catostomus)* and absent from 35 redside shiners *(Richardsonius balteatus)* examined. Gills of cutthroat trout from the Yellowstone River, below the Upper and Lower Falls, had another parasitic protozoan which was new to this fish.

Linton (1891), Woodbury (1934) and Bangham (1951) gave lists of parasites for Yellowstone Lake fishes while Scott (1935), Simon (1935) and Cope (1958) published short reports concerning some of the known fish parasites from this lake. There is little information on the protozoan parasites of fishes from this locality. Bangham (1951) reported one *(Myxosporidia)* from two of 291 cutthroat trout examined. Hoffman (1967) lists only five protozoan parasites for cutthroat trout from all localities.

Trichophyra (Claperede and Lachman 1859) was found in the gills of all adult cutthroat trout examined from Yellowstone Lake and was the organism selected for further study. *Haemogregarina* (Danilevski 1885)

was present in only one fish and *Trichodina* (Ehrenberg 1831) in one of 13 specimens examined below the Upper and Lower Falls of the Yellowstone River.

Butschli (1889) reported *Trichophyra* in perch *(Perca)* and pike *(Esox)* from Europe and assigned the species name *T. piscium*. Davis (1937, 1942) was the first to report *Trichophyra* in the Northern Hemisphere. He assigned the names *T. micropteri* and *T. ictaluri* for the gill parasites of smallmouth bass *(Micropterus dolomieui)* and channel catfish *(Ictalurus punctatus)* respectively. No name was given for *Trichophyra* in brook trout *(Salvelinus fontinalis)*. He also was the first to suggest that it may have a pathogenic effect. Chen Chih-leu (1955) and Prost (1952) added to European records by assigning *T. sinensis* to infected white and black Amur fishes and *T. intermedia* to infected salmon-fry *(Salmo salar)*. Lom (1960) added to the host record for *T. intermedia* by including brown trout *(Salmo trutta)* and three other fishes in Czechoslovakia. Culbertson and Hull (1962) summarized all host records of *Trichophyra* and suggested that *T. piscium* be used for all species found in fishes. This suggestion was followed by Sandeman and Pippy (1967) who reported on four salmonids of New Foundland infected with *Trichophyra*. Hoffman (1967) stressed the need for further taxonomic study of trichophyran species and their symbiotic effects.

Trichophyra belongs to the Subphylum Ciliophera (Doflein, 1901), Class Ciliatea (Perty, 1852), Subclass Suctoria (Haeckel, 1866), Order Suctorida (Claperede and Lachmann, 1858) and Family Dendrosomatidae (Fraipont, 1878) (Honigberg 1964 and Kudo 1966). Suctorian parasites are not unique to fishes. Other hosts include horses (Hsuing, 1928), bathynellid worms (Masuzo, 1962), isopods of the Sahara desert (Delamare-Deboutiville, 1959) etc.

The objectives of this study were: to determine the distribution and comparative morphology of *Trichophyra* in Yellowstone Lake fishes and to examine the host-parasite relationship. Study on this project extended from June 1968 to September 1969. To date there has been no ultrastructural description of *Trichophyra*. Meyer (1966) questioned the parasitic nature of *Trichophyra ictaluri* and stated the main effect may be mechanical interference with respiration. Davis (1967) reported heavy loss among fingerling and adult smallmouth bass, raised in hatcheries, due to *T. micropteri*. These were attached to the gills by a broad base, closely applied to the epithelium, causing hyperplasia and necrosis of host tissue.

MATERIALS AND METHODS

Distribution and Morphology

Fishes, ranging in total length from 3.5 to 45.7 cm, were obtained from several sites in Yellowstone Lake and Yellowstone River (Fig. 1). Each fish was examined, immediately after sacrificing, for external and internal parasites. The gills, fins and viscera were removed and placed in finger bowls containing physiological saline. The content of each finger bowl was examined using a dissecting microscope. Each organ, the surface of each appendage and the body surface was scraped with a scapel and part of the material was placed on a depression slide containing a drop of distilled water for observation using a compound microscope. Material, from each scraping, was also placed on glass slides and stained with methyl green-pyronin Y (Jordon, 1955). Blood smears were duplicated from the heart and peripheral circulatory system of each fish. After the blood smear had air-dried, it was fixed with methyl alcohol and stained with Giemsa. Each slide was observed with high dry and oil immersion objectives. A record was kept for each fish indicating length, weight, sex, location of sample, hematocrit and hemoglobin percent, parasites observed and organ infected.

Intact gills, infected with *Trichophyra*, were scraped and the macerate was examined using the following vital stains; Giemsa, acidified methylene green, aqueous nigrosin, and Nolands stain modified by Farley (1965). Infected gills were also fixed with 10% formalin for permanent

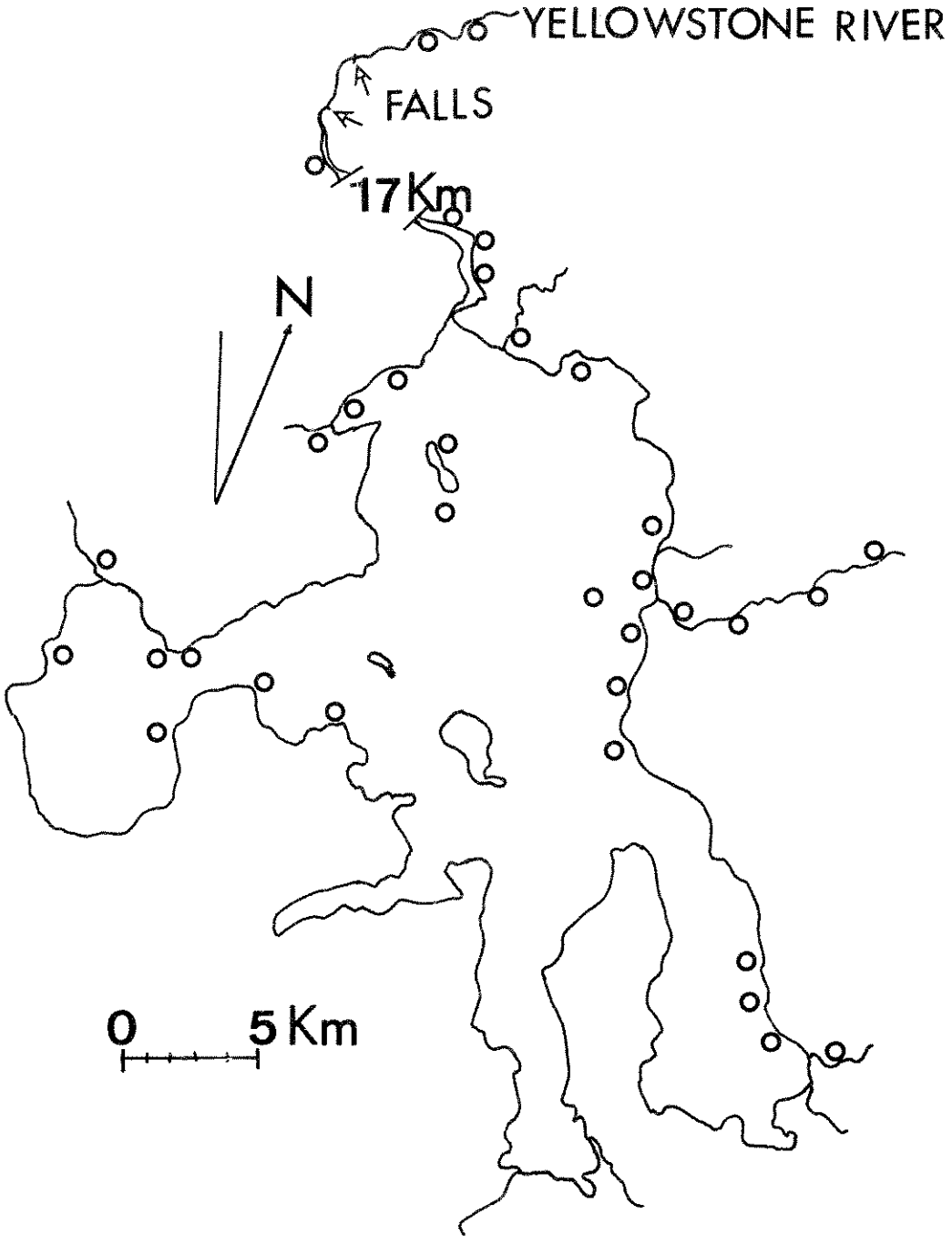


Fig. 1. Location of fish samples from Yellowstone Lake and Tributaries

o Sample Location

histological preparations. Part of the gill macerate was also preserved with formalin for future observations. Standard methods (Davenport, 1960) were used in preparing gill tissue for sectioning and staining. Paraffin embedded gills were sectioned at 8-10 microns with a microtome. Sections were spread on glass slides and histochemically stained with the following: Harris hematoxylin and eosin, periodic acid Schiff, (McManus, 1956), mercuric bromphenol blue (Mazia, 1953), five dye stain (Greenstein, 1961), Schiffs reagent (Davenport, 1960) and Bodians protargol (Bodian, 1936).

Fishes were sampled during the summer of 1969 from the following counties in Montana; Gallatin, Park, Madison, Petroleum, Broadwater and from Fremont County, Idaho to determine the extent of the trichophyran infection.

Electron Microscopy

Infected gill macerate and single gill filaments were placed in small plastic vials containing 2.5% gluteraldehyde buffered with potassium phosphate (0.1 M. pH 7.3). Post fixation was accomplished with 1% osmium tetroxide in the same buffer system. This material was dehydrated with graded series of acetone and 100% propylene oxide. Araldite epoxy resin blocks (Luft, 1961 and Mollenhauer, 1964) were prepared from the dehydrated material and sectioned with a Reichert OM U2 ultramicrotome using glass knives. Sections were mounted on 300

mesh, uncoated copper grids and post-stained with uranyl acetate (Watson, 1958) and Reynolds lead citrate (Reynolds, 1963; Karnovsky, 1961). The grids were observed with a Carl Zeiss EM9A electron microscope. Pictures were taken using Kodalith LR film (Estar base) and printed on Polycontrast F paper using a 3.5 Polycontrast filter. To determine the histochemical nature of magnified structures, the staining procedure was varied in the following manner; no post-stain, uranyl acetate only and Reynolds lead citrate only. Fixed gill macerate and filaments were sent to Florida State University for scanning electron microscope analysis.

Immunology

To determine the possible existence (Papermaster, 1964) within the host of a specific antibody against *Trichophyra*, an antigen was prepared by isolating approximately 12,000 organisms and fixing them with 0.4% formalin. This material was sonicated 10 minutes at 1.0 to 1.2 amperes using a Ratheon Sonic Oscillator (Summerfelt 1966). The preparation was checked for complete disruption by observing an aliquot with a compound microscope.

Two methods were used in testing for natural antibodies; the Ouchterlony (Campbell, 1964) and microprecipitation or the ring-interface technique (Ascoli, 1902 and Powell, 1968). The Ouchterlony method was modified by using glass slides placed in a humidity chamber and by

staggering antigen-antibody wells from 3 mm to 30 mm. Blood was collected from infected fish and centrifuged. The sera were used for the above two methods.

Cutthroat trout, of approximately equal size from Yellowstone Lake and a fish hatchery, were anesthetized with MS 222 (Tricaine methanesulphonate) and given two intramuscular and intracardial inoculations of the prepared antigen. The injections, spaced 14 days apart, varied from 2.0 ml to 0.5 ml per inoculation site. The fish from Yellowstone Lake were infected with *Trichophyra* while those from the fish hatchery were not. The fish were sacrificed 7, 10, and 14 days after the second inoculation. Sera from each were checked for antibody activity using the two methods previously described.

Tracer Study

A tracer experiment was conducted with four infected cutthroat trout of approximately equal size from Yellowstone River. Each fish was anesthetized with MS 222 and injected intracardially with 5 microcuries of ^{14}C-D-glucose (U). Previous to the injection, blood samples were checked for glucose using chromatography. The fish were sacrificed 1, 2, 4 and 8 hours after injection. The gills were removed and washed in physiological saline then fixed in 10% formalin. Samples of 25 *Trichophyra* and 0.4 grams of gill filament were analyzed from each fish using liquid scintillation counting (Arnoff 1960 and Chase 1962). The

suctorians and gill filaments were placed in liquid scintillation vials containing 0.5 and 1.0 ml respectively of hydroxide of Hyamine (Rohm and Haas) for 12 hours to disrupt the cell membranes. Scintillation fluid was added and each sample was counted for a period of 20 minutes. The gill parasites were removed from the host by teasing them free and then sucking each one up into a small microcapillary column with an inside diameter approximately the size of the organism. Trichophyrans were evacuated from the column into a vial filled with 10% formalin and washed three times with changes of formalin.

The wet film method for autoradiography (Pelc, 1947 and MacDonald, 1948) was used to corroberate the data from liquid scintillation counting. Gills, from the injected fish, were prepared histologically (Davenport, 1960) and sectioned at 20 microns. Duplicate thin sections from each fish were spread on glass slides which were immersed in water along with unexposed film (Fuji plate film; ET2F-9327). A strip of film was then removed from the plate and placed over the tissue sections. The tissue-film preparation was then removed from the water, air dried and stored in light-tight film boxes. The preparation was developed and stained with Mayer's hematoxylin after 2, 4, 6 and 8 weeks exposure (Shigematsu 1969a, 1969b). It was then observed with a compound microscope.

Experimental Infection

Lahonton cutthroat trout *(Salmo clarki henshawi)* and Yellowstone cutthroat trout *(Salmo clarki lewisi)* were both obtained from fish hatcheries. These fish were examined for *Trichophyra* by scraping the gill filaments of six individuals and observing the stained macerate with a compound microscope. There were no trichophyrans in the gills of hatchery fish. The fish were divided into experimental and control groups and maintained in separate tanks. Viable gill macerate, whole excised gills and intact fish infected with *Trichophyra* were added to the experimental tank. Fish from both groups were sacrificed after 3, 6, 20 and 30 days. A gill sample was taken from each fish and checked for *Trichophyra* by staining and observing the gill macerate. Blood was taken for hematocrit (Hesser, 1960), oxyhemoglobin (Collier, 1955) and nonprotein nitrogen (Bullock, 1954) determinations by incising the sinus venosus (Steucke, 1967). Citrated blood was used for oxyhemoglobin and nonprotein nitrogen values (0.5 ml 5% sodium citrate per 0.4 ml blood). Average values were obtained for oxyhemoglobin and nonprotein nitrogen by using a total of 30 infected trout from Yellowstone Lake and the Yellowstone River. Seventy-five samples were taken for an average hematocrit value.

The following media and broths were selected to culture *Trichophyra* in a partially defined medium; nutrient broth, brain-heart infusion,

tryptophan broth, sucrose broth, lettuce and hay infusion, blood agar, blood agar overlayed with fish mucus, agar, agar with fish mucus, fish mucus, and three cultures (different species) of free-living protozoa (Difco Manual, 1964). Each was inoculated with viable organisms and maintained at both 4 and 20 degrees Celsius.

RESULTS

General Survey

The parasites of Yellowstone cutthroat trout taken during two summers are given in Table I. The average weight of all trout examined was 350 grams (range, 1 to 820 grams) and average total length was 36.8 cm (range, 2.5 to 45.7 cm). Male and female trout showed no difference in the number of parasitic species present. All fish from Yellowstone Lake were infected with at least three species of parasites. Six different parasites were found in some fish and some individuals had thousands of parasitic protozoa and hundreds of metazoan parasites. One unusual example taken from the West Thumb of Yellowstone Lake had thousands of *Trichophyra* inhabiting the gills, 22 copepods *(Salmincola* sp.*)* and 17 leeches *(Piscicola salmositica)* attached to the fins, 59 nematodes *(Bulbodacnitis scotti)* in the pyloric caeca, 36 trematodes *(Crepidostomum farionis)* in the gall bladder and intestine, and 88 cestodes *(Diphyllobothrium* sp., plerocercoid stage*)* attached to the viscera and other organs throughout the abdominal cavity. One fish from the east side of Yellowstone Lake had over 400 plerocercoids attached to the viscera and internal body wall. The average number of parasites for fish examined was; 6 *Salmincola* sp. (range 1 to 23), 4 *P. salmositica* (range 1 to 24), 36 *B. scotti* (range 1 to 219), 25 *Diphyllobothrium* sp. (range 1 to 450), 38 *C. farionis* (range 1 to 230) and approximately 42,000 *Trichophyra* (range 1,000 to 53,000). Ten of

TABLE I. Parasites of 263 Yellowstone Cutthroat Trout *(Salmo clarki lewisi)* from Yellowstone Park

Parasite	Number of Fish Infected	Location of Parasite	Percent Infection (Adult Fish)
Protozoa			
Trichophyra clarki	250	gills	95
Myxosporidia sp.	2	gills	0.8
Haemogregarina sp.	1	blood	0.4
Trichodina truttae	1	gills	0.4
Costia pyriformis	5	gills	19
Cestoda			
Diphyllobothrium sp. (Plerocercoid)	241	kidney, spleen, gills, liver digestive tract, mesenteries musculature, air bladder pyloric caeca, gonads	92
Acanthocephela			
*Neoechinorhynchus rutili**	1	intestine	0.4
Nematoda			
Bulbodacnitis scotti	250	intestine, pyloric caeca	95
Copepoda			
Salmincola sp.	205	gills, fins, body surface buccal cavity	80
Hirudinia			
Illinobdella sp.	1	fins	0.4
Piscicola salmositica	47	fins, body surface	18
Trematoda			
Crepidostomum farionis	250	gall bladder, intestine, pyloric caeca	95

* Reported by Linton (1891) as *Echinorhynchus tuberosus*

the specimens had what appeared to be furunculosis *(Bacterium salmonicida)* on fins, gills and body surface.

A single *C. farionis*, which usually occupied the entire lumen of the gall bladder, was in each of 40 fingerling trout (2.5 to 5.0 cm in total length) examined from two tributaries of Yellowstone Lake. *Trichophyra, C. farionis* and plerocercoids of *Diphyllobothrium* sp. were found in mature trout (12.7 to 15.0 cm in total length) from Yellowstone River. *Trichophyra* was not present in adult trout examined from Yellowstone River below the Upper and Lower Falls. This suctorian was present in 60% of the longnose suckers examined from Yellowstone Lake but was absent from all redside shiners. Fourteen percent of the latter had another gill parasite *(Trichodina,* Fig. 36) which was also in one adult cutthroat trout taken below the Upper and Lower Falls of the Yellowstone River. Two other parasitic protozoa, *Costia pyriformis* (Fig. 37) and *Hemogregarina* (Fig. 38) were found in cutthroat trout.

Fish species from areas other than Yellowstone Lake were examined for gill parasites (Table II). *Trichophyra* was found in brown trout *(Salmo trutta)* and rainbow trout *(Salmo gairdneri)* from the Madison river, Madison County, Montana and from the Snake River (Fremont County, Idaho). *Trichophyra* was absent from fishes examined in other areas of Montana which included cutthroat trout from Yellowstone River near Livingston. Three other parasitic protozoa were found in gills of fishes examined. *Chilodenella* was present in the gills of longnose suckers from the Yellowstone River, *Apiosoma* was present in 12% of the rainbow

TABLE II. Fishes Examined for Gill Parasites During 1969. (All from Montana unless designated)

Location	Fish Species	Number	Parasites Observed	% Infected
West Gallatin River (Gallatin Co.)	*Prosopium williamsoni*	22	0	
	Salmo trutta	18	0	
	Salmo clarki	8	*Trichodina*	12
	Salmo gairdneri	21	0	
	Salvelinus fontinalis	3	0	
Bacon Rind Creek (Gallatin Co.)	*Salmo clarki*	8	*Trichodina*	50
Spanish Creek (Gallatin Co.)	*Salmo gairdneri*	3	0	
	Salvelinus fontinalis	3	0	
East Gallatin River (Gallatin Co.)	*Catostomus catostomus*	8	*Trichodina*	25
	Catostomus commersoni	3	*Trichodina*	66
	Catostomus platyrhynchus	20	*Trichodina*	60
	Prosopium williamsoni	4	*Trichodina*	25
	Rhinichthys cataractae	10	*Trichodina*	20
Hyalite Dam (Gallatin Co.)	*Salmo clarki*	15	0	
	Salvelinus fontinalis	5	0	
	Thymallus arcticus	4	0	
Fish and Game Ponds (Gallatin Co.)	*Salvelinus fontinalis*	8	0	
	Salmo gairdneri	10	0	
Madison River (Madison Co.)	*Salmo gairdneri*	12	*Trichophyra*	50
			Apiosoma	12
	Salmo trutta	12	*Trichophyra*	33
Snake River (Fremont Co., Idaho)	*Salmo gairdneri*	3	*Trichophyra*	100

TABLE II. (Continued)

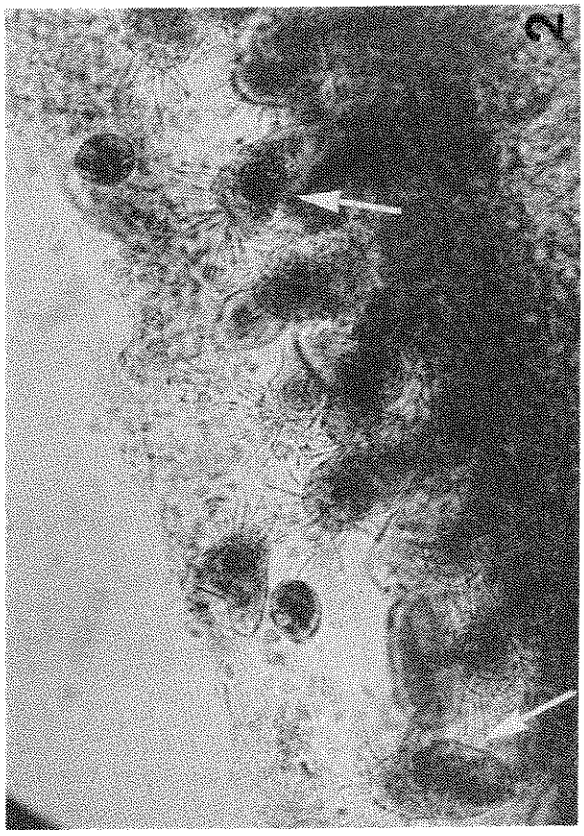
Location	Fish Species	Number	Parasites Observed	% Infected
Yellowstone River (Park Co.)	*Salmo trutta*	8	0	
	Salmo gairdneri	5	0	
	Salmo clarki	8	*Trichodina*	25
	Catostomus catostomus	1	*Chilodenella*	100
			Trichodina	100
	Prosopium williamsoni	28	0	
	Catostomus platyrhynchus	1	*Trichodina*	100
Shields River (Park Co.)	*Prosopium williamsoni*	1	0	
	Salmo gairdneri	1	0	
Mission Creek (Park Co.)	*Catostomus commersoni*	4	*Trichodina*	50
	Salmo trutta	3	0	
Missouri River (Broadwater Co.)	*Salmo gairdneri*	1	0	
	Cyprinus carpio	3	0	
	Cottus sp.	8	0	
Warehouse Lake (Petroleum Co.)	*Micropterus salmoides*	12	*Trichodina*	8

trout checked from the Madison River and *Trichodina* was found in several areas of Montana from the following hosts; mountain whitefish *(Prosopium williamsoni)*, largemouth bass *(Micropterus salmoides)*, cutthroat trout, longnose suckers, white suckers *(Catostomus commersoni)*, mountain suckers *(Catostomus platyrhynchus)* and longnose dace *(Rhinichthys cataractae)*.

Trichophyra

Light Microscopy

Morphology Methyl green-pyronin Y was the best vital stain for the morphological studies of *Trichophyra* and for differentiation of other gill parasites. This stain produced good differentiation between cell organelles of the infected gill macerate (Fig. 2) and emphasized the protozoa surrounded by host tissue. Nolands and nigrosin stains were good for tentacle morphology and number but unsatisfactory for emphasizing internal organelles. Acidified methylene green and Giemsa were unsatisfactory. The morphological measurements of *Trichophyra* from cutthroat trout and longnose suckers are summarized in Table III. There are distinct differences in the mensural data. *Trichophyra* from cutthroat trout (Fig. 2) is larger than *Trichophyra* from longnose suckers (Fig. 5)and 95% of the latter possess auxilliary tentacles which are absent in 65% of the former. On the basis of mensural data, host specificity, and electron microscopy, the species names assigned for



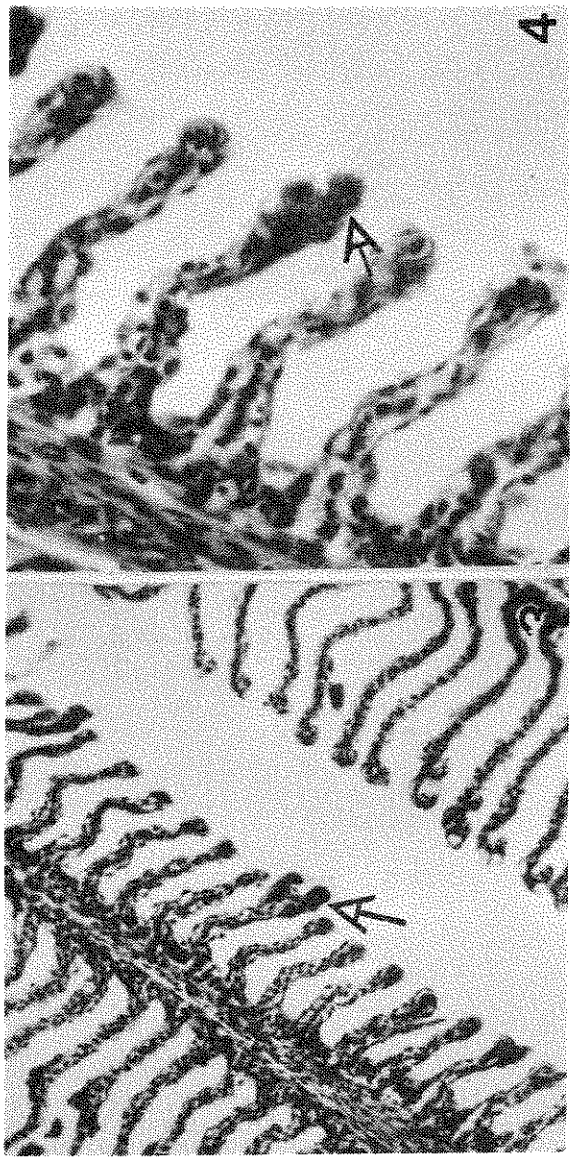


Figure 2. Whole mounts of *Trichophyra clarki* (methylene green-pyronin Y)
Scale, 100 microns ⊢——⊣

Figures 3, 4. Infected cutthroat trout gills, *T. clarki* at tip of arrows.
Hematoxylin and eosin.
Scale, 100 microns ⊢——⊣ (Fig. 3)
⊢————⊣ (Fig. 4)

TABLE III. Measurements (microns) of 100 *Trichophyra* from both Longnose Suckers and Cutthroat Trout (Ranges in parentheses)

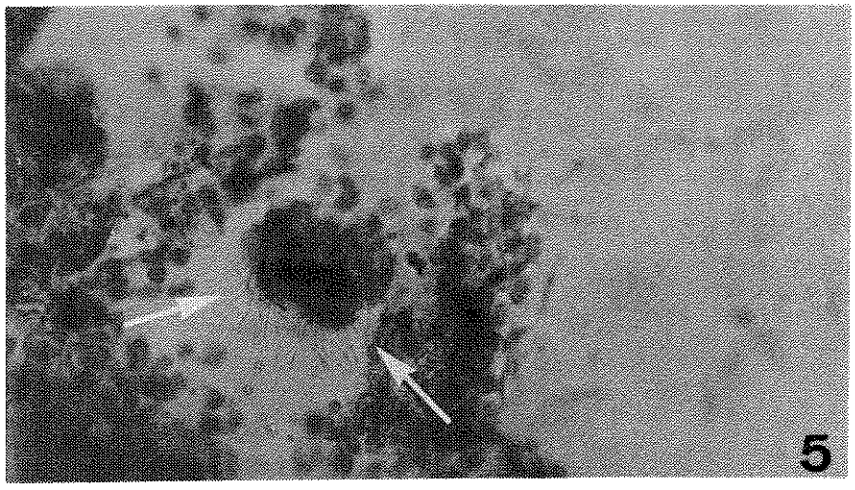
Parasite	Average Cell Size Length	Average Cell Size Width	Macronucleus Size Length	Macronucleus Size Width	Tentacle Length	Percent with Auxiliary* Tenacles
Trichophyra clarki	80.7 (41-118)	40.6 (30-62)	24.9 (8-40)	14.3 (9-23)	26 (12-48)	35
Trichophyra catostomi	43.6 (21-68)	25.2 (18-38)	14.7 (10-24)	9.7 (6-14)	18 (12-27)	94

* Auxilliary tentacles; trichophyrans characterized by two fascicles of tentacles at opposite poles.

TABLE IV. Liquid Scintillation Counts for *Trichophyra* and Gill Sections from Fish Injected with ^{14}C-D-Glucose (U). (Counts in disintegrations per minute).

Fish	Time Elapsed (hours)	*Trichophyra*	Counts minus check*	Gill Section	Counts minus check**	Percent Increase (activity)
1	1	111	0	188	0	0
2	2	111	0	193	5	3
3	4	111	0	545	357	190
4	8	111	0	272	84	45

* Check disintegrations per minute for *Trichophyra*; 111
** Check disintegrations per minute for gill section; 188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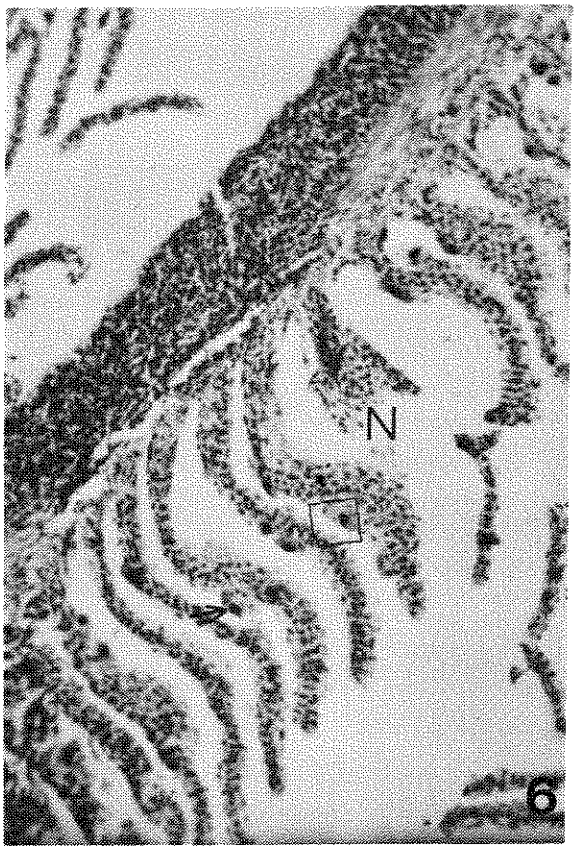
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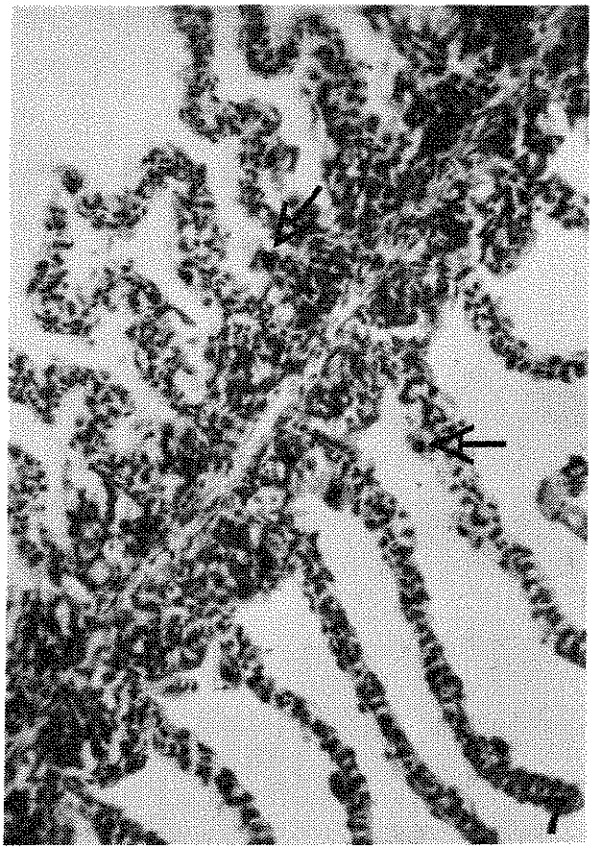


Explanation of Figures:

Figure 5. Whole mounts of *Trichophyra catostomi* (methylene green-pyronin Y). Note tentacles at both poles of cell (arrows). Scale, 100 microns ⊢——————⊣

Figure 6. Pathological damage to longnose sucker gills. *T. catostomi* located in box and at tip of arrow, with hyperplasia around the parasites. Necrotic areas shown (N). Scale, 100 microns ⊢——⊣

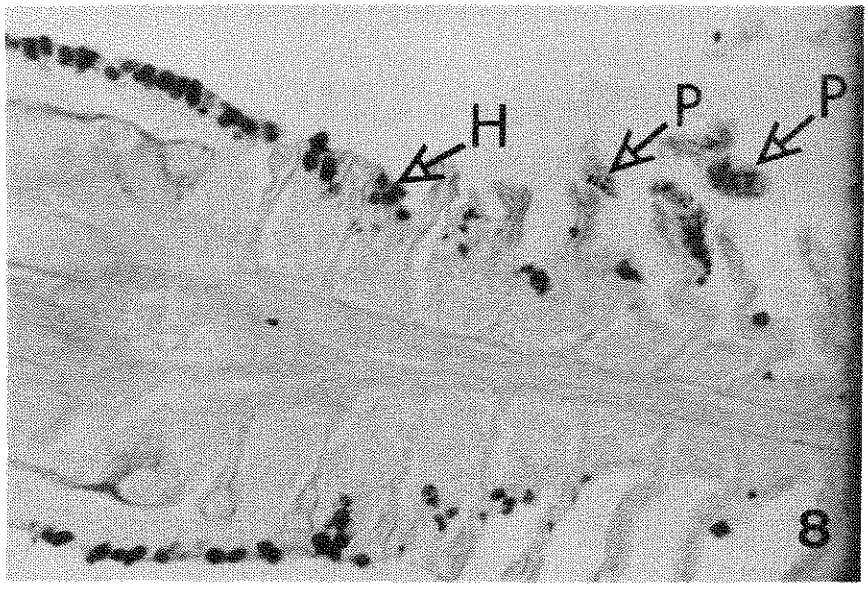
Figure 7. *T. catostomi* (tip of arrow) in infected longnose sucker gills. Hematoxylin and eosin. Scale, 100 microns ⊢——⊣

the gill parasites are; *Trichophyra clarki* (host, *Salmo clarki lewisi*) and *Trichophyra catostomi* (host, *Catostomus catostomus*).

Histology The suctorian parasites on cutthroat trout are usually concentrated on the lamellar tips of the gill filament (Fig. 3, 4) where they are closely attached to the epithelial cells. Hematoxylin-eosin and five dye stain were satisfactory for general cytological study. Cutthroat trout samples, with the largest number of trichophyrans, had 7.1% of the gill epithelium covered by the parasite. In one 36 cm trout, there was an average of 31 suctorians per gill filament totaling about 42,000 organisms. *T. catostomi* is located along the sides and at the base of the gill lamellae. Concentrations of *T. catostomi* within host gills are similar to *T. clarki*.

Histochemistry The macro- and micronucleus of *Trichophyra* were Feulgen positive (Schiffs reagent) and the mercuric bromphenol stain showed an intense blue area between the parasite and the epithelial host cell. There were similarities in stained particles within the protozoan and the surface of the epithelial cells when periodic acid Schiff stain was used (Fig. 8). This indicates that similar complex polysacchride deposits exist in both areas. There are no argentophilic structures in *Trichophyra* but nerve fibers and other structures within the gill filament were intensified with protargol.

Pathology Sections of gills from cutthroat trout had no apparent pathological damage while longnose sucker gills were definitely affected



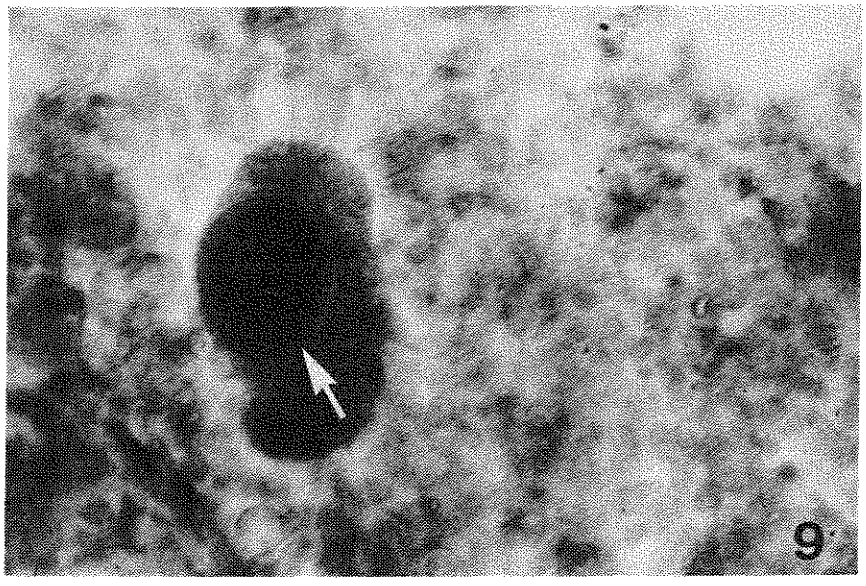


Figure 8. Periodic acid Schiff preparation of cutthroat trout gills infected with *T. clarki* which shows particles of similar stain intensity within the parasite (P) and gill epithelium (H). Scale, 100 microns

Figure 9. Endogenous budding (tip of arrow) within *T. clarki* for asexual reproduction of organisms. Scale, 100 microns

by the parasite. *T. clarki* causes no damage to the host epithelium or other gill cells. There was definite damage to the gill lamellae due to *T. catostomi*. It causes hyperplasia and hemorrhaging of the immediate host tissue with subsequent necrosis (Fig. 6, 7).

Conventional Electron Microscopy

Trichophyra clarki The ultrastructural characteristics of *T. clarki* are similar to those of other suctorians (Bardele, 1967, 1968a, 1968b and Paulin, 1969). Microanatomical structures of this protozoan are shown in Figures 10, 11. The average mitochondrion is 1.8 microns in length and 0.4 microns in width and is morphologically similar to those described for other protozoa (Pitelka, 1963). Myelin-like structures (Anderson, 1967) and endoplasmic reticula are found throughout the cytoplasm. The trichophyran cell membrane (Fig. 10), composed of four to six unit membranes and many micropores (Garnham, 1961), is unique for animal cells with double unit membranes. *Trichophyra* has a trilaminar limiting membrane (Desportes, 1969). Dense staining bodies of unknown function and two nuclei are present in each trichophyran cell. The macronucleus (Fig. 11) often occupies 30-35% of the internal cell space and is surrounded by a double unit membrane. The internal macronuclear structure contains numerous dark staining bodies and microtubules (Rudzinska, 1956). The micronucleus is also limited by a double unit membrane but lack dark staining bodies and microtubules. The concentrated dark granules within the

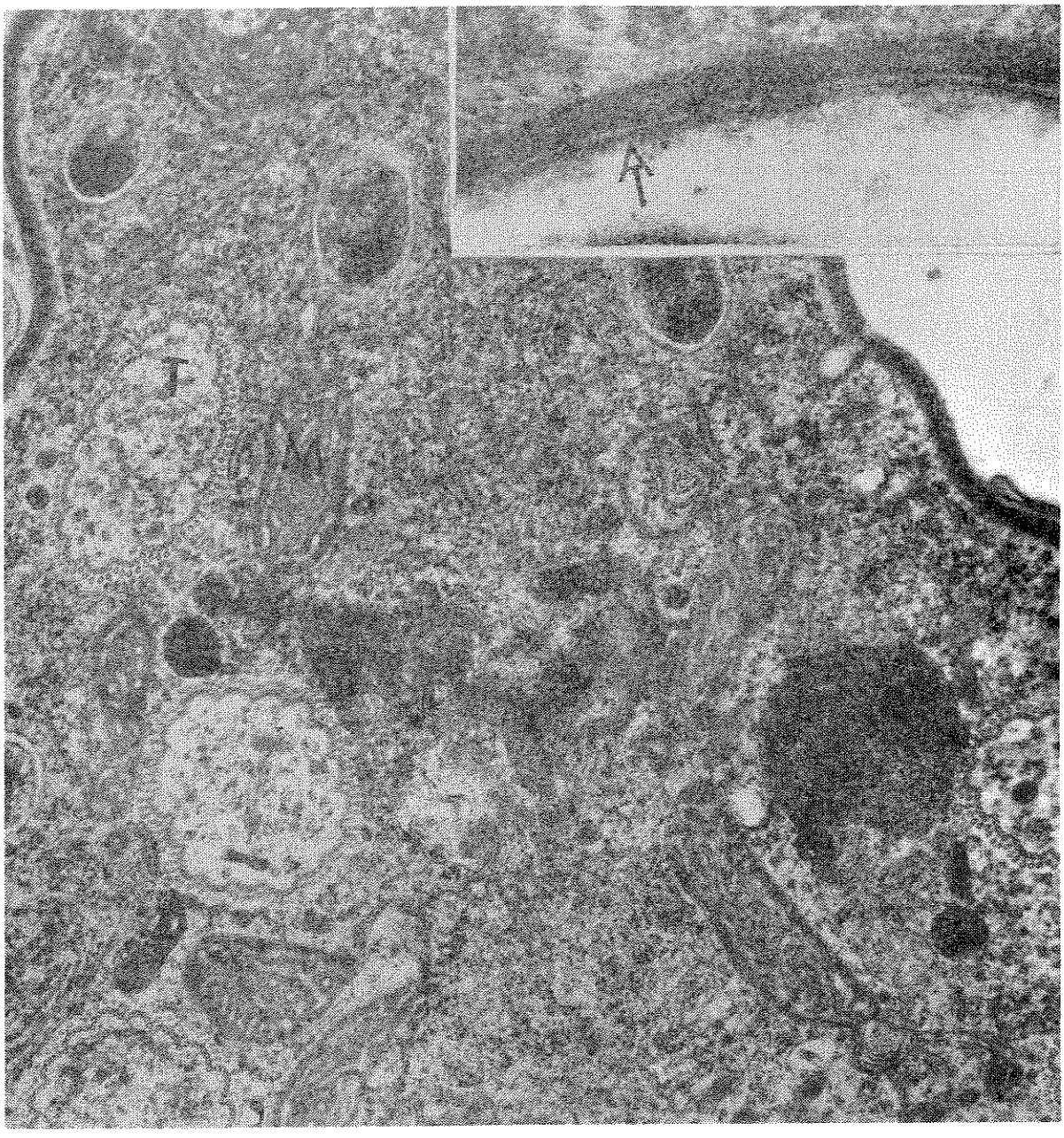


Figure 10. Electron photomicrograph of *Trichophyra clarki* showing mitochondria (M), myelin-like bodies (m), tentacles with inner and outer rings of microtubules (T) and trilaminar cell membrane.
Scale: 0.5 microns

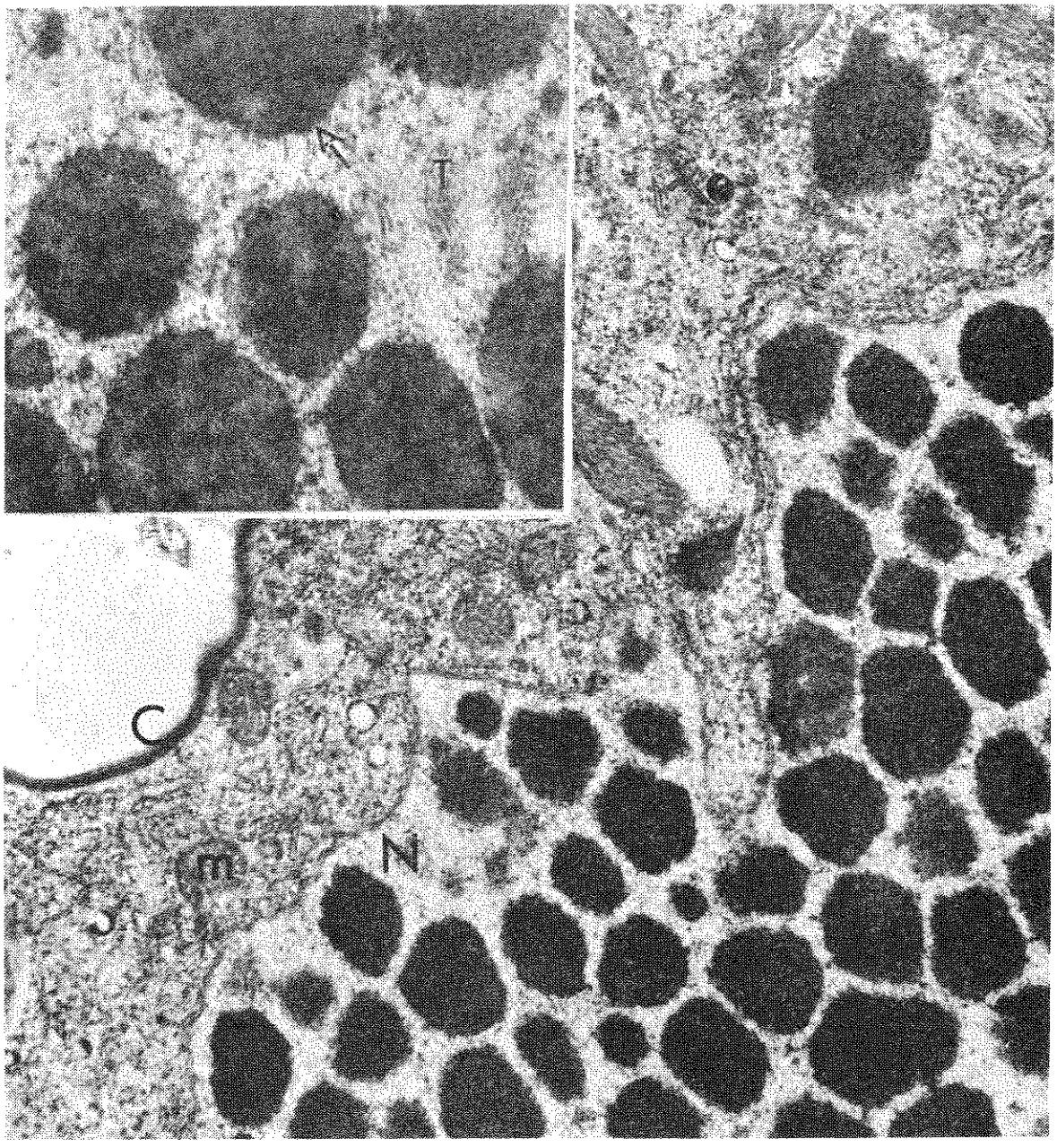


Figure 11. Electron photomicrograph of *Trichophyra clarki* showing mitochondria (m), macronucleus (N) with dense staining bodies (arrow) and microtubules (T) (Inset). The trilaminar cell membrane (C) is also visible.
Scale, 0.5 microns

micronucleus are chromatin masses (Fig. 12). The tentacles are delimited by a trilaminar membrane (Fig. 13-16) and originate in the cytoplasm. These are represented by microtubules (0.02 microns in diameter) that course throughout the length of the tentacle. Tentacle diameters average 0.6 microns at the base and 0.3 microns proximal to the expanded tip. The cross section of each tentacle (Fig. 17) shows two rings of microtubules which have a definite pattern throughout. The outer ring has 84-86 while the inner is composed of 110-112 arranged in 17 semicircles, each with 6-7 microtubules. Fine filaments connect adjoining microtubules. The expanded tip of the tentacle (Fig. 14, 15) has phialocysts (Battise, 1966) or missle-like bodies (Lom, 1967), dense staining bodies and small canals which may function in directing the flow of food to the center of the organism. In one preparation the outer limiting membrane shows an expansion into the milieu of the cell (Fig. 18) and may be the formation of a cytopyge for waste disposal.

Trichophyra catostomi The major difference between the ultrastructure of *T. catostomi* and *T. clarki* is the microtubule arrangement in the tentacle (Fig. 19). *T. catostomi* has 56-58 microtubules in the outside ring (30 less than *T. clarki*) and 10-12 semicircles for the inside ring each with 5-6 microtubules for a total of 58-64 (48-52 less than *T. clarki*). This ultrastructural difference between the two species of *Trichophyra* represents one basis for giving each a different name. The

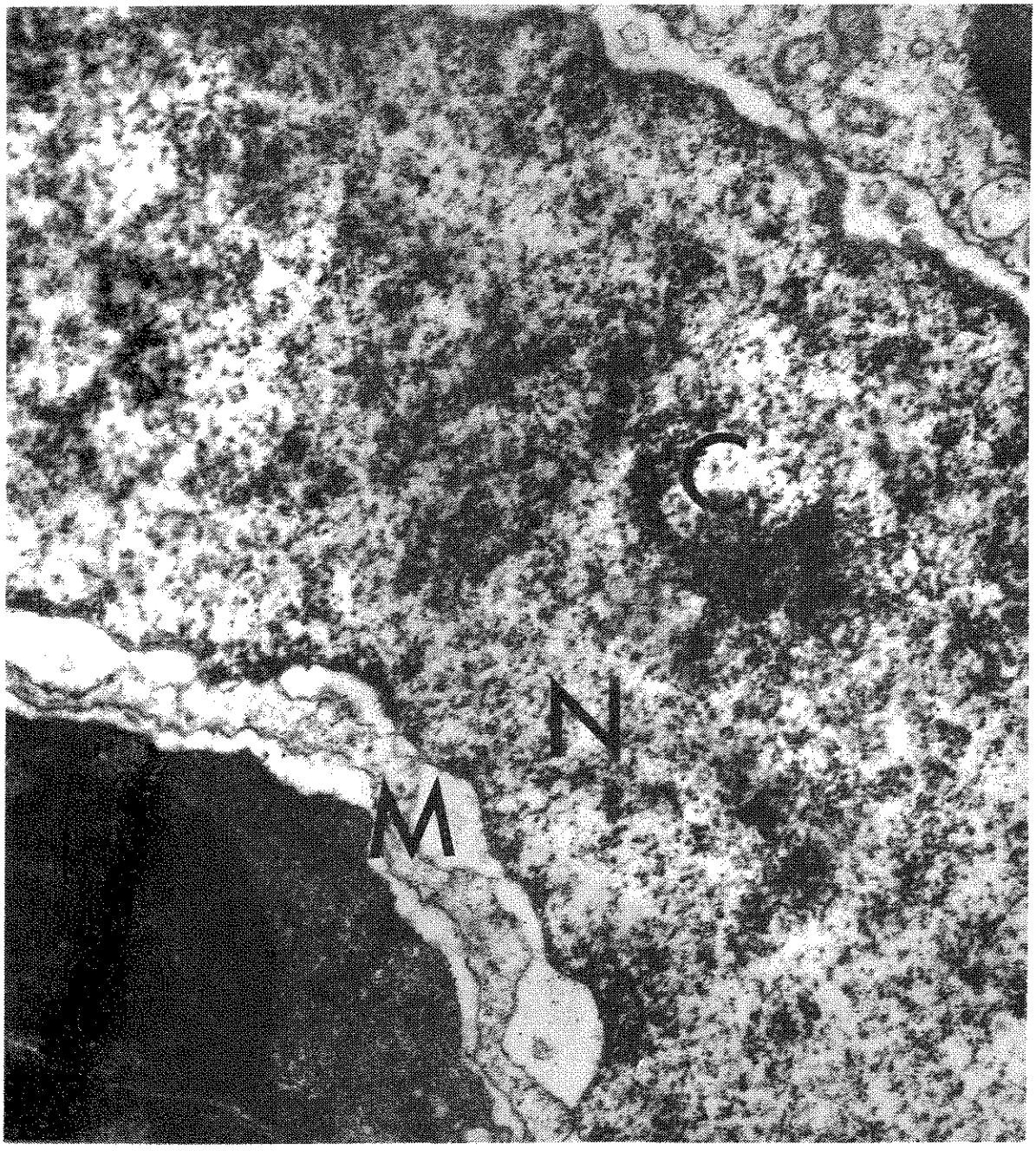


Figure 12. Micronucleus (N) of *T. clarki* showing concentrated dark staining masses (C) and an outer limiting membrane (M). Scale, 0.5 microns ⊢——————⊣

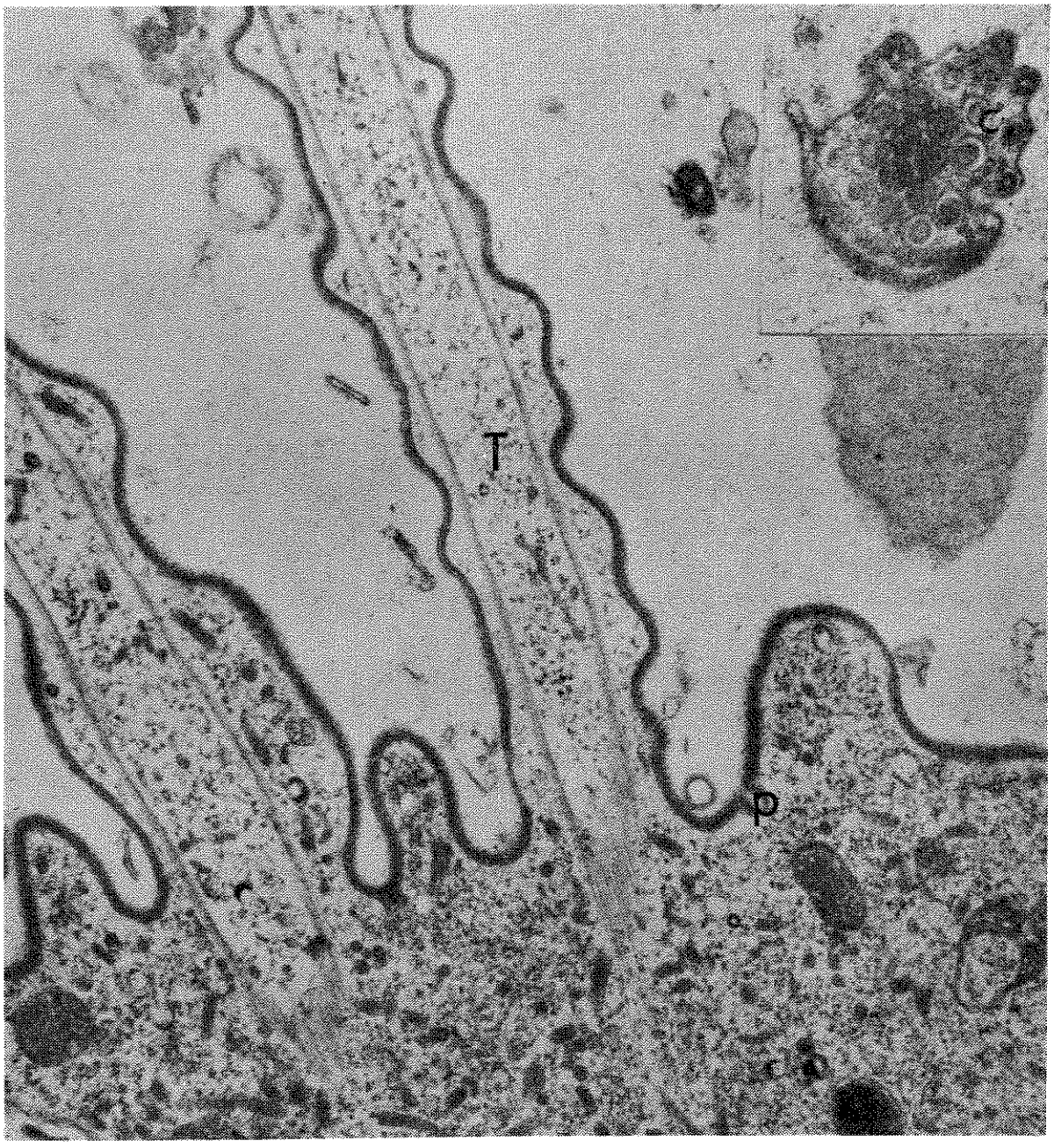
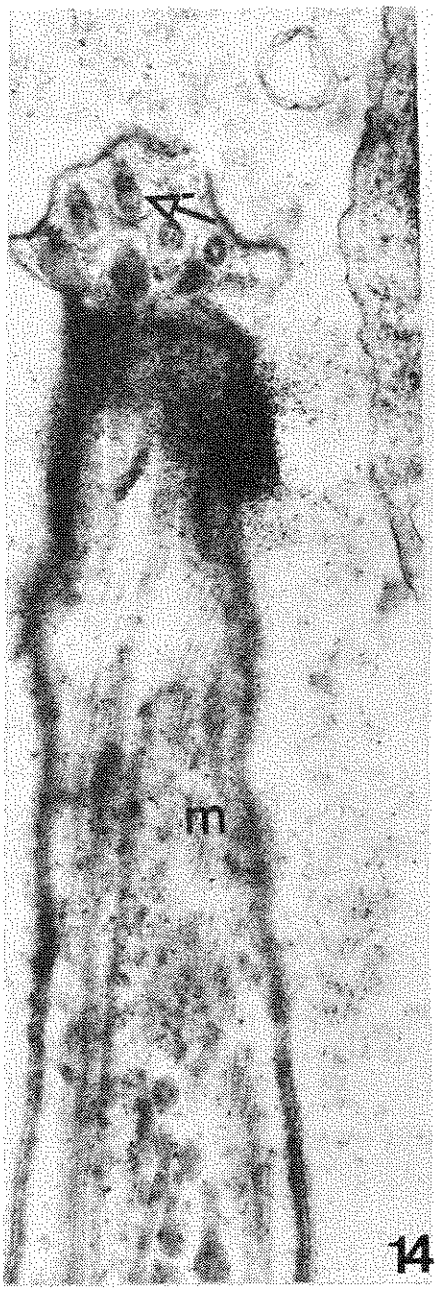
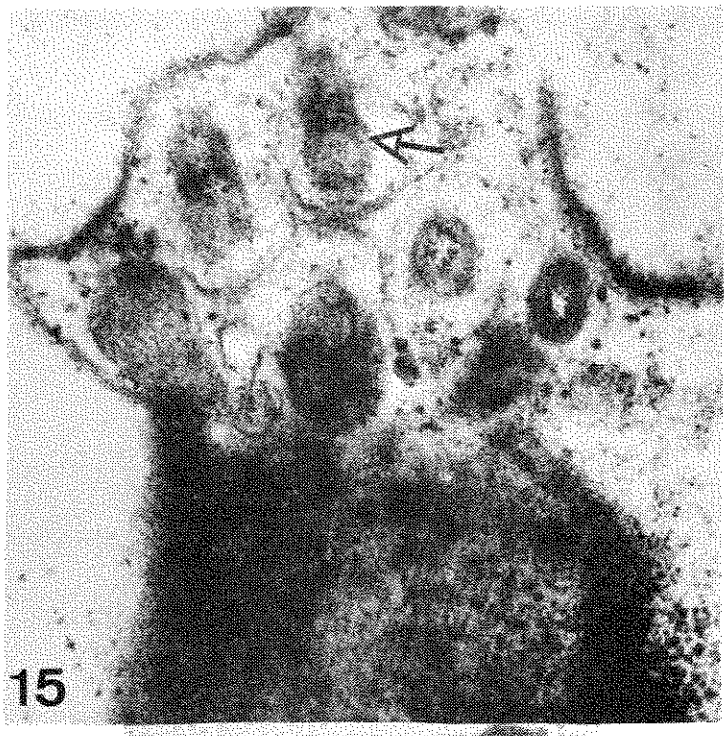


Figure 13. Longitudinal section of *T. clarki* showing the fine structure of tentacles (T) and their origin. Micropores (p) occur commonly in outer limiting membrane. Inset, cross section of tentacle tip. Scale, 0.5 microns





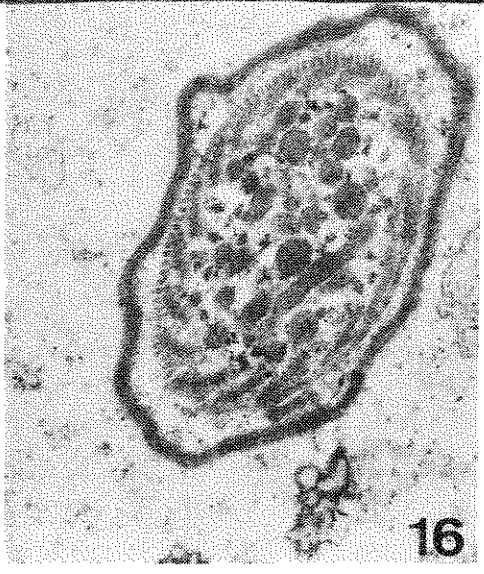


Figure 14, 15, 16. Fine structure (longitudinal and cross sections) of a tentacle showing microtubules (m), phialocysts (tip of arrow) and persistence of the microtubule pattern (16).
Scale, 0.5 microns

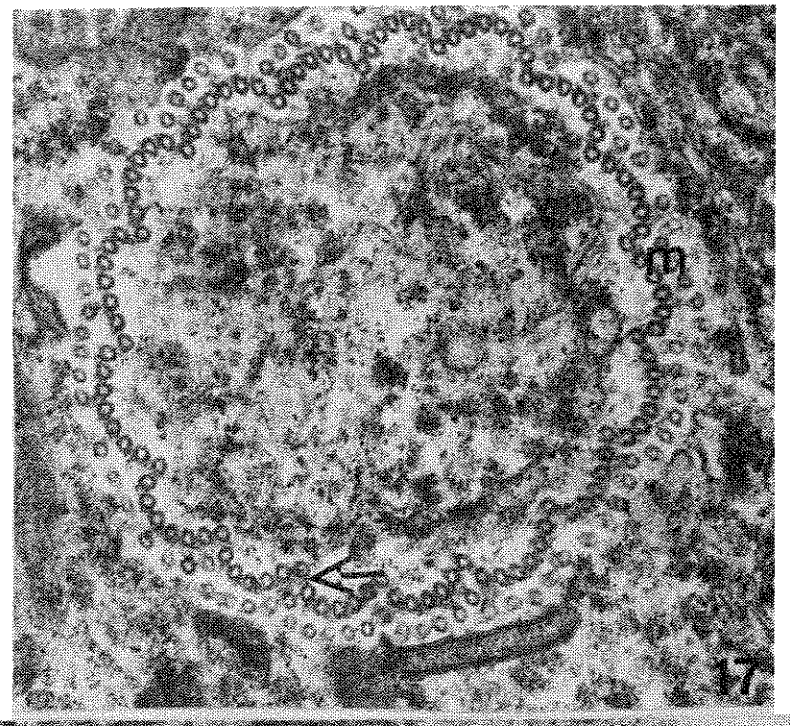


Figure 17. Cross section of *T. clarki* showing microtubules (m) and fine filaments connecting (tip of arrow). Scale, 0.5 microns

Figure 18. Fine structure of possible cytopyge formation (tip of arrows) for *T. clarki*. Scale, 0.5 microns

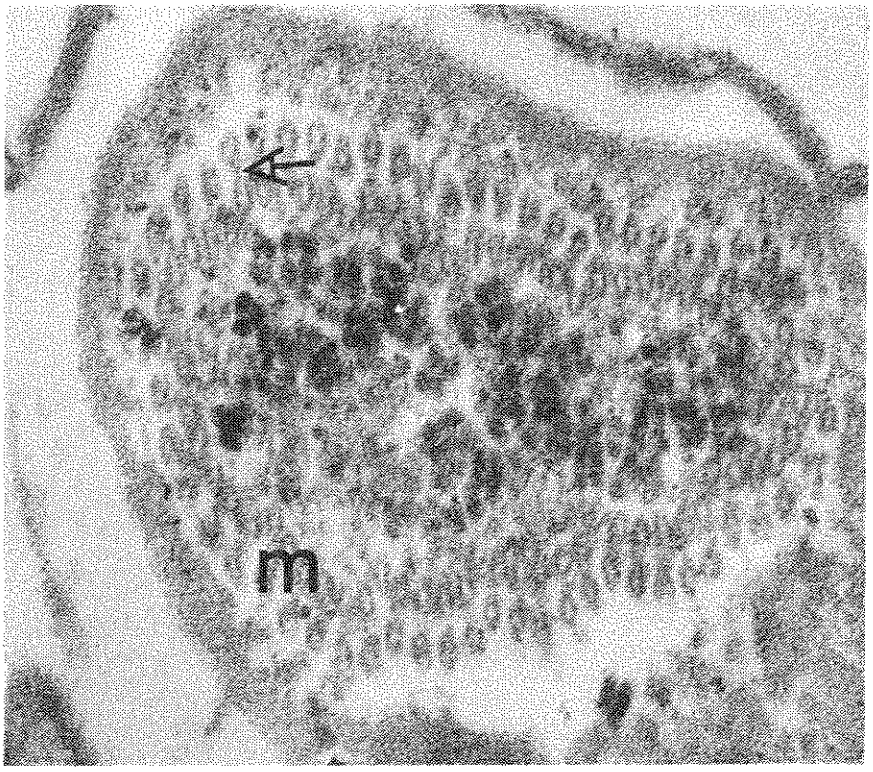


Figure 19. Fine structure of *Trichophyra catostomi* tentacles showing microtubules (m) and fine filaments (arrow) connecting adjoining tubules. Scale, 0.5 microns ⊢——————⊣

other cytological structures (Fig. 20) are similar for both trichophyrans. The ribosome has prominent fine structure in *T. catostomi*. These were seen as polysomes (groups of 3, 4, 6, 8, etc.) and as ribosome helices (Wooding, 1968). Vacuoles were found throughout the cytoplasm and longitudinal sections show a deep cytoplasmic origin and the early development of tentacles.

Host Cells No published work of the characteristics of gill epithelial cells of cutthroat trout was found. The electron photomicrographs (Fig. 21, 22) are representative for animal ultrastructure in general. The nucleus is surrounded by a two unit membrane with randomly spaced pores. The cell membrane has a two unit structure and is extensively convoluted increasing the surface area. Mitochondria, showing internal cristae, and endoplasmic reticulum (agranular and granular) are scattered throughout the cells. The mitochondria average 1.3 microns in length and 0.5 microns in width and are shaped like rods and spheres. The Golgi apparatus (Fig. 23) is often duplicated in each epithelial cell and has flattened cisternae with vesicles being pinched off either tip. Lysosomes and desmosomes (darkened areas along cell boundaries Fig. 24) are present. Chloride cells (Fig. 25) are present in gill epithelium and are characterized by numerous mitochondria (2.5 microns maximum length) and agranular endoplasmic reticulum. Their ultrastructure is similar to that described by Philpott (1963, 1965) and Kessel (1960) for chloride cells in three species of *Fundulus*. Mucous

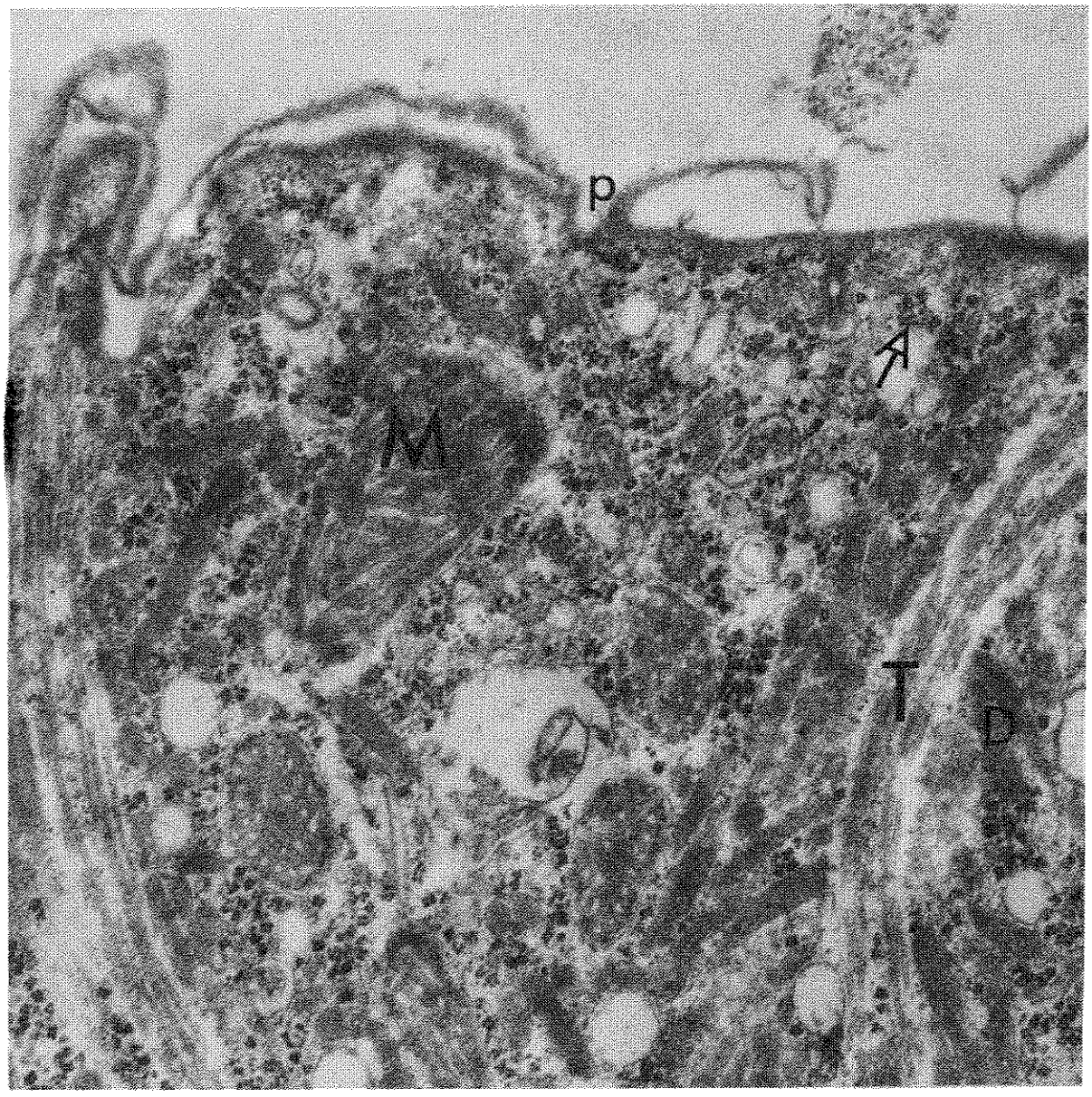


Figure 20. Fine structure of *Trichophyra catostomi* showing vacuoles (V), tentacles (T), dense staining bodies (D), mitochondria (M), and micropores (p) in the trilaminar cell membrane. Ribosome packets are visible at tip of arrow. Scale, 0.5 microns ⊢——⊣

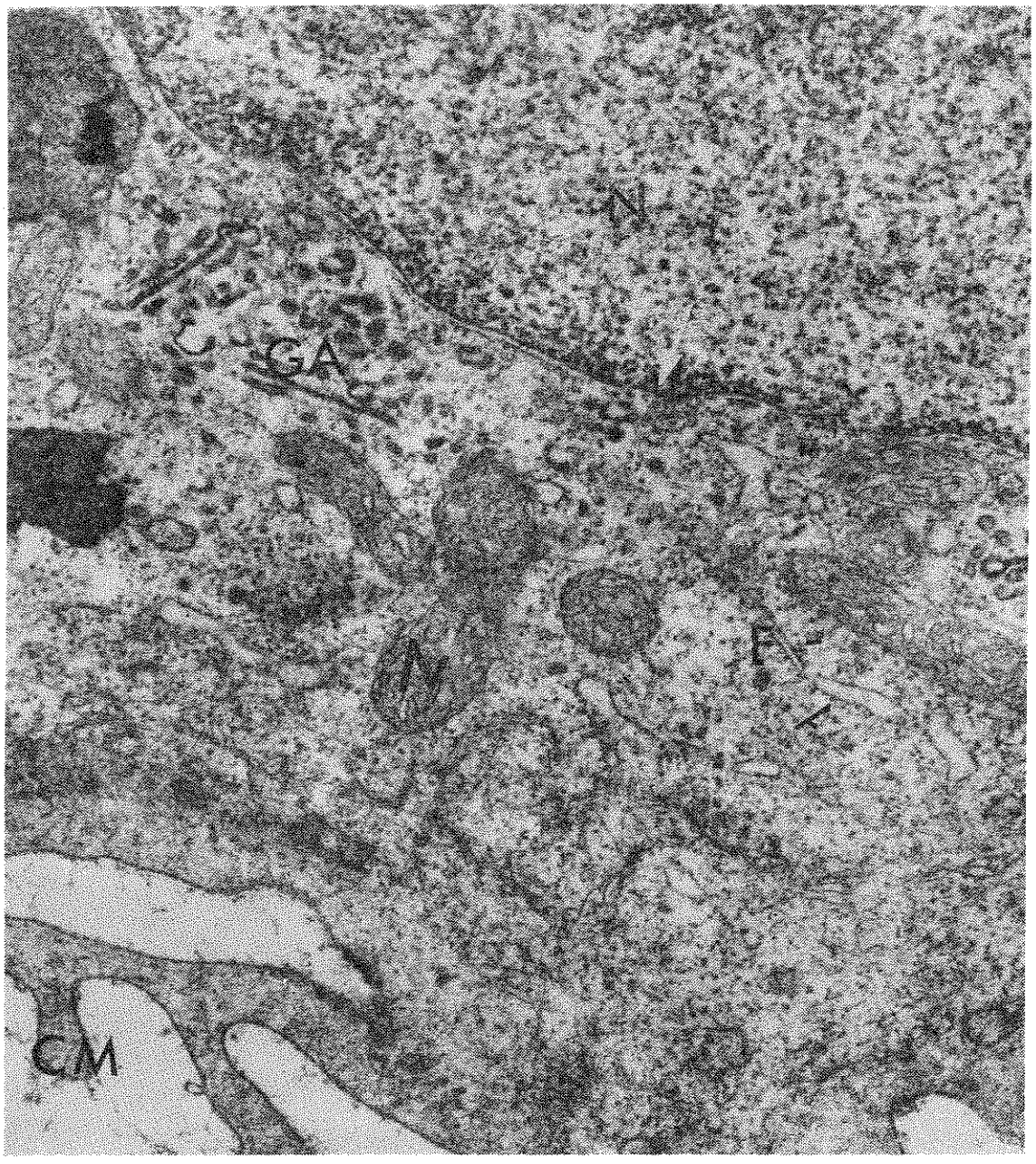


Figure 21. Fine structure of cutthroat trout gill epithelial cell showing convoluted cell membrane (CM), numerous mitochondria (M), Golgi apparatus (GA), nucleus (N) with a two unit membrane (ARROW) and endoplasmic reticulum (E) with ribosomes (arrow). Scale, 0.5 microns ⊢——⊣

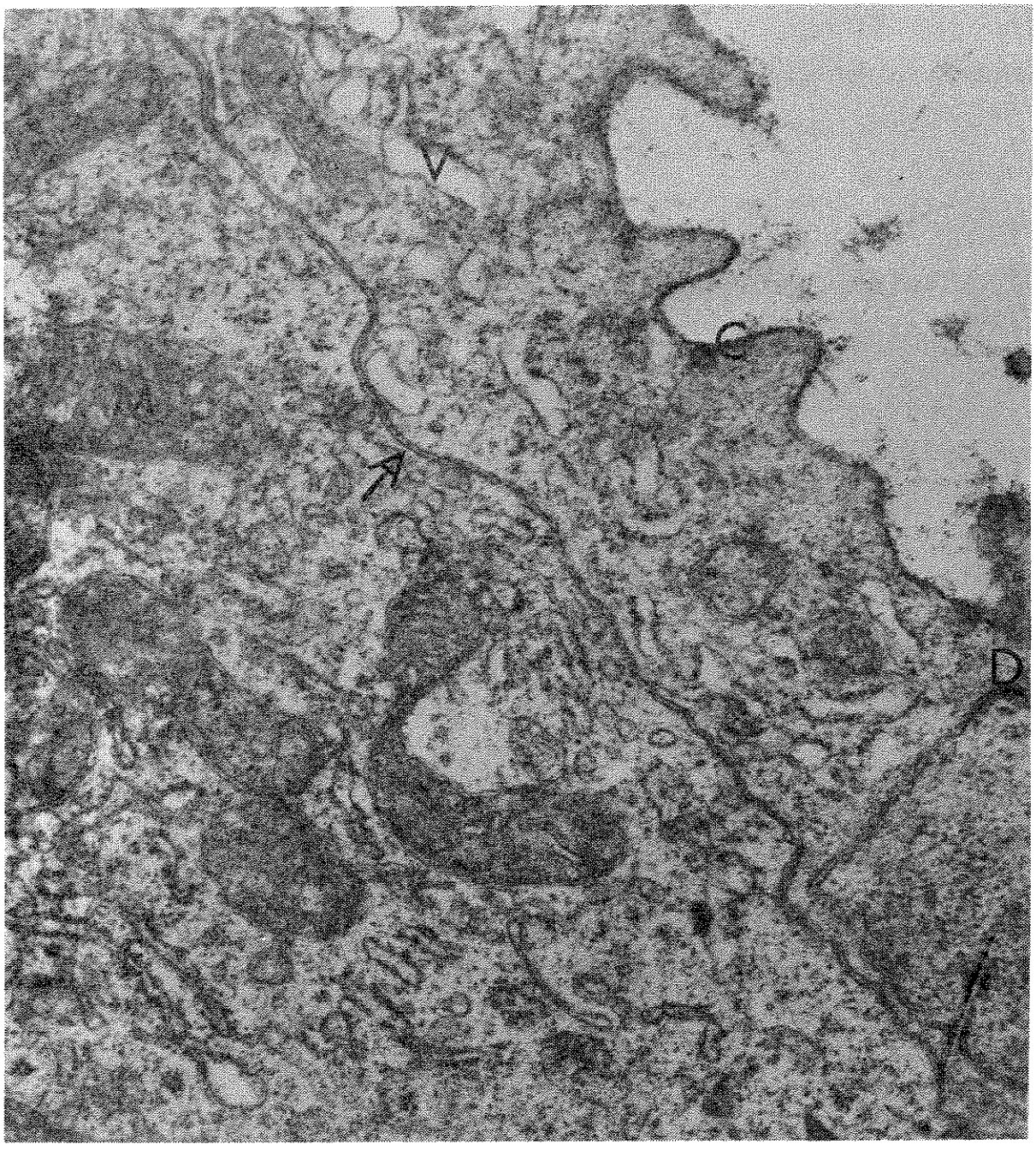
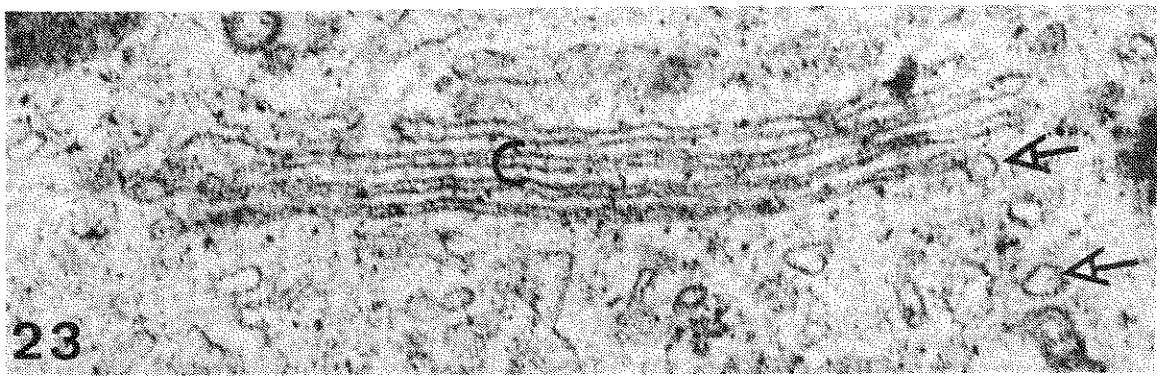


Figure 22. Gill epithelial cell fine structure showing cell limiting membrane between cells (arrow), vacuoles (V), desmosomes (D), mitochondria (M), endoplasmic reticulum (E) and convoluted cell membrane (C). Scale, 0.5 microns



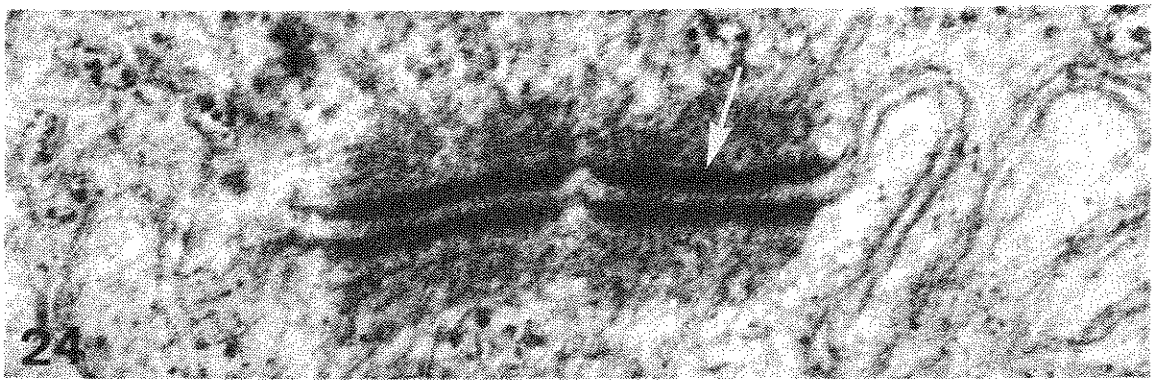


Figure 23. Fine structure of Golgi Apparatus from a gill epithelial cell showing flattened cisternae (C) and vesicles (arrows). Scale, 0.5 microns ⊢———⊣

Figure 24. Fine structure of a desmosome (arrow) representing an area along the limiting membrane between cells. Scale, 0.5 microns ⊢———⊣

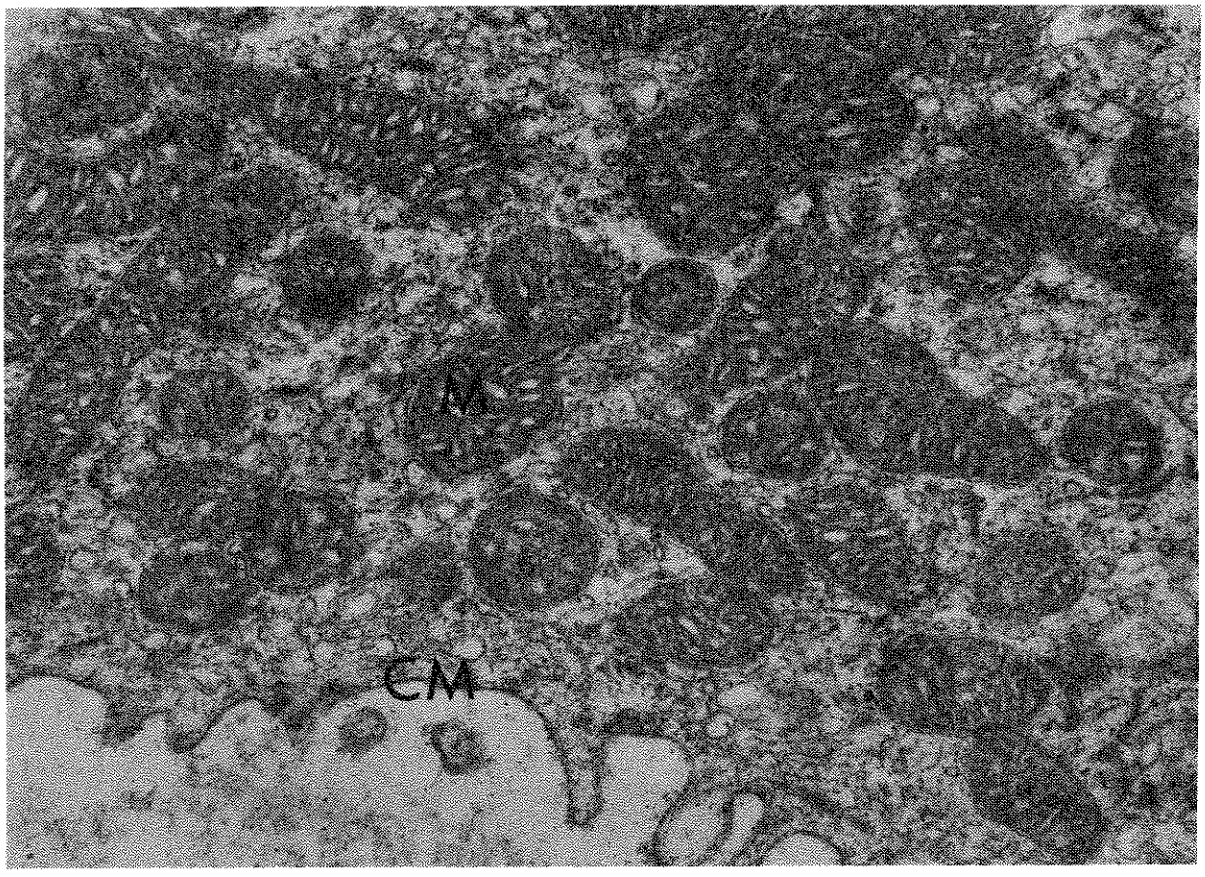


Figure 25. Fine structure of a chloride cell showing numerous mitochondria (M) and convoluted cell membranes (CM). Scale, 0.5 microns ⊢——⊣

cells, with extensive vesicles and endoplasmic reticulum (Henrickson, 1968a, 1968b, 1968c) were also observed.

Parasite-Host Relationship Sections of the interface between the host epithelial cell and parasite were prepared. A helical structure in the interface attaches the parasite to the gill epithelium (Fig. 26). This structure (referred to as attachment helix) has the following measurements; length, 0.52 microns (range 0.20-0.82 microns), width 0.04 microns (range 0.30-0.06 microns) and is solid in cross section (Fig. 27). Previous descriptions of this structure have not been found. The attachment helix is found only on the side of the protozoan next to the host cell (Fig. 28, 29). It originates as a cleft in the outer wall of the protozoan. The protozoan membrane, in the cleft, moves into the space between host cell and parasite and expands into a long fine filament. The filament then contracts to form the helix (Fig. 27). The origin of the attachment helix was corroborated by histochemistry. This structure is osmophilic and mercuric bromphenol blue positive. Lipid material, found in the unit membranes, has an affinity for osmium while protein stains blue with bromphenol blue.

A series of electron photomicrographs show damage to the epithelial cells of cutthroat trout due to *T. clarki*. The damage is detected as the number of host cell mitochondria decrease and disappear (Fig. 30, 31, 32). Similar damage was observed for *T. catostomi* in longnose suckers. This latter parasite probably uses necrotic host epithelial

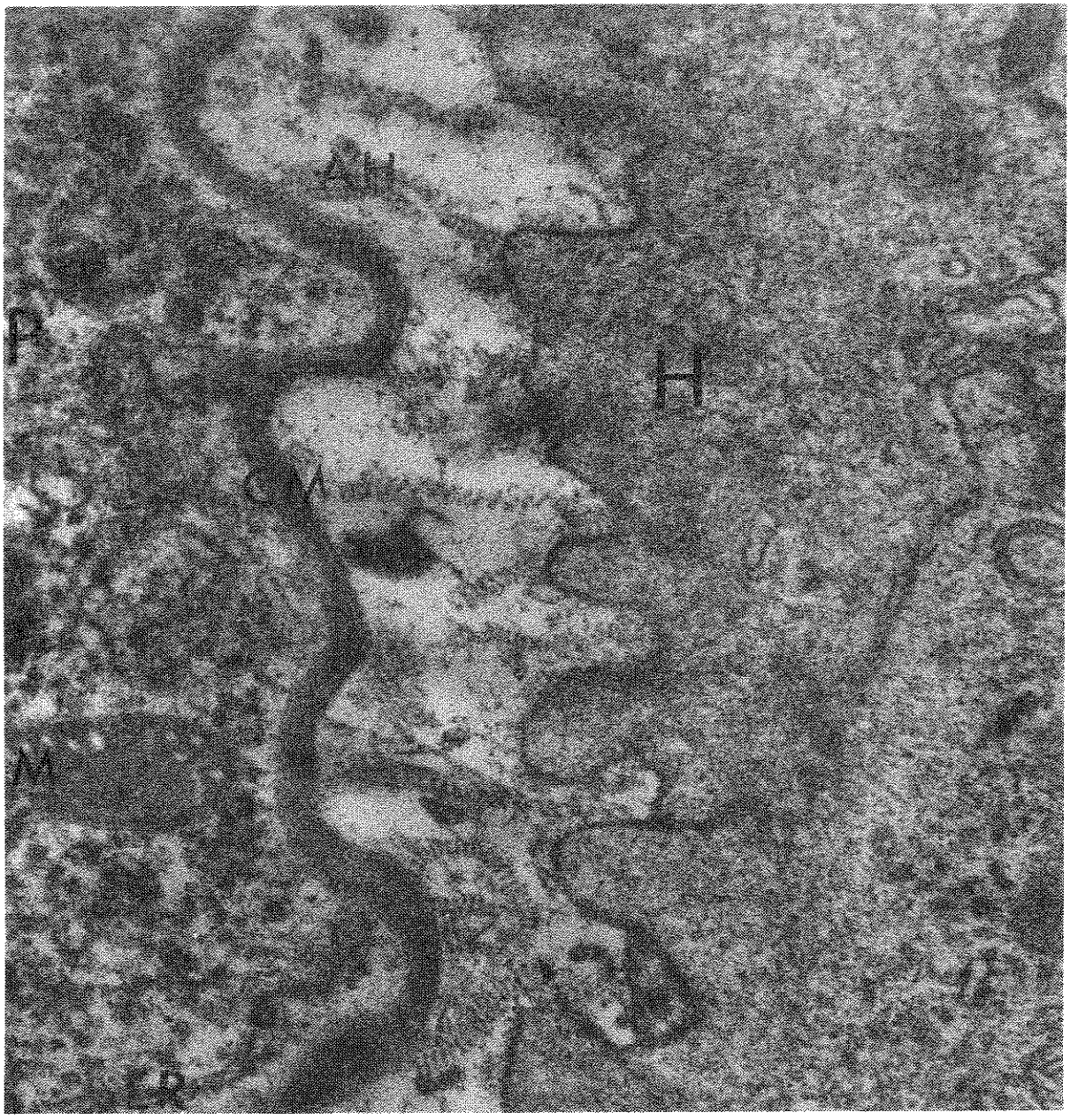


Figure 26. Interface between parasite (P) and host (H) cells showing helical structures (AH) attaching the two cells. Endoplasmic reticulum (ER), mitochondria (M) and cell membrane (CM) labeled for parasite. Scale, 0.5 microns

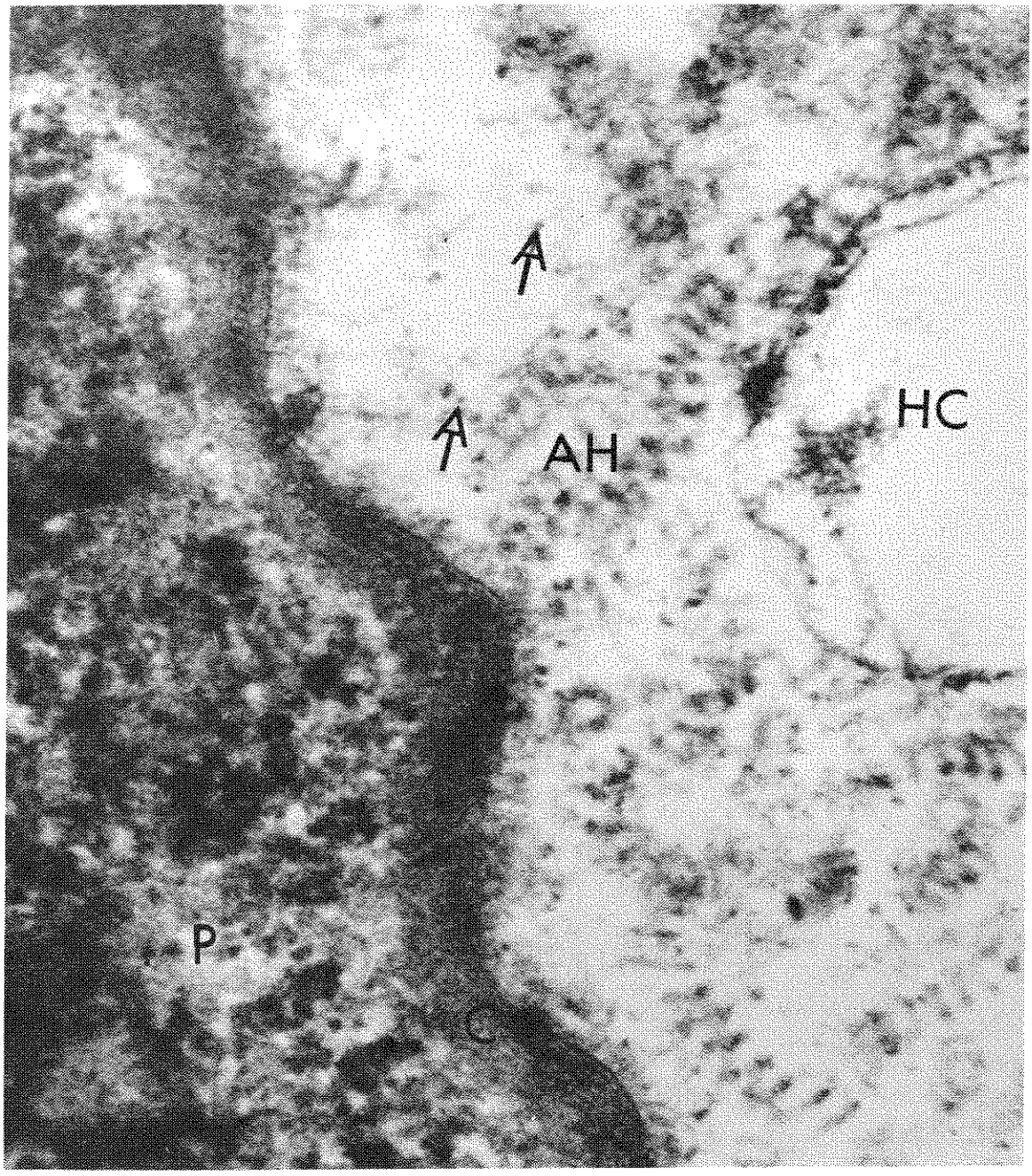
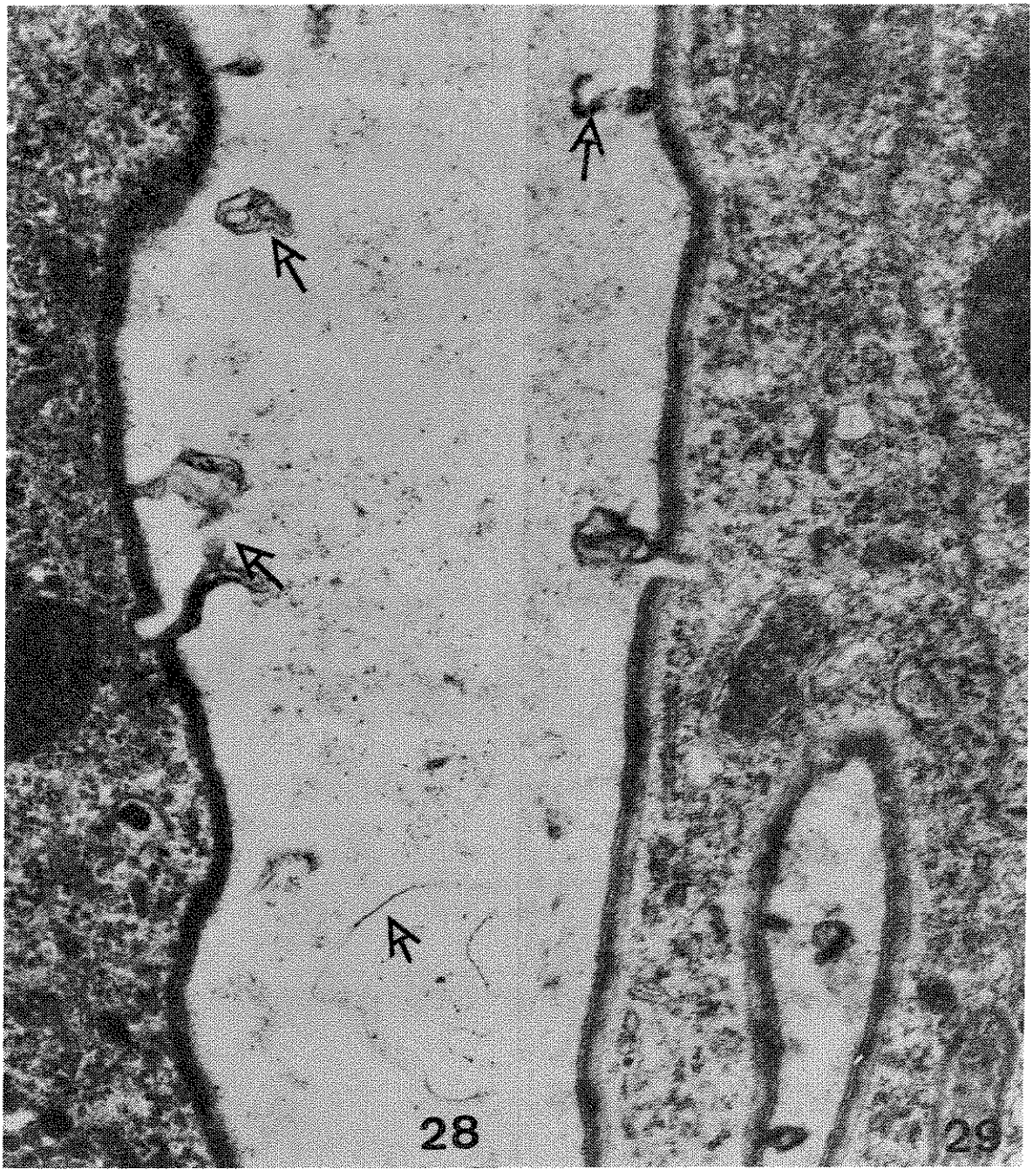


Figure 27. Fine structure of attachment helices (AH) showing solid nature (arrows) in cross section. Host cell (HC) and parasite (P) labeled. Scale, 0.5 microns



Figures 28, 29. Origin of attachment helices. Arrows indicate cleft, moving away of cell membrane and fine filament formation. Scale, 0.5 microns

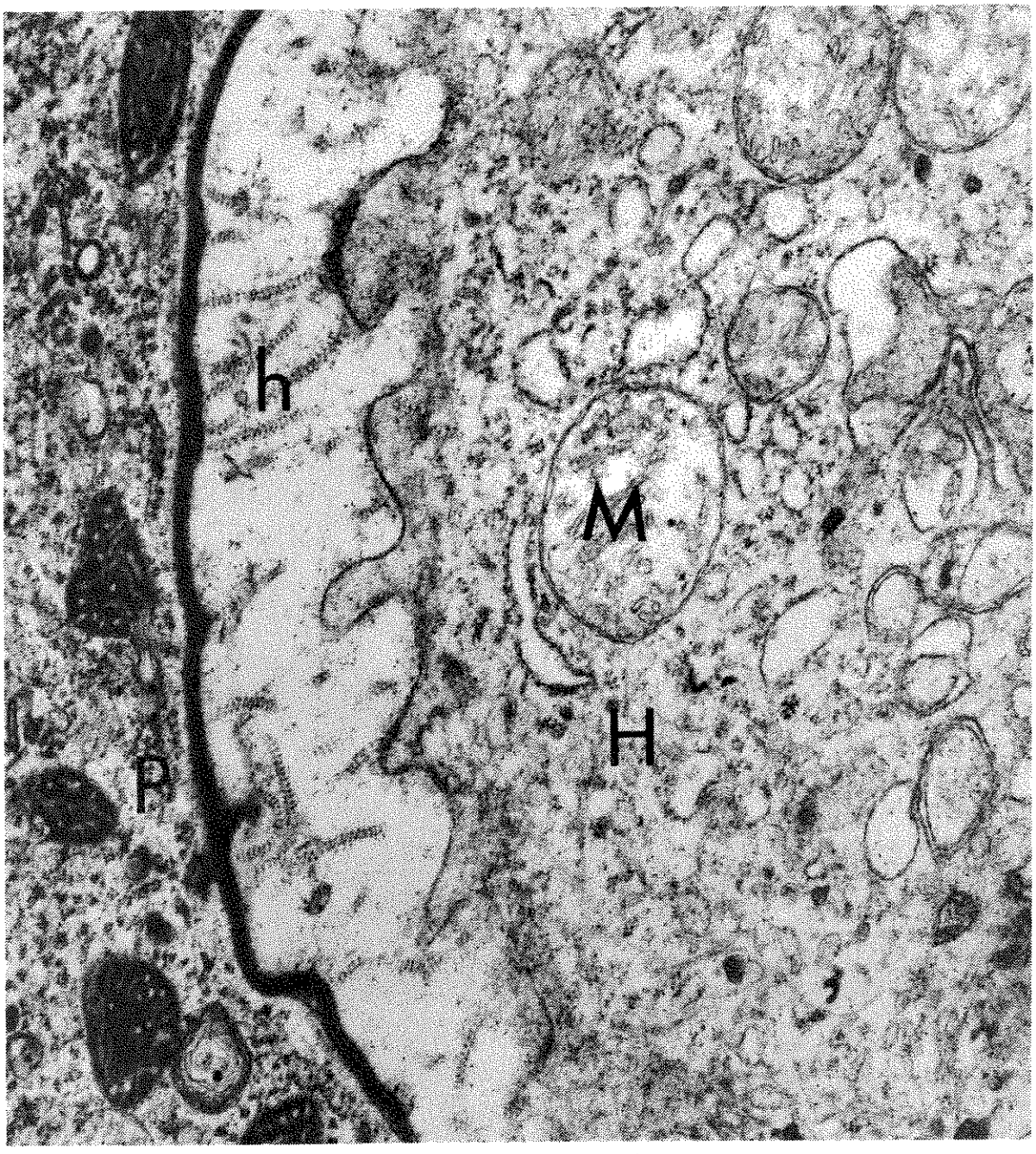


Figure 30. Electron photomicrograph showing damage to immediate host cells (H) by adjoining parasite (P). There is a decrease in host cell mitochondria (M) and then disappearance. (h) attachment helices. Scale, 0.5 microns

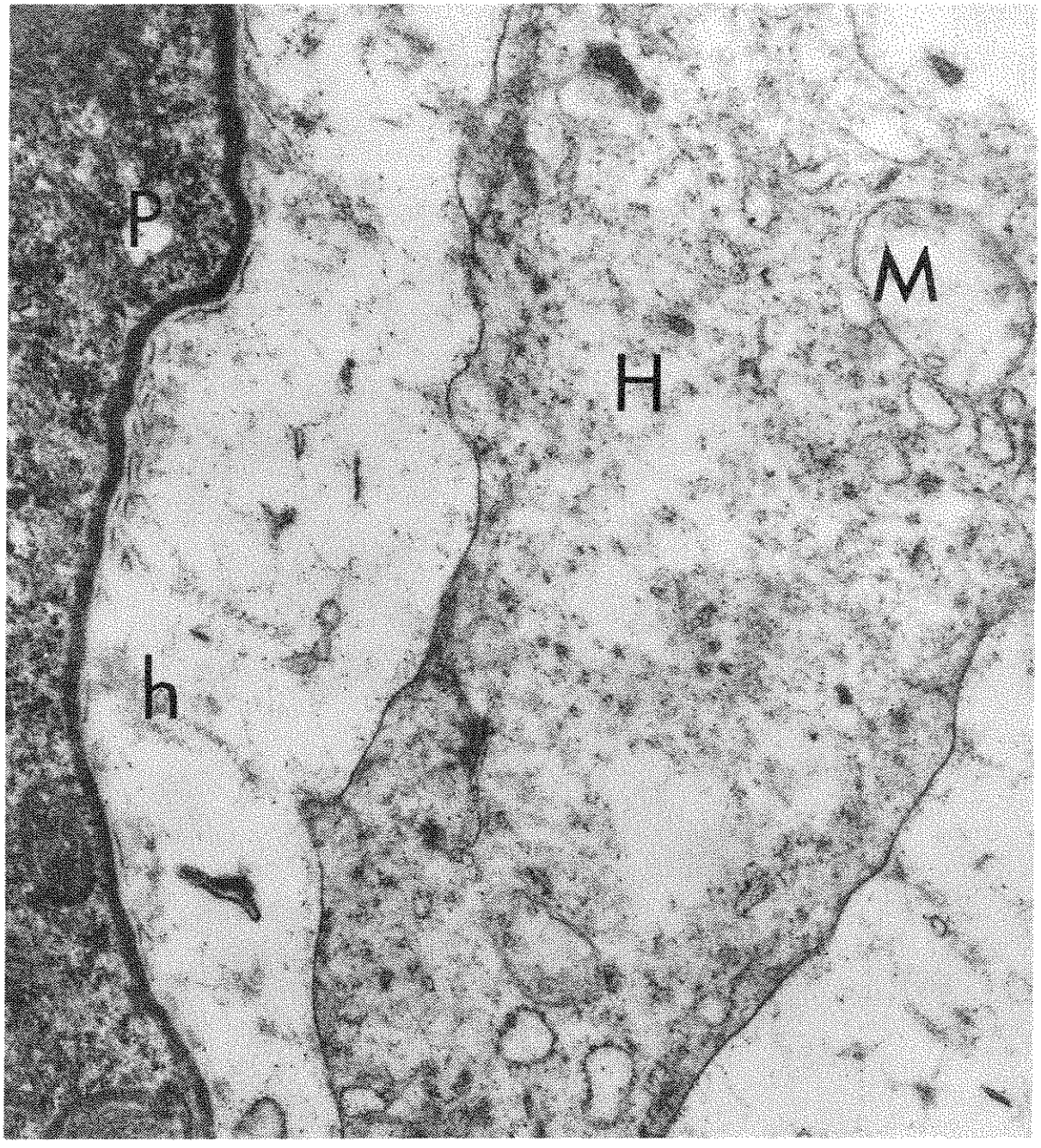


Figure 31. Electron photomicrograph showing damage to immediate host cells (H) by adjoining parasite (P), There is a decrease in host cell mitochondria (M) and then disappearance. (h) attachment helices. Scale, 0.5 microns

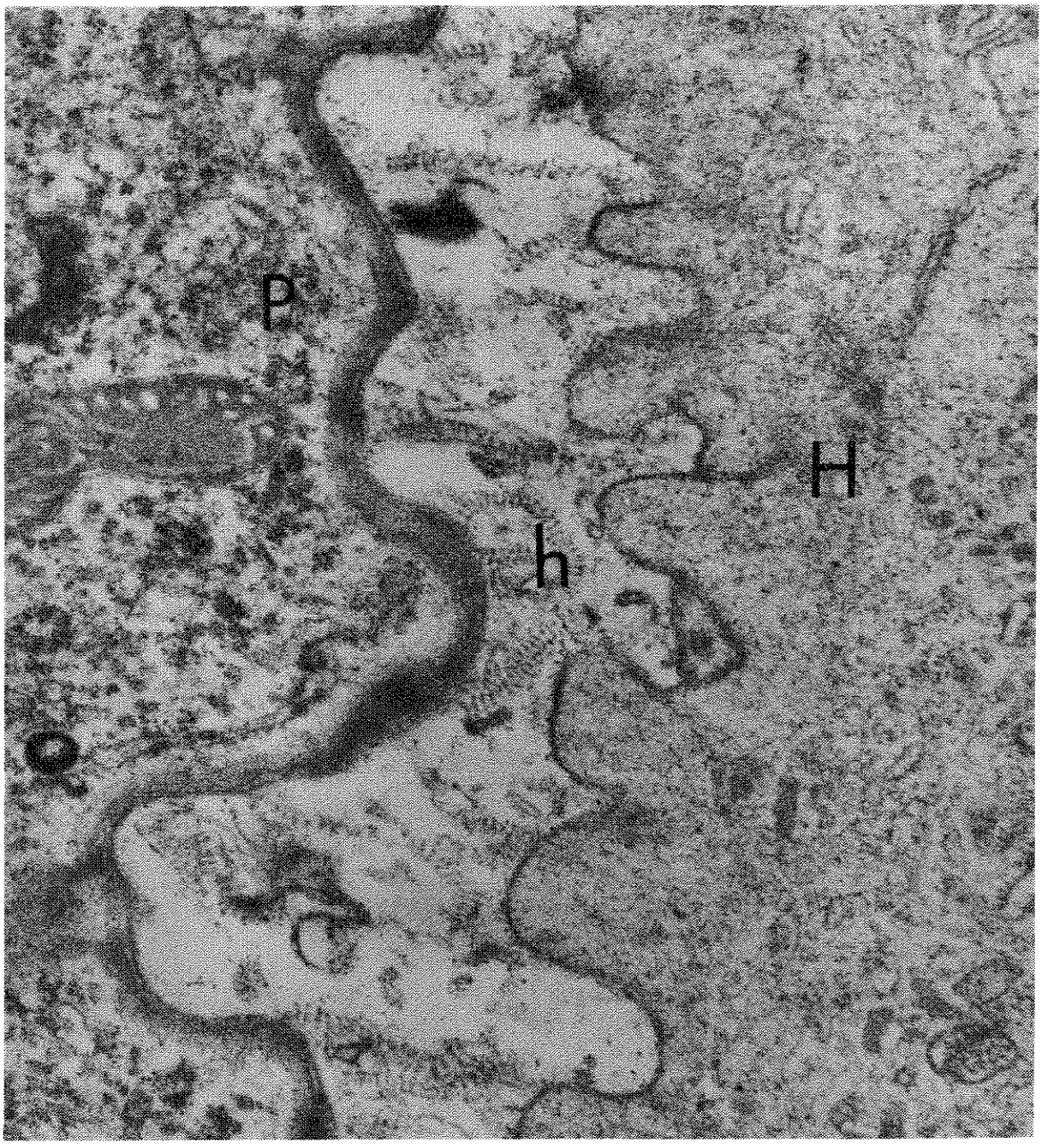


Figure 32. Electron photomicrograph showing damage to immediate host cells (H) by adjoining parasite (P). There is a decrease in host cell mitochondria (M) and then disappearance. (h) attachment helices. Scale, 0.5 microns

tissue for sustenance (Fig. 33). The tentacles extend into the necrotic tissue and granules of similar stain intensity were observed in both host and parasite cells.

Scanning Electron Microscopy

Trichophyra clarki Scanning electron microscopy show (Fig. 34) this suctorian to be saucer-shaped with the convex surface attached to the gill epithelium. There are fine filaments (Fig. 35) between the suctorian and the host cells which are probably aggregations of attachment helices.

Immunology

There is no detectable specific antibody in cutthroat trout against *Trichophyra clarki* as determined by the Ouchterlony and Ascoli methods. Four fish (total length 35 cm) given doses of 1.0, 1.5 and 2.0 ml of the prepared antigen both intramuscularly and intracardially died shortly after the inoculation. Injections of 0.5 ml were not lethal but the area around the intramuscular inoculation turned black and persisted for 10 to 14 days. Injected fish showed the following symptoms: muscles next to the intramuscular inoculation remained contracted; a definite bend occurred in the fishes body; they swam in circles for 4 to 6 hours with the injected side always facing the inside. These symptoms were not observed after the second inoculation. Ten days after the second inoculation experimental fish produced antibodies

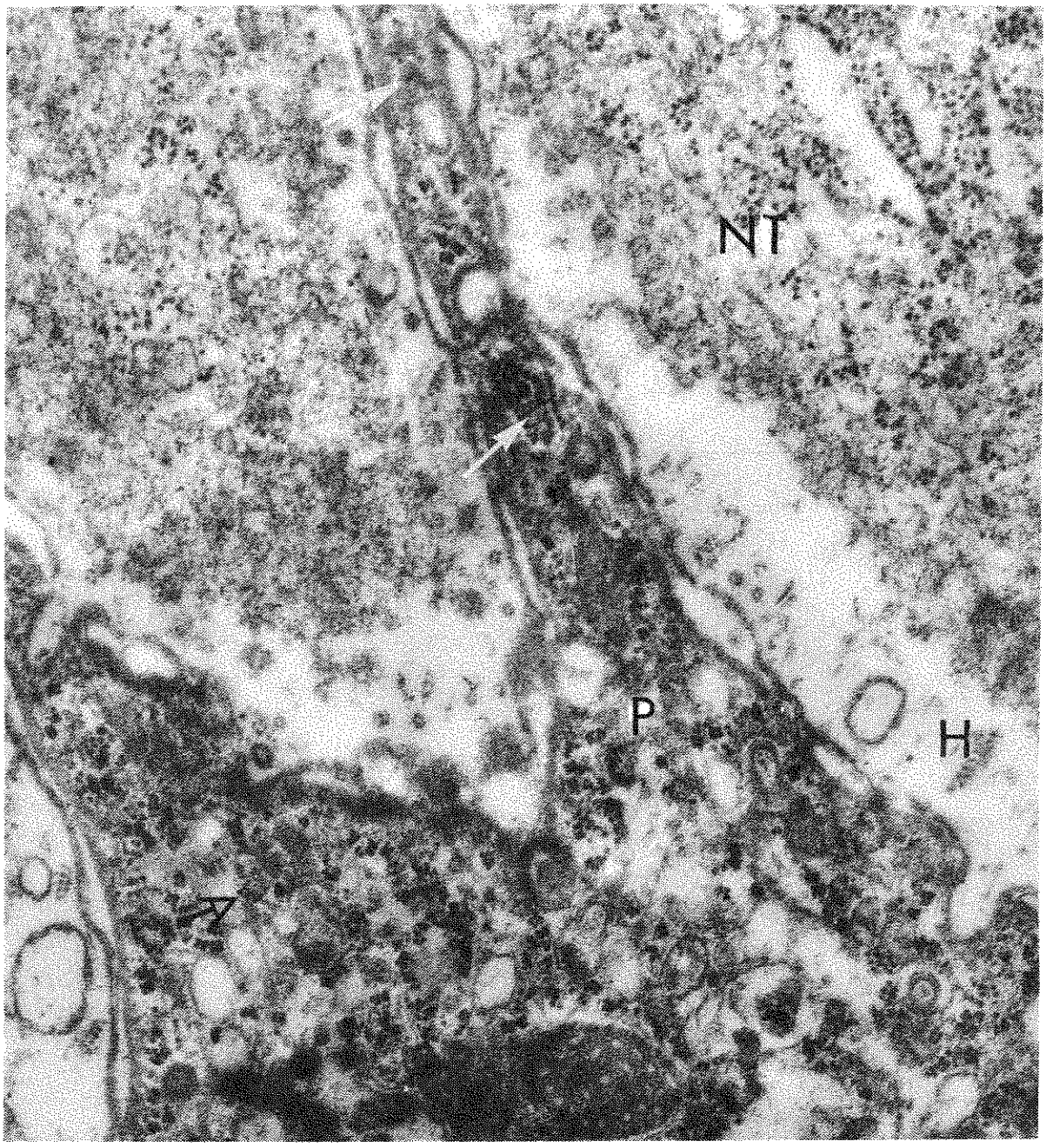
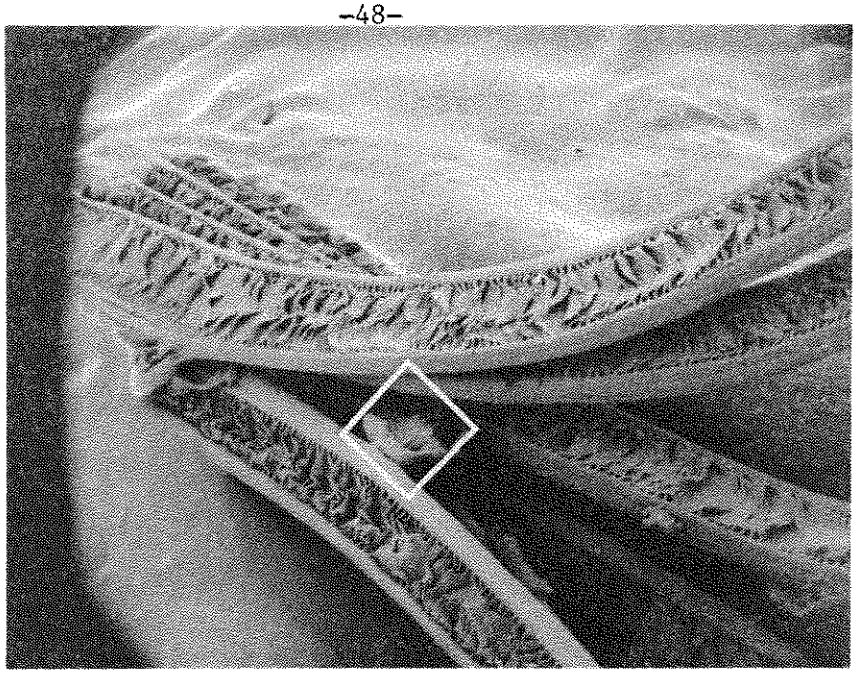


Figure 33. Electron photomicrograph of *T. catostomi* (P) showing part of the cell extending into necrotic host tissue (NT) with attachment helices (H) still present. White arrows show aggregations of dark stained particles in parasite similar to those in necrotic tissue. Scale, 0.5 microns



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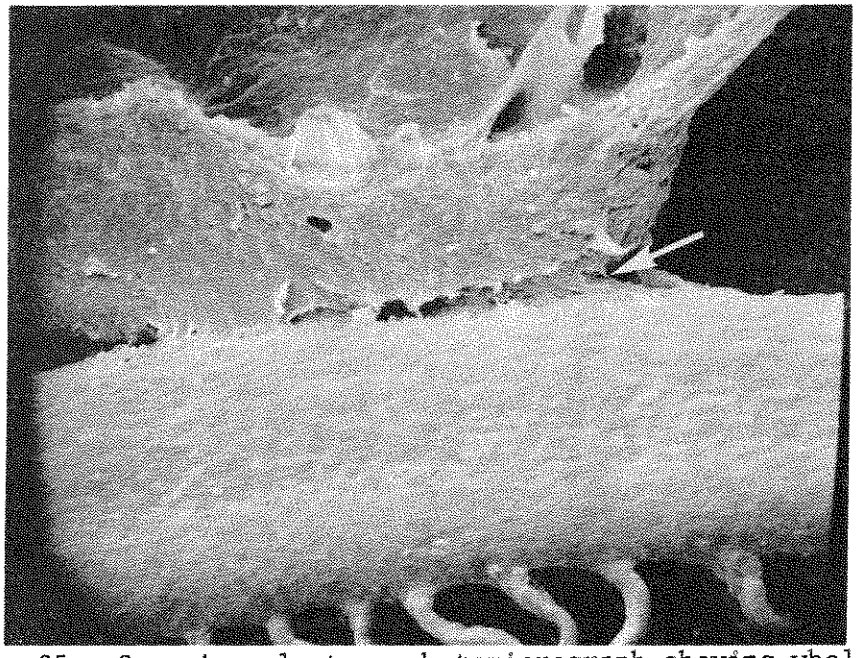

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Figures 34, 35. Scanning electron photomicrograph showing whole mount of *Trichophyra clarki* (box) and attachment filaments (arrow) next to host epithelium.
Scale, 100 microns

detected by microprecipitation (Ascoli method) of fish sera. There was no zone of precipitation for any of the inoculated fish using the Ouchterlony method. One reaction appeared between the 10 day sample and the prepared antigen but was not duplicated on the other Ouchterlony plates. Those fish containing *T. clarki* previous to injection had no suctorians after the second inoculation of the prepared antigen.

Tracer Study

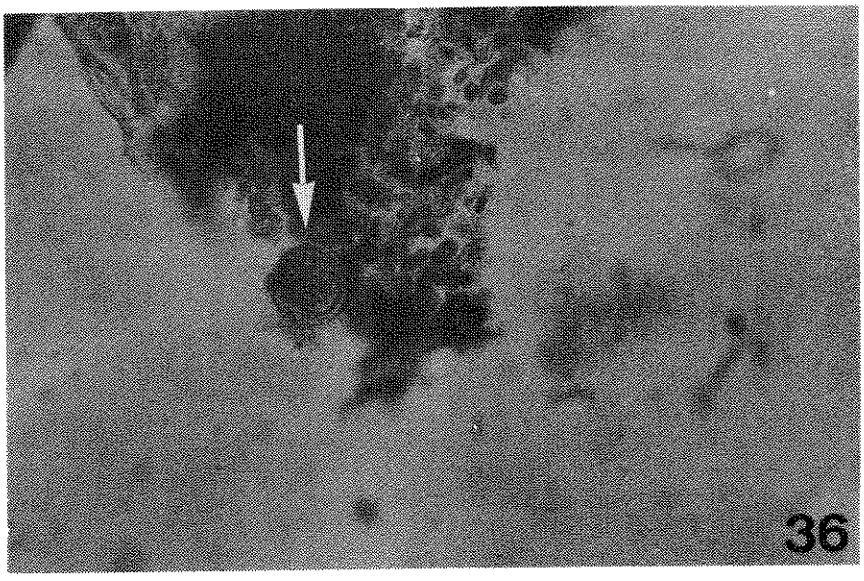
Liquid scintillation counts for both *Trichophyra* and gill samples from injected fish are given in Table IV. There is no uptake of ^{14}C by *Trichophyra* after eight hours following injection. The isotope continues to be present in the gill epithelium after two hours and was presumably available to parasites using sustenance directly from the host. Silver grains were exposed from all four samples after four weeks (autoradiography technique). Samples from fish sacrificed at one and two hour intervals after ^{14}C injection had exposed silver grains only above the lumen of blood vessels and capillaries of the gills. The four-hour sample demonstrated radioactivity in these same regions and also in the epithelial cells of the gill filament. The eight-hour sample was similar but of less intensity (50% fewer grains visible).

Experimental Infections

Both hatchery *(S. clarki henshawi* and *clarki lewisi)* and wild cutthroat trout (Yellowstone Lake) were placed in the experimental tank.

These fish fed on the viable trichophyran macerate and excised infected gills without contracting a patent trichophyran infection. Gill samples were taken periodically for 30 days after exposure. Even the presence of infected fish from Yellowstone Lake in the experimental tank was insufficient to start an infection. Examination of gills from hatchery fish showed only one or two adult trichophyrans in 5 of 35 specimens. Both hatchery and wild trout contracted costiasis in the laboratory tanks. *Costia pyriformis* (Fig. 37) was found in the gills of all specimens. Three blood parameters (oxyhemoglobin, nonprotein nitrogen and hematocrit) were taken from each hatchery and wild fish to determine the effect of parasitism on the circulatory system (Table V). There was no significant statistical difference in oxyhemoglobin and hematocrit for hatchery fish but nonprotein nitrogen was 37% greater for control fish. Parameters of wild trout versus hatchery trout show statistical differences for oxyhemoglobin and nonprotein nitrogen with wild trout having higher values. Hematocrit values were 37.5% packed-cell-volume for wild trout and 42.5% for hatchery fish. The average total length for wild trout was much higher (36 cm) than for the hatchery fish (29 cm, *henshawi* and 28 cm, *lewisi*).

Nutrient broth and brain-heart infusion (4 degrees Celsius) supported *Trichophyra* for 10 days without division. There was no growth and maintenance in other media at 4 or 20 degrees Celsius.



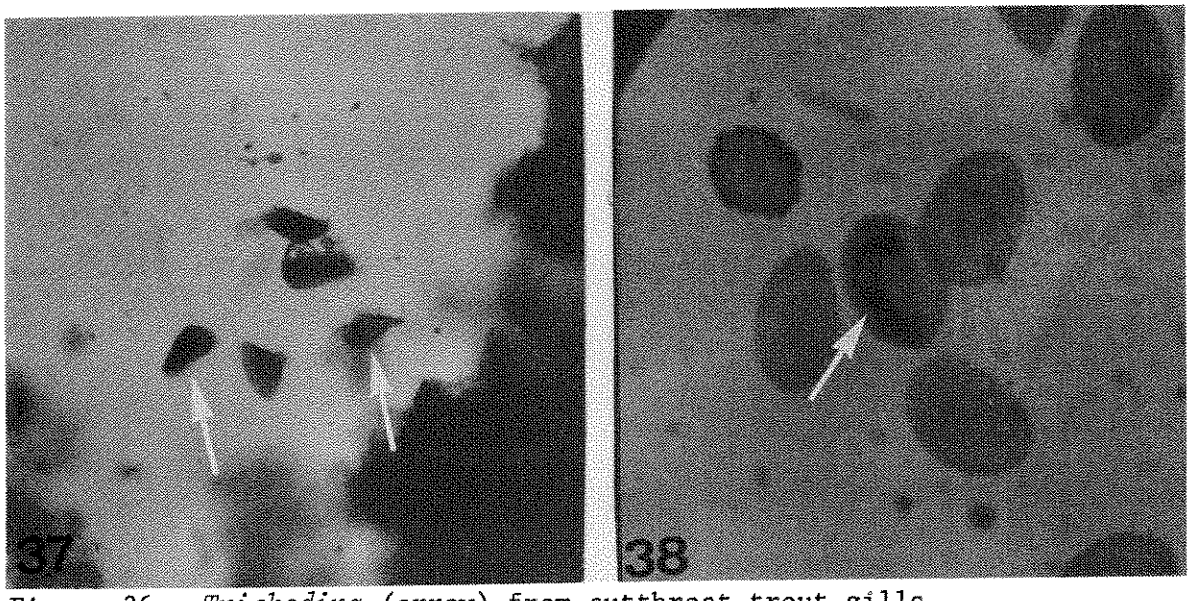


Figure 36. *Trichodina* (arrow) from cutthroat trout gills.
Scale, 10 microns ⊢——⊣

Figure 37. *Costia pyriformia* (arrow) from cutthroat trout gills.
Scale, 10 microns ⊢——⊣

Figure 38. *Hemogragarina* (arrow) from cutthroat trout blood.
Scale, 10 microns ⊢——⊣

TABLE V. Blood Parameters for Wild and Hatchery Cutthroat Trout. Percent Transmittance Given (Absorbance in Parentheses)

Hatchery Fish					
Control			Experimental		
Oxyhemoglobin	Nonprotein Nitrogen	Hematocrit	Oxyhemoglobin	Nonprotein Nitrogen	Hematocrit
18 (.75)	35 (.46)	41	12 (.91)	17 (.75)	41
8 (1.5)	35 (.46)	44	13 (.90)	16 (.80)	42
13 (.88)	15 (.84)	42	6 (1.5)	16 (.80)	40
12 (.91)	17 (.75)	42	18 (.75)	8 (1.5)	37
23 (.64)	23 (.64)	40	11 (.95)	38 (.42)	43
27 (.56)	16 (.80)	30	10 (.97)	19 (.73)	47
10 (1.0)	10 (1.0)	48	12 (1.0)	19 (.83)	42
10 (1.0)	9 (1.1)	50	22 (.68)	14 (.85)	41
5 (1.7)	20 (.70)	40	12 (.91)	29 (.53)	47
14 (.99)	21 (.76)	43	20 (.70)	50 (.30)	40
18 (.75)	20 (.70)	35	10 (.99)	40 (.40)	36
11 (.97)	25 (.60)	48	14 (.85)	49 (.31)	49
20 (.70)	6 (1.2)	42	7 (1.6)	17 (.76)	41
21 (.68)	32 (.50)	41	17 (.76)	19 (.73)	37
14 (.84)	31 (.51)	50	5 (1.7)	19 (.73)	36
14 (.84)	13 (.90)	44	10 (1.0)	16 (.80)	39
20 (.70)	20 (.70)	51	25 (.61)	32 (.50)	45
11 (.95)	20 (.70)	50	10 (.97)	42 (.38)	46
3 (1.5)	8 (1.5)	40	21 (.68)	64 (.20)	39
28 (.55)	16 (.80)	31	25 (.61)	35 (.45)	36
5 (1.4)	8 (1.5)	64	22 (.65)	40 (.40)	39
8 (1.2)	14 (.85)	45			
16 (0.8)	15 (.84)	42			
9 (1.1)	13 (.90)	54			
14 (.96)	17 (.50)	50			
Means 14 (.97)	19 (.82)	44	Means 14 (.97)	28 (.68)	41

TABLE V. (Continued)

Wild Fish

Females			Males		
Oxyhemoglobin	Nonprotein Nitrogen	Hematocrit*	Oxyhemoglobin	Nonprotein Nitrogen	Hematocrit
38 (.42)	57 (.24)	38, 34, 34	12 (.92)	25 (.61)	38, 41, 42
27 (.57)	26 (.57)	45, 36, 33	10 (1.0)	32 (.49)	40, 35, 35
18 (.75)	16 (.81)	38, 40, 30	17 (.78)	70 (.16)	43, 40, 48
13 (.90)	10 (.98)	42, 42, 35	17 (.78)	44 (.36)	36, 38, 36
44 (.36)	57 (.24)	41, 30, 35	35 (.46)	37 (.44)	46, 48, 30
24 (.62)	58 (.23)	41, 32	46 (.34)	54 (.27)	37, 30, 34
13 (.86)	48 (.31)	38, 33	18 (.74)	55 (.26)	44, 42, 36
17 (.78)	26 (.58)	40, 40	25 (.60)	60 (.24)	44, 30, 30
13 (.90)	24 (.62)	40, 44	45 (.35)	55 (.26)	38, 29, 36
19 (.72)	25 (.61)	51, 37	24 (.62)	21 (.68)	35, 38, 20
36 (.45)	43 (.38)	36, 17	21 (.68)	13 (.88)	39, 42
23 (.64)	37 (.44)	42, 40	34 (.47)	17 (.76)	43, 37
22 (.65)	38 (.42)	35, 45	23 (.64)	46 (.34)	39, 40
33 (.48)	12 (.91)	38, 32	28 (.55)	15 (.81)	39, 40
18 (.75)	11 (.95)	39, 38	26 (.66)	22 (.70)	40, 36
Means 23 (.63)	32 (.53)	37	Means 25 (.63)	37 (.44)	40

* Additional hematocrit samples only were taken without determining the other two parameters.

TABLE V. (Continued) Analysis of Variance of Data

Source of Variation	Sum of Squares	d.f.	Mean Square	F.	$F_{.95}$	$F_{.99}$
Wild Trout (Oxyhemoglobin and Nonprotein Nitrogen)						
Total	12385	59				
Among-Means	1835	3	611	3.2*	2.76	4.13
Within-Samples (or Error)	10550	56	188			
* Significant at .95 level (d value 8.0)						
Wild Trout (Hematocrits)						
Total	1801	69				
Among-Means	45	1	45	.88	4.10	
Within-Samples (or Error)	1756	34	51			
Hatchery Trout (Control vs. Experimental, All Parameters)						
Total	27253	125				
Among-Means	18209	5	3442	46.0**	2.29	3.17
Within-Samples (or Error)	9044	120	75			
** Significant at .99 level (d value 10.9)						
Wild versus Hatchery (All Parameters)						
Total	33847	179				
Among-Means	16691	5	338	2.96*	2.21	3.02
Within-Samples (or Error)	17156	150	114			
* Significant at .95 level (d value 10.3)						

DISCUSSION

Taxonomy and Distribution

Culbertson and Hull (1962) lumped all *Trichophyra*, found in fishes, into one species *(T. piscium)*. They studied trichophyran specimens from five species of fishes comparing the following criteria; mensural data, morphological characteristics and host specificity. I used the same criteria and in addition, ultrastructure of the organism to describe two new species of *Trichophyra*. The pattern and number of microtubules in the tentacles of *T. clarki* (host, cutthroat trout) are distinctly different in *T. catostomi* (host, longnose suckers). Microtubule patterns have been used by various authors (Table VI) for differentiating genera of suctoria but no published records have been found where this character has been used for distinguishing species. The microtubules may originate from the macronucleus. Microtubule aggregations were observed in the macronucleus of *Trichophyra* and large bundles have been seen in the same area for *Acineta* (Bardele, 1968a, 1969) and *Tokophyra* (Heckmann, 1967).

All cutthroat trout 14 cm in total length from Yellowstone Lake were found to be infected with trichophyrans. The smallest specimen examined containing gill suctorians was 12 cm in total length. On the other hand, fry and fingerling bass are commonly infected with *T. micropteri* (Davis, 1947, 1967). The gill lamellae of cutthroat trout may have to be a certain size before suctorians can attach. There were no

TABLE VI. The Microtubule Counts for Suctorian Tentacles

Organism	Numbers for each Ring Outer	Inner	Reference
Tokophyra infusionum	21	28	Rudzinska, 1967
Cyathodinium	50-54	64-76	Paulin, 1969
Phalacrocleptes verruciformis	0	16	Lom, 1967
Podophyra parameciorum	22	35	Jurand, 1965
Acineta tuberosa	24	32	Bardele, 1968b
Ephelata gemmipara	115-130	624-672	Batisse, 1966
Lernaeophyra capitata	33	40	Batisse, 1967b
Trichophyra clarki	84-86	110-112	
Trichophyra catostomi	56-58	58-64	

suctorians on the gills of redside shiners from Yellowstone Lake which have small lamellae. *T. clarki* and *T. catostomi* were found in Yellowstone Lake, its tributaries and Yellowstone River above the Upper and Lower Falls. Other areas were checked (Table II), including the Yellowstone River immediately below the Falls and approximately 144 km downstream, without finding these suctorians. The gill samples from 23 fish taken below the Falls were free of trichophyran infections. The reason for this is inknown but the small number of samples may have been insufficient to detect low incidence of trichophyran infection. *Trichophyra* was found in other salmonids from Madison River (Montana)

and the Snake River (Idaho).

Host-Parasite Relationship

Trichophyra clarki and *T. catostomi* are parasitic to cutthroat trout and longnose suckers respectively. There was no uptake of ^{14}C or specific immunity by *T. clarki* but electron microscopy disclosed definite changes in the organelles of host epithelial cells. The mitochondria decrease in number and disappear which is probably due to the masking effect the parasite has on respiratory activity (Davis, 1942 and Meyers, 1966). Strobel (1965) observed a reciprocal response by mitochondria for hydrating spores of *Puccinia striiformis* which was also related to respiration. The pathological symptoms of infected longnose suckers are more extensive. Infected gill tissue shows areas of hyperplasia and necrosis, which is visible with light microscopy, in addition to the previously described fine structures.

The exclusive use of free-living protozoa as food for fish suctoria has been questioned (Davis, 1942). The tentacles of suctoria are used in obtaining food, immobolizing prey and transporting cytoplasm to the central body (Hull, 1961a, 1961b). Phialocysts (Batisse, 1967a, 1967b) or haptocysts (Bardele, 1967) at the tip of the tentacles are used to hold and impale prey. Rudzinska (1954, 1965, 1966) described *Tokophyra infusionum* feeding on living ciliates using its tentacles as described. No other protozoa were observed in the fish gills or on the trichophyran tentacles in infected fishes (*T. intermedia*) of Czechoslovakia (Lom,

1960). Prost (1953) suggested that *T. intermedia* feeds on host necrotic tissue. In my study no free-living protozoa were impalled on the tentacles of the two suctorian species and I observed that *T. catostomi* may feed on the necrotic gill tissue of longnose suckers. The mucous layer on the surface of gill epithelium may also be a source of food. Periodic acid Schiff preparations show particles (complex polysacchrides) of similar stain intensity both in *T. clarki* and on the surface of host epithelium. *Trichophyra*, added to cultures of free-living protozoa, failed to divide or be maintained.

The attachment helix is an organelle which functions in holding the parasite next to the epithelial host cell. This structure may hold the parasite in position when water flows across the gill surface. Other structures in the interface of the parasite and host have been described. Uspenskaja (1966) found small cytoplasmic extensions ("rootlets") from *Myxidium* into the urinary bladder epithelium of *Esox lucius*. He considered these to function primarily as absorption organelles rather than for attachment. Scholtyseck and Hammond (1966a, 1966b) noted ribbon-like extensions (150 angstroms by 2 microns) from *Eimeria* macrogametocytes into host cells and postulated that their function was ingestion of nutrients. Beams (1959), Reger (1967) and Stebbens (1968) reported cell membrane folds between parasite and host, in mature gregarine trophozoites, which function for both attachment and absorption.

Life History

The life cycle of *Trichophyra* involves single and multiple endogenous buds (Fig. 9) which escape, via a birth pore, and form ciliated larvae (Bykhovskaya-Pavlovskaya, 1964). The larval stage is free-living and continues for an undetermined period after which it attaches to a host, loses the cilia and forms tentacles (Hoffman, 1967). The infraciliature remains intact when the organism reaches adult stage. Sexual reproduction by conjugation has also been reported (Davis, 1942 and Kudo, 1966). In my study, one reason for unsuccessful trichophyran cultures, lack of a patent infection in experimental fish and absence of infected fish below the Falls of Yellowstone River may be the necessity of an intermediate host in the life history. Samples of aquatic invertebrates (six species) from two tributaries of Yellowstone Lake were checked for larval and adult suctorians. A suctorian of undetermined taxonomy was found in the external gills of caddis-fly larvae (Order Trichoptera) which was smaller than *T. clarki* but of similar morphology. There may be a maturation process required by the ciliated larvae before they can become viable to the definitive host. Perhaps the 30 day period of exposure was insufficient for the suctorian to divide and attain a patent infection in cutthroat trout. Most ciliated protozoa require 12-48 hours to reach maximum numbers under ideal conditions (Kudo, 1966).

Experimental Immunity

Injections of sonicated trichophyrans above 0.5 ml were lethal to fish. There are two possible explanations for this: drastic displacement or blood dilution following the intracardial injection; presence of toxic materials within the trichophyran. Smith (1966) gave averages of 5.4 and 6.9 ml blood per 100 grams body weight for blood volumes of two species of salmon and brook trout respectively. Using these data, injections of 1.0 ml of antigen into the heart of cutthroat trout would increase blood volume 7% while 2 ml would increase it 14%. The capacity of the bloodstream buffer systems may be exceeded resulting in a lethal pH change. Toxic substances are quite common in protozoans (Kudo, 1966) and trichophyrans may have poisons which are released into the antigen preparation. Surviving injected fish produced detectable antibodies which were sufficient to repel attached trichophyrans. Antibodies were detected by microcapillary columns but not with Ouchterlony plates which corroborates the work of Powell (1968).

Blood Changes

Three blood parameters were used to see if *Trichophyra* had a physiological effect on the cardio-vascular system of cutthroat trout. Low degree of parasitism did not disturb respiratory and excretory functions of the fish in any detectable manner. Reichenbach-Klinke (1966) demonstrated that damage to the blood due to parasitism takes place when regulatory mechanisms are not successful. Schumacher (1956)

reported one example of the above, brook trout infected with furunculosis doubled sedimentation readings after 60 minutes as compared to control fish.

Blood data for male wild cutthroat trout had higher average hematocrits than females (40 and 37). Snieszko (1961) reported sexual differences for hematocrits of rainbow trout (male 50.0, female 42.2), brown trout (male 44.0, female 38.3) and brook trout (male 45.2, female 38.0). The range of hematocrits for mountain whitefish was 28.5 to 62.2 (average 43.5) (McKnight, 1966). She also noted differences for sex and size. The hematocrit range for hatchery cutthroat trout was 30.0 to 64.0 (average 42.5). There was a significant difference between the hematocrit values of wild and hatchery? cutthroat trout (37.5 and 42.5).

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