STUDIES ON THE ULTRASTRUCTURE, ONTOGENY AND TRANSMISSION OF MYXOSOMA PHARYNGEUS AND M. CYPRINI (PROTOZOA: MYXOSPORIDA)

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STUDIES ON THE ULTRASTRUCTURE, ONTOGENY AND TRANSMISSION OF *MYXOSOMA PHARYNGEUS* AND *M. CYPRINI* (PROTOZOA: MYXOSPORIDA)

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CHAPTER I

INTRODUCTION

The Myxosporida are all parasites in lower vertebrates, principally fishes. They have been known to man for nearly a century but, despite the existance of hundreds of species descriptions, their phylogenetic relationships remain unclear. Many descriptions, along with quantitative data on seasonal variability or host and organ specificity, are incomplete. Research on such fundamental topics as transmission and ontogeny, chemical composition and ultrastructure, nutrition and metabolism, or _in vitro_ culture either has been superficial in scope or has not been conducted at all.

The history of revisions and opinions concerning the taxonomy of the Myxosporida was given by Stunkard (1969) and stated simply, the position of this group within the Protozoa has been transitory and remains unsettled. Ulrich (1950; in Levine, 1969) questioned if the Myxosporida were Protists because of the multicellular origin of their spores. Grell (1956) excluded the Myxosporida from the Protozoa. Dunkerly (1925) presented evidence that myxosporidan spores were physiologically analogous to the infusoriform embryo of the Mesozoan, _Dicyema_, although he also recognized that the Mesozoa probably had ciliated ancestors. To the contrary, Shulman (1966) and others have argued that the Myxosporida arose from multinucleated parasitic amoebae. Finally, Lom (1969) and Lom and Vavra (1964, 1965) have aligned the Myxosporida with the Coelenterata (Metazoa) because of the parallel development of polar capsules with cnidocysts.

During September, 1969, an undescribed species of Myxosporida infecting *Gambusia affinis*, the mosquitofish, was found in Oklahoma (Parker, Spall and Warner, 1971). Extensive collections of *G. affinis* from throughout Payne County plus limited collections from Logan, Noble and McCurtain Counties indicated that the parasite, *Myxosoma pharyngeus*, is ubiquitous in these areas and that it is present in all seasons. Furthermore, the host is easily cultured under laboratory conditions, thus establishing this host-parasite combination as a satisfactory model for studying the biology of the Myxosporida.

The purposes of the present studies were to:

1. Accomplish experimental transmission of *M. pharyngeus* to *G. affinis*, and to determine the role of transfer hosts and the effects of "aging" of spores and environmental oxygen levels on transmission.
2. Describe the development of the vegetative and reproductive stages of *M. pharyngeus* from either natural or, preferably, experimental infections.
3. Give an account of the ultrastructure of *M. pharyngeus* spores.

More recently, a congener, *M. cyprini* sp. n., was found infecting the gills of *Notropis lutrensis* and *Notemigonous crysoleucas* in this locality (Spall, in press) and ultrastructural features of the two species were compared.

The terms used to describe life-cycle stages and development of the Myxosporida do not always have the same meanings as when they are applied to descriptions of the Sporozoa for which they were originally intended. To minimize ambiguity, the following definitions will be used in this paper.

cyst: the growing vegetative individual in histozoic Myxosporidans;

plasmodium: equivalent to "cyst", applied to both histozoic and coelozoic species;

plasmogamy: the union of the cytoplasm of two or more cells while the nuclei of each remain distinct;

plasmotomy: equivalent to "internal budding" of the older literature. Used to denote the process whereby vegetative nuclei within the plasmodium or cyst acquire their own cytoplasm and limiting plasma membranes;

sporont: a cell whose immediate division products are sporoblasts (Sprague and Vernick, 1971, p. 564);

sporoblast: a multicellular stage which is destined to develop into one (monosporoblast) or more (pansporoblast) spores;

spore: the mature individual; the stage which is transmitted;

sporogenesis: the process by which spores are developed from a sporoblast;

sporulation, sporogony: equivalent to sporogenesis;

trophozoite: the sporoplasm (zygote) after it is released from the spore and while it is invading new host tissues.

CHAPTER II

ATTEMPTS TO TRANSMIT *MYXOSOMA PHARYNGEUS* IN LABORATORY AQUARIA

Introduction

It long has been supposed that life cycles of the Myxosporida were direct from fish to fish, that the route of infection was oral, and that the infectious stage was the spore (Plehn, 1904, Kudo, 1966; Schaperclaus, 1954). According to the traditional hypothesis, after ingestion of the spore by the host, the sporoplasm leaves the spore, penetrates intestinal mucosa and migrates via the lymphatics and systemic circulation to a target organ. However, none of these latter points has been verified experimentally and successful transmission of a histozoic myxosporidan by feeding spores was reported only once (Uspenskaya, 1963; pers. comm. in Hoffman and Putz, 1969). However, Uspenskaya's results are questionable since numerous attempts to duplicate her work have failed (e.g., Hoffman and Putz, 1968; Schafer, 1968; Fryer, 1971). The only reproducible transmission experiments conducted to date with histozoic species of Myxosporida (*Ceratomyxa shasti* and *M. cerebralis*) involve placing susceptible (i.e., young) salmonid fish in screened cages into streams, lakes or ponds where the disease occurs (Hoffman and Putz, 1968; Schafer, 1968) or into aquaria or raceways which contain at least three inches of bottom sediments contaminated by spores (Hoffman and Putz, 1969; Fryer, 1971; Schafer, 1968; Tidd and Tubb, 1970).

The mode of transmission in these studies was not established. With M. cerebralis, aging of spores for at least three to four months is critical for transmission (Hoffman and Putz, 1968, 1969, 1971; Tidd and Tubb, 1970). Schafer (1968) showed that infection by C. shasti did not depend on ingestion of food organisms, that association of non-infected trout with infected trout in the same tank produced no infection in the former group, and that force-feeding of infected viscera containing schizonts, trophozoites and spores to non-infected trout failed to produce infection. Salmonids maintained for as long as six months in the presence of fish (carcasses) which had died from C. shasti did not become infected (Schafer, 1968; Fryer, 1971).

There is additional evidence to that given by Schafer suggesting that routes other than oral are important in the transmission of histozoic myxosporidans. Putz and Hoffman (1966) and Putz and Herman (1970) reported that they successfully transmitted M. cerebralis to embryonic (prehatched) trout and prefeeding sac fry by placing eggs overnight into aquaria contaminated by spores and by exposing one-day-old fry to contaminated aquaria for periods of at least 40 minutes.

In contrast to histozoic myxosporidans for which controlled experimental transmission has failed, Kudo (1930) reported results of Auerbach (1909) who transmitted the coelozoic form Myxidium bergense to young Gadus virens by feeding spores. Walliker (1968) was unsuccessful in attempts to transmit Myxidium by feeding spores. Kudo (1930) also reported that Erdmann (1912) fed spores of coelozoic Chloromyxum leydigi in gelatine capsules to host fish and found that two-thirds of 33 fish thus fed became infected.

Transplantation of the histozoic (gill) myxosporidan Myxosoma ovalis from Ictiobus bubalus to Notemigonus crysoleucas was achieved by hypodermal and intramuscular injection of spores (Wagh, 1961). Schafer (1968) transferred C. shasti from infected to noninfected rainbow trout (Salmo gairdneri) by abdominal inoculation of peritoneal fluid containing schizonts, sporoblasts and spores. Schafer described development as "possibly regressive" because the visceral cavity contained spores and early schizonts and the intestinal tissue had veen invaded by schizonts. No trophozoites or pansporoblasts were found.

In view of the paucity of reliable data concerning transmission of the Myxosporida, and the need for infections of known duration to trace the course of development of trophozoites and sporogenic stages, the following experiments were conducted.

Methods and Materials

Aqueous suspensions of 200-1000 M. pharyngeus spores were pipetted into the stomach of noninfected, laboratory-reared mosquitofish. These spores were orally administered immediately after their removal from naturally infected hosts or were given after "aging" up to three months in either an aerobic or an anerobic (microaerobic) aqueous environment. Aged spores were prepared in the following manner.

A total of seventeen intact cysts were removed from 10 infected, wild-caught mosquitofish, placed in sterile saline (0.12 M NaCl) containing 0.05 M glucose and macerated with a glass rod. The resulting spore suspension was filtered through cotton gauze overlaid by a thin, loose mesh of spun glass to remove the small amounts of host tissue adhering to the cysts. Spores were collected from this filtrate on a 5µ

Millipore filter, resuspended in fresh saline, and concentrated by centrifugation (2000 rpm for 5 min.). The resulting pellet was resuspended in 8 ml of sterile, freshly-boiled and cooled tapwater (to remove gases) containing 0.05 M glucose as an exogenous energy source for the spores, if needed.

The spore suspension was divided into four lots (A, B, C and D) of approximately two ml each. Two (A and B) were "aerated" by shaking in presterilized 100 ml Pyrex tissue-culture bottles. The remaining two lots (C and D) were pipetted into shell vials, overlaid with paraffin oil and capped. Spores in these latter two vials were considered as having been maintained in an anerobic or microaerobic environment. All lots of spores were kept at room temperature. To compute estimates of dosage, samples of spores were counted in a Bright Line[1] hemacytometer.

Laboratory transmission of *M. pharyngeus* was attempted also by intramuscular (I.M.) and intraperitoneal (I.P.) inoculation of total cyst contents (spores and sporogenic stages). Cysts contents which were injected I.M. and I.P. into experimental fish were aspirated directly from infected tissues into tuberculin syringes via 27 ga. needles, mixed with a small amount of saline and immediately transferred by the prescribed methods to uninfected mosquitofish.

The final means whereby laboratory transmission of *M. pharyngeus* was attempted involved the use of mosquitofish natural food organisms as potential vectors. These included mosquito larvae (*Culex*) and cladocerans (*Daphnia*). Both fresh and aged spores were used. Fresh spores were obtained from naturally infected fish, macerated in tapwater which had been allowed to stand in an open container for at least 24 hours and, along with bits of infected tissue, fed to the invertebrates by mixing the spore suspension in one-gallon aquaria containing these organisms. Aged spores used in vector experiments were obtained from lots A-D described above.

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[1] American Optical Company, Buffalo, New York.

Cultures of mosquito larvae, originally obtained from the Oklahoma State University Insectory, were hatched from eggs and raised in 500 ml beakers of water. They were fed pelleted rabbit food which had been pulverized in a mortar. Adult mosquitoes were kept in screened cages and provided chicken blood-meals and a sucrose solution as nutrients. Egg rafts were collected in beakers of water placed in these cages. Cladocerans were cultured in one gallon aquaria and provided a diet of powdered yeast and dry milk. Mosquitofish used in these experiments were offspring of uninfected parents. They were raised in concrete-block, 10 m^3 pools, transferred to 40-liter aquaria and maintained on a diet of crumbled trout chow, brine shrimp (Artemia) and mosquito larvae.

Spores were administered to lots of mosquitofish, in the following manner:

I. Oral transmission; spores in aqueous suspension pipetted into stomach (except "Group K", see below).

Group A.	30 fish, each of which received 0.1 ml of an aqueous suspension containing 1000 spores freshly removed from naturally infected hosts.
B thru G.	30 fish, each of which received 1000 spores aged aerobically in water for 1, 6, 14, 30, 63, and 90 days, respectively.

H thru J. 30 fish, each of which received 500 spores aged in the absence of air for 6, 14, and 30 days, respectively.

K. 30 fish which were allowed to feed _ad libitum_ on freshly killed, minced fish tissues containing _M. pharyngeus_ cysts (with ca. 7 x 10^4 spores).

L. Control; 38 fish, each of which received a sham oral inoculum (0.1 ml water).

II. _Inoculation_; spores in saline suspension, 0.1 ml injected hypodermally, I.M. or I.P.

Group M. 30 fish, each of which received 200 fresh spores I.P.

N. Similiar to M.; dose = 1000 spores

O. Similiar to M.; I.M. and/or hypodermal

P. Similiar to O, dose = 500 spores aged aerobically in water for 23 days.

Q. Control; 16 fish each of which received a sham I.P. injection (0.05 ml saline).

III. _Vector_; spores "fed" to cladocerans and mosquito larvae which subsequently were washed several times in running water and fed to mosquitofish.

Group R. 700 _Daphnia_ allowed to feed for six hours in medium contaminated by fresh spores and subsequently were fed to 30 mosquitofish.

S. Similiar to R, 80 _Culex_ larvae substituted for _Daphnia_; experiment repeated with same group of fish three days in succession.

T. 20 mosquitofish; allowed to feed *ad libitum* on both *Daphnia* and *Culex* larvae which had been cultured for 1 to 5 days in water containing spores aged aerobically for 1 to 10 weeks.

IV. Contaminated environment (Group U): 30 fish placed in same aquarium which had housed a total of 37 naturally-infected *Gambusia* for 1 to 13 weeks prior to entry of experimental fish.

The procedures used to evaluate the effectiveness of inoculation or transmission efforts and to diagnose expected infections were as follows:

1. Systematic sampling of two (usually) fish from each experimental unit for histological demonstration of plasmodia at approximately 10 minutes, 1, 4, 8, 24, 48, 96 hours and 1, 2, 4, 8, 12 and 16 weeks (termination point) post exposure. Histological techniques included fixation of whole fish or selected tissues in ethanol-formalin-acetic acid solution (AFA), decalcification (where necessary) in 10% EDTA, embedment in paraffin, serial sectioning at 7-10μ and staining with Delafield's hematoxylin-eosin or Mallory's aniline-blue connective tissue reagents. Tissues selected for examination included those at the site of inoculation (gastrointestinal tract, muscle, etc.) and those at the expected locus of development of the parasite (pharyngeal tissues), along with those possibly involved with the migration or transport of the amoebula (liver, mesenteries, blood vessels, kidney, cardinal sinus). Smears of cardiac blood taken from each sampled fish were fixed in methanol and

stained with Wright's stain. Sections and blood smears were scanned at 150X and 675X.

2. Weekly, gross inspection of skin and pharynx of fish.
3. Examination of serial sections (prepared as above) of suspected vectors for evidence of ingestion of spores.

Results and Discussion

No patent infections of *Gambusia* experimentally exposed to *Myxosoma pharyngeus* were found during the 16-week, postinoculation observation period. Even cyst contents injected I.M., I.P. and hypodermally failed to produce detectable infections. No parasite stages could be demonstrated in paraffin sections of tissues from the locus of inoculation beyond the 30th day following injection. Prior to then, the number of spores originally present in the inoculated tissues gradually diminished. Sporoblasts or other sporogenic stages were not seen in fishes sacrificed after the second day postinoculation. The numbers of host reactive cells which appeared at the site of inoculation were minimal.

Examination of tissue sections and blood smears from mosquitofish which had spores pipetted into their stomachs revealed no trophic stages of the parasite. Spores, detectable in the alimentary canal of recipient fish for up to 72 hours post inoculation, underwent no visible changes.

Histological examination of the invertebrates used in feeding experiments showed that both *Culex* larvae and *Daphnia* ingested *M. pharyngeus* spores along with other food items, but subsequent ingestion of these contaminated invertebrates by *Gambusia* produced no detectable myxosporidan infections in the fish. The spores appeared to undergo no morphologic changes in the gut of either Culex or Daphnia.

The results of the present experiments, and those of Hoffman and Putz (1969), Fryer (1971), Walliker (1968) and others indicate defects in the arguments presented in support of the central hypothesis of direct transmission of myxosporidan parasites from fish-to-fish via ingestion of spores. The incongruities in experimental results supporting this hypothesis, of course, in no way disprove its validity. However, alternative explanations for transmission of histozoic myxosporidans deserve more attention.

Observations on natural gill infections by Henneguya exilis in channel catfish, Ictalurus punctatus, and M. cyprini in red and golden shiners support the hypothesis of vector transmission by ectoparasitic copepods.

These observations include:

1. The Myxosoma and Henneguya cysts are not uniformly distributed within gill tissues, but rather are clumped. That is, many plasmodia occur together in one or more small, restricted areas of the gill. If the infective stages reached the gill via the circulation, one might expect random and nearly uniform distribution of plasmodia.
2. Observations made with an electron microscope confirm that, in the case of M. cyprini, early plasmodia develop in peripheral epithelial tissues of secondary gill lamellae rather than within the confines of the capillary compartment.
3. H. exilis occurs concomitantly with ergasilid copepod infections in channel catfish. Ergasilids feed by browsing on gill

epithelium and connective tissues (Einszporn, 1964) and commonly move from host-to-host (Kabata, 1971).

Although experimental proof is lacking, this circumstantial evidence indicates that some myxosporidans may be transmitted by the bites of externally-parasitic copepods.

CHAPTER III

ULTRASTRUCTURE OF THE PLASMODIUM, SPOROGENIC STAGES AND SPORES OF MYXOSOMA

Introduction

Numerous and often detailed descriptions of the structure and development of the Myxosporida based on light microscope observations have been reported (Auerbach, 1910; Erdmann, 1917; Kudo, 1920; Noble, 1944; Chakravarty and Basu, 1948; Willis, 1949). However, it was not until the electron microscope was utilized that more consistant concepts of their morphology and morphogenesis developed.

Description of the fine structure of certain myxosporidan life-cycle stages began in 1960 when Grasse recorded his observations of the plasmodium of Sphaeromyxa. His electron micrographs showed "microvillosities" on its surface, a highly vacuolated endoplasm and characteristic of generative cells. Cheissin, Shulman and Vinnitchenko (1961), Lom and Vavra (1964, 1965) and Lom (1965) presented electron micrographs of the polar capsule and its filament (including morphogenesis) of Myxobolus and Henneguya. Lom and dePuytorac (1965a, b) described the principal features of the plasmodia and sporogenic stages of Myxobolus, Henneguya, Zschokkella, Myxidium and Choloromyxum. Later studies on Henneguya by Schubert (1968), on Sphaeromyxa by Lom (1969) and on Myxidium by Uspenskaya (1969) complemented the previous work on sporogenesis in the Myxosporida. However, none of these workers obtained good quality thin sections of mature spores, a major goal of this study.

The present study describes for the first time the ultrastructure of members of the genus Myxosoma and gives an account of certain morphologic features previously unreported from the Myxosporida. Included with the descriptions are inferences about sporogenesis in this genus.

Methods and Materials

At the time of their collection, host fish were put in iced water to minimize metabolic changes in the study material during transit to the laboratory. This also effected an analgesic-like state in the fish which facilitated inspection and dissection. Selected tissues containing cysts of M. pharyngeus and M. cyprini were removed from the hosts (Gambusia affinis and Notropis lutrensis, respectively) and were fixed 1 to 4 hours in a cold 2 percent glutaraldehyde solution. The fixative also contained 0.5 percent acrolein and 4.0 mM calcium chloride and was buffered by 0.2 M sodium cacodylate to pH 7.4. Osmolarity of the complete fixative as ca. 500 mOs. Tissues were demineralized when necessary for 30 min. in 0.1 M disodium ethylenediaminetetraacetate (EDTA). Cysts were rinsed in 0.16 M sucrose in 0.07 M cacodylate buffer and post-fixed for 1 hour in buffered 1 percent osmium tetroxide. After again rinsing in sucrose-wash solution, the tissues were embedded in Spurr's low viscosity embedment[2] using the "hard" modification. The embedding steps included dehydration through a graded ethanol series and infiltration in propylene oxide, propylene oxide-embedment (1:1) and pure embedment. Sections ca. 60nm (silver-to-gold interference colors) were cut on a Porter-Blum ultramicrotome (Sorvall MT-2) and mounted on naked 200-mesh grids of either nickel or copper. Sections were either doubly stained in uranyl acetate and lead citrate according to the schedule of Venable and Coggeshall (1965) or were stained in hot alcoholic uranylacetate (60 C) and viewed and photographed at 60 or 80 KV with Phillips Model 200 microscope. Measurements were made from photographic plates. Magnification of photographed images was calibrated with a germanium-shadowed, carbon replica of a 54,864 line-per-inch diffraction grating (spacing of grating = 463 nm).

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[2] Polysciences, Inc.; Paul Valley Industrial Park, Warrington, Pa. 18976

Satisfactory micrographs of mature spores could only be obtained from freeze-substituted tissues. Cysts were infiltrated with glycerol in ascending concentrations (0 to 60 percent) in cacodylate buffer at 0 C and quickly submersed and frozen in 2-methylbutane at -160 C. Frozen tissues were placed in absolute ethanol at -85 to -75 C for 3 days (substitution step) followed by one change of alcohol for 2 hours at -35 C. Tissues were next infiltrated with alcohol-embedment (1:1) for 1 hour at -35 C and pure embedment for 1 hour at 0 C. The embedment was polymerized at room temperature for 6-8 hours, at 37-40 C for 10-15 hours and at 70 C for 4 hours. Sectioning and staining were as described above.

A modification of the above technique which produced more contrast with membraneous organelles in the finished sections was also employed. This method required the use of 1% OsO_4 in absolute ethanol rather than ethanol alone to substitute for the frozen glycerol. The remaining steps of the procedure were the same.

For comparative purposes, 0.5 to 1.0 μ sections were stained (1) on a hot plate with 0.5% toluidine blue in 1% sodium borate, (2) with Shiff's reagent after hydrolysis with periodic acid or 0.1 *M* HCl, or (3) with 0.1% methyl green and viewed with the light microscope.

Results and Discussion

Plasmodia of *M. pharyngeus* occur in at least three different forms, depending upon their location in the host. One form is compact and multilocular. These cysts are the largest (diameters to 3 mm) and grossly appear as white, ovate bulges beneath the integument. They are found within the connective tissues which support the scales and epithelium along the general body surface. Microscopically, the borders of these cysts are seen to be interrupted by host-tissue trebeculae.

A second type of plasmodium of *M. pharyngeus* is the pharyngeal vesicular cyst. Vesicular cysts are unusual in that they do not invade host tissues but rather are found free in protected regions of the pharyngeal cavity such as along the lateral margins of the isthmus between adjacent branchial arches. They are covered by a thin host-tissue epitheloid and are loosely connected to the pharyngeal tissues by a narrow suspensory pedicel of collagen and epithelial cells. Vesicular cysts are compact but lack the intrusions of host-tissue trabeculae and thus are more simple in design than integumentary plasmodia. Vesicular cysts are also smaller (diameter ca. 300 μ) than those of the integument.

A third morphologic variant of *M. pharyngeus* is a diffuse, "metastatic" cyst found in the subepithelial connective tissues which rest upon the toothed pharyngeal bones. These bones include the pharyngobranchials and the fifth branchial arches. Grossly, these cysts present a circular profile and appear as a grey mass. In section, they are observed to ramify diffusely among the connective tissue cords entwining the pharyngeal teeth. Free spores also were observed to have infiltrated nearby connective tissues, a condition noted in other histozoic myxosporidans (Kudo, 1930).

Cysts of _M._ _cyprini_ occur in epithelial tissues of the secondary gill lamellae of red and golden shiners. These plasmodia are all spherical, compact and simple. They are quite small (average diameter ca. 200 μ).

Sporogenesis of both species is asynchronous and all developmental stages may be found within a given cyst. However, in the multilocular dermal cysts of _M._ _pharyngeus_, isolated pockets of sporogenic cells result from the intrusion of host-tissue trabeculae into the enlarging plasmodium. Cells within individual locules do tend toward synchrony in their development.

The ultrastructure of the plasmodium, sporont, sporoblast and mature spore of _Myxosoma_ is described in the following section. The figures are arranged in a sequence which suggests a possible developmental cycle for spores of this genus. Descriptions generally apply to both _M._ _pharyngeus_ and _M._ _cyprini_. Exceptions to this are noted in the text.

Structure of Plasmodia

Plasmodia of both _Myxosoma_ species are bounded by plasmalemmae which are devoid of microvilli observed in other myxosporidan genera (e.g., _Sphaeromyxa_, _Myxidium_, _Chloromyxum_) but which do possess two types of inward-directed microtubules (Figs. 1 to 6). One type consists of closely-packed cylindrical tubules of nearly uniform diameter (ca. 40 nm) which occur as dense, discontinous clusters or bands (Figs. 3 and 4). Microtubules of this type are particularily abundant in dermal cysts of M. pharyngeus and effect a 400-fold amplification in the surface area of the plasmalemma. The second type is either cylindrical or pyriform-shaped, variable in length and more evenly distributed along the entire plasmalemma (Fig. 2). Great numbers of pinocytotic vesicles appear along the medial ends of both kinds of microtubules indicating a high rate of synthesis and internalizaion of the plasma membrane (Figs. 2 and 3). In cross section, clusters of microtubules are encompassed by a granular, electron-dense ectoplasmic matrix (Fig. 4). This material is PAS-positive, toluidine blue-positive and stains only weakly with osmium. It does not incorporate either lead or uranium salts in the absence of osmium fixation. After osmium fixation, sections doubly-stained with uranyl acetate and lead citrate are moderately electron-dense in the region of the supporting matrix. These observations indicate that this material is composed largely of protein and that it lacks significant amounts of lipid or carbohydrate.

Other features of the ectoplasm include large and small vesicles, the contents of which are poorly defined (Fig. 4), and scattered osmophilic inclusions. Mitochondria and generative cells are absent from this layer. Neither free nor bound ribosomes are observed in the ectoplasm of either species.

The endoplasm of the M. pharyngeus plasmodium is either diffuse with scattered generative cells (Fig. 8) or uniformly dense with closely packed sporogenic stages (Figs. 1 and 10). The endoplasm of M.

cyprini plasmodia is of the dense type only (Figs. 7 and 9). Numerous mitochondria with branched tubular or flattened cristae are present in the interior of plasmodia of both species. Heterogeneous collections of dense and clear membrane-bound vesicles, randomly arranged cisternae of smooth endoplasmic reticulum, occasional fibers and unbound granules are also present in both species (Figs. 1, 2, 9 and 11). Myelinic figures and Golgi bodies observed in the plasmodium of Henneguya by Lom and dePuytorac (1965b) were not seen in our preparations. The absence of myelinic figures is probably of no consequence sine they are thought to be glutaraldehyde fixation artifacts (J. Venable, pers. comm.).

Structure and Development of Early Sporogenic Stages

The presence of sporonts, two-celled sporoblasts and isolated nuclei, possibly "vegetative" cells, marks the outer limit of the endoplasm of Myxosoma (Figs. 1 and 2). The interior of the plasmodium is occupied by more advanced stages. The presence of pseudopodia and finger-like folds in the plasmalemmae of some early stages suggests that they may wander within the plasmodial cytoplasm (Fig. 13). Cytoplasmic organelles of early sporogenic stages include numerous mitochondria, tubular cisternae of smooth endoplasmic reticulum and closely-packed granular or vermiculated inclusions of electron-dense substance (Fig. 2 and 8). These inclusions resemble the dense material seen in glycogen-rich mammalian cells. Mitochondria of the generative cells are dissimilar to their counterparts in the plasmodial cytoplasm. The former have fewer cristae than mitochondria of the plasmodium and possess unusual dense membraneous bodies (compare Figs. 11 through 15). In generative cells, the mitochondrial matrix often is flocculated rather than granular as is that of plasmodial mitochondria.

Nuclei of the early generative cells are constant in size and appearance, regardless of the degree of cytoplasmic development. Each contains a single, densely-staining nucleolous (Fig. 8). Nuclei of *M. pharyngeus* are acentrally located; those of *M. cyprini* are centered within the cells. More heterochromatin is seen in the nuclei of early stages of *M. cyprini* than in those of *M. pharyngeus* (Fig. 9).

Single-celled stages and generative nuclei are often clumped rather than uniformly distributed within the plasmodium. Mitotic figures were not observed with single, unassociated cells even in young plasmodia. Differences in morphology are not observed between the various single-cell stages present in plasmodia of *Myxosoma*. They are perhaps bipotent at this stage and can become either sporonts or envelope cells. The origin of sporonts, envelope cells and vegetative cells is still in doubt.

Certain cell associations observed in *Myxosoma* (Figures 16 to 20), provide evidence for the presence of envelope cells similar to those described by Lom (1969). Figures 19 and 20 show apparent enveloped cells (= the "sporont") which are undergoing division. Four chromosomes are identified for *M. pharyngeus* (Fig. 20). The enveloping cell was never observed undergoing division. Multicellular sporoblasts and pairs of maturing spores are almost always bound together by a separate enveloping plasmalemma. It closely adheres to the plasma membranes of the enclosed cells (Fig. 10).

Structure and Development of Late Sporogenic Stages

Development of undifferentiated sporoblasts which contain homogeneous, spherical cells (Figs. 2 and 10) into sporoblasts which contain identifiable cell types (Figs. 7 and 10) is evidently very rapid. Advanced sporoblasts nearly always outnumber lesser developed forms. Myxosoma is usually disporoblastic, i.e., spores develop as pairs within a single membrane, the envelope cell. Polarity in the location of cells within pansporoblasts is evident early during their development as is the embryonic fate of each sporogenic cell (Fig. 21).

Valvogenic Cells. The four valvogenic cells differentiate into elongate cells with peripheral expansions of their cytoplasm (Fig. 22). Their position within the pansporoblast is established early and they serve to segregate members of the two pairs of capsulogenic cells and the single pair of sporoplasm cells into two adjoining monosporoblasts (Fig. 22). The valvogenic cells thus unite the internal elements of individual spores allowing each to continue to develop as an independent unit.

The sutural junction between opposing valvogenic cells forms during the early development of the valves (Fig. 23). The junction first appears as a local thickening of adjacent plasmalemmae, each ca. 15 nm wide by 650 nm long in transverse section (Fig. 23). A bundle of parallel-arranged fibrils forms in each cell adjacent to the sutural thickening (Fig. 23 and 24). These fibrils, ca. 10 nm in cross section, probably reinforce the junction. They are unidirectional and oriented anteroposteriorly. As valve cell development progress, the 20 nm space originally present between the adjacent thickened pellicles becomes obliterated by electron dense, "fuzzy" material (Fig. 24). The junction remain relatively constant in appearancne during further differention of the valvogenic cells.

Other features of developing valvogenic cells include the presence of enlarging, extremely dense inclusions in the region which becomes the sutural ridge (Fig. 24). Large inclusions apparently result from the coalescence of smaller bodies (Fig. 25). They eventually fill the peripheral cytoplasm of the entire valve cell (Fig. 26). These electron dense inclusions coincide with PAS-positive material seen in comparable sections with the light microscope.

Valvogenic cells are the only cells of Myxosoma sporoblasts to contain Golgi-like bodies (Fig. 23). Similar results are apparent for Henneguya (Shubert, 1968). One function of the Golgi-bodies of vertebrate cells is that of synthesizing carbohydrate (Neutra and LeBlond, 1969). In this capacity, in Myxosoma they probably are responsible for making the carbohydrate moiety of the PAS positive, electron-dense cortical substance of the valves.

Another contribution of the valvogenic cells in Myxosoma is that of making the "cork" (plug) or "stopper substance" of the filament-discharge channel (Lom, 1964; Lom and dePuytorac, 1965b). The developing cap of M. cyprini is much thicker (200 nm; Fig. 27) than that of M. pharyngeus (< 60 nm). The substance of this plug is dense and granular and is closely applied to the anterior tip of the maturing polar capsule in the region of the filament discharge channel (Fig. 27). Laminar periodicity in the plug material is sometimes seen. Neither the chemical nature nor cell organelle from which the plug originates is known. Lom (1964) reported that the plug is proteinaceous because it was bromphenol-blue positive and denatured in urea. Presumably, the function of the cap is to prevent autoextrusion of the polar filament by hydrostatic forces within the polar capsules (Lom, 1964). In _M_. _cyprini_, the adjoining plasmalemmae of the capsulogenic and valvogenic cells in the region of the dense cap are thickened (Fig. 27). Similar membrane specialization is seen in the formation of the valve suture already described (Fig. 23).

Nuclei, electron-lucent vesicles and mitochondria observable in early valvogenic cells are absent from mature valves (Figs. 28 and 29). Mature valves are smoothly contoured structures composed of a trilaminar pellicle about 36 nm thick on both the inner and outer surface. The pellicle encloses a homogenous, osmophobic matrix. This matrix stained deeply with lead in freeze-substituted preparations in which no osmium was used (Fig. 30). The inner matrix is only about 15 nm thick except in the region of the valves which support the intervalvular suture. The matrix is about 10^3 nm thick in the region of this sutural ridge (Figs. 29 and 30).

The anterior-most part of each valve is perforated by a groove or tube directly in front of the anterior tip of the paired polar capsules (Figs. 29 and 31). This is the channel through which extruding polar filaments leave the spore. No furrow such as that seen in _M_. _cerebralis_ by Lom and Hoffman (1971) is seen on the external surface of mature valves of _M_. _pharyngeus_. The valves of _M_. _pharyngeus_ are entirely smooth (Figs. 28 to 30, 50, and 51).

Capsulogenic Cells. The cell type which differentiates earliest in the pansporoblast is the capsulogenic cell. These cells are first recognized by a loss of nucleoli and formation of rough-surfaced endoplasmic reticulum. Also appearing during the early development of these cells is the capsular primordium (Fig. 32 and 33). The primordium begins as a pear-shaped body with a homogeneous central matrix and an electron-dense cortex (Fig. 33). With further development, the primordium consists of a long, entwining external tube plus a bulbous precapsule. As the capsule increases in size, the nucleus and cytoplasmic organelles of the capsulogenic cell usually disappear. During the process of elongation, dense granules appear in the central matrix of both the external tube and the capsular body (Fig. 34 to 36). These granules coalesce into a central dense zone within a more expansive, less-dense, finely-granular matrix. This matrix is circumscribed by an electron-lucent area which is enclosed by the capsuler cortex (Figs. 37 and 38). It appears as though the central, electron-dense zone gives rise to the coiled polar filament of the mature capsule and the finely-granular matrix becomes the subcortical wall (Figs. 40 to 42).

Coincident with the appearance of the coiled polar filament in the capsule is the disappearance of the external tube. This observation lends support to the hypothesis of Lom and dePuytorac (1965b) that the external tube contributes most of the polar filament. Subsequent to the formation of the filament, the external tube shortens and forces the polar filament into the capsule.

Approximately thirty microtubules (diameter ca. 17nm) are arranged concentrically around and slightly oblique to the long axis of the external tube in M. cyprini (Fig. 27). Microtubule formation precedes formation of the external tube in this species. Porter (1964) alleged that microtubules contribute to cytoplasmic movement and thus might account for the shortening of the external tube. Slautterback (1963) presented micrographs of small cytoplasmic tubules circumferentially arranged around developing nematocysts in cnidoblasts of hydra. He proposed that these tubules function in the transport of water, ions or small molecules. These microtubules closely resemble those found in *M. cyprini* capsulogenic cells. Microtubules are not observed around the external tube or capsule primordium in *M. pharyngeus*.

Events leading to formation of the mature polar capsule in *Myxosoma* are essentially the same as those described by Lom and dePuytorac (1965b) and Lom and Vavra (1965) for other myxosporidans. Homologous development was observed in fine structural studies of nematocyst formation in hydrozoan coelenterates (Slautterback, 1963; Westfall, 1966) and in polar capsule formation of the actinomyxidan *Aurantiactinomyxon eiseniellae* (Ormieres, 1970). This homologous development suggests that all three groups have common ancestry.

When mature, the paired polar capsules occupy the entire anterior half of the spore. Each contains a polar filament ca. 60 µ long when freshly extruded (Parker, Spall and Warner, 1971). The filament, ca. 250 nm in diameter, is wound in approximately eleven coils within the capsule of *M. pharyngeus* (Fig. 41). Figure 42 shows that the coiled filament is also tightly twisted. Lom and Vavra (1965) observed that the polar filament of *Henneguya* was tightly twisted, which they regarded as *a priori* evidence supporting their hypothesis that the filament everts like the finger of a glove during extrusion. Lom and Vavra reasoned that, if the inverted filaments were not twisted as described above, they would maintain their eleven windings after extrusion because the intracapsular pressure would be insufficient to completely straighten out the relatively thick filament. My observations of polar filament extrusion in Myxosoma using phase microscopy do not support their viewpoint. The polar filament does not appear to evert during extrusion. There are about 4 times as many twists as there are coils in the filament of M. pharyngeus (Figs. 45 and 46).

Development of the sporoplasm. The cell which becomes the sporoplasm in the mature spore is first recognized during the early development of the pansporoblast by the presence of two classes of cytoplasmic granules which persist throughout its development (Figs. 43 to 49). The first class of granules are spherical, membrane bound and usually electron-dense (Figs. 44 and 47). In M. pharyngeus, these granules are 130 to 170 nm in diameter. In M. cyprini, however, they range from 100 to 450 nm in diameter. In both species these granules often have a haloed appearance similar to the secretory granules of mammalian Paneth cells. The second class of granules are smaller (ca. 10 nm), electron-dense and often are densely aggregated (Figs. 44 and 49). The chemical nature of neither type is known but they probably represent nutrient reserves. The smaller granules resemble the abundant, dense bodies seen in glycogen-rich mammalian tissues.

Another feature characteristic of the sporoplasm cell is its lobed appearance. Often it is bilobed in longitudinally-sectioned sporoblasts (Figs. 43 and 46). Usually, the nuclei and most of the large dense granules reside in the same lobe. The other lobe is smaller and contains many of the glycogen-like granules and smooth membraneous cisternae (Figs. 46 to 48).

The sporoplasm commonly, if not always, possesses two nuclei, although both did not always lie in the same plane of section. They are always adjacent to one another (Figs. 48 to 50). Serial or closely spaced sections indicate that one lacks a nucleolus and thus appears not to take part in directing cellular metabolism (Figs. 48 and 49). The origin of the second nucleus remains obscure since neither mitotic figures nor plasmogamy occurring in the sporoplasm of Myxosoma were observed.

Mitochondria decrease in number in the sporoplasm of more advanced sporoblasts. Free ribosomes are abundant. Membrane-bound ribosomes are usually restricted to the nuclear envelope or nearby cisternae of endoplasmic reticulum. The sporoplasm of mature spores occupies the entire space in the posterior half of the spore (Figs. 50 and 51).

Summary and Conclusions

When trying to apply interpretations of electron micrographs to a description of the ontogeny of a species, the variability that exists between samples and the static nature of this technique must be considered. Inability to achieve experimental transmission in the present study caused me to rely on observations of natural Myxosoma infections of unknown age. Further difficulty was encountered in preparing satisfactory thin sections of mature spores. These were obtained only with freeze-substituted tissues.

Development of the trophozoite of the polysporous genus Myxosoma includes the formation of at least six discrete cell types: the trophozoite itself, envelope cells, and sporonts which give rise to valve cells, capsule cells and the diplokaryotic sporoplasm. This sets the Myxosporida apart from the remaining groups of Protozoa except the Actinomyxida. The present study also corraborates the opinions of others that the Myxosporida are unlike the Microsporida and Haplosporida with which they have been frequently classified. The sole basis for including them with these Cnidosporans is their possession of polar capsules and filaments. But, as has been shown, the ontogeny, fine-structure, and function of Myxosporidan polar capsules are unlike those of other Cnidosporans except the Actinomyxida. The Myxosporida also are not intermediate between Protozoa and Mesozoa; the latter group are thought to be degenerate Echinoderms or Trematodes (Manter, 1969).

The series of events leading to the formation of the mature capsule described by Lom and Vavra (1964) and others and supported by the present study closely parallel the development of cnidocysts in the Coelenterata described by Westfall (1966) and polar capsule formation in the Actinomyxida (Ormieres, 1970). Homologous development among these groups indicates probable common ancestry. Since coelenterates are thought to originate from flagellate stock (Meglitsch, 1970) and myxosporidans from multinucleate amoebae (Shulman, 1966), the suggested homology substantiates the close relationships thought to exist between the flagellates and sarcodines.

The present study revealed that Myxosoma plasmodia are unique among the Myxosporida thus far studied with regard to the extensive microtubular system along the inner cell surface. Coelozoic genera such as Myxidium, Zschokkella and Chloromyxum lack any such structures. Instead, the absorptive surface of plasmodia of these genera is increased by the presence of branching microvilli (Lom and dePuytorac, 1965b). The plasmodial surface of the histozoic genus Henneguya does show abundant pinocytic activity (Lom and dePuytorac, 1965a) but lacks the organized dense zones of microtubules seen in my preparations.

Sporogenesis in Myxosoma is essentially the same as that described for other myxosporidans (Lom and dePuytorac, 1965b; Lom, 1969). Myxosoma is disporoblastic. Each mature spore contains two valves, two polar capsules and a binucleate sporoplasm. Sporogenesis begins when a generative cell, the sporont, is enclosed within a second cell, the envelope cell. The sporont and its progeny undergo division until ten cells occur within the enveloping membrane. Two of these cells eventually become diplokaryotic. These cells represent the germ plasm of the next generation. The remaining eight cells differentiage into four valves and four polar capsules. A possible schema of multiplication is shown in Figure 52.

Valvogenesis occurs concomitantly with capsulogenesis and maturation of the sporoplasm. A local thickening known as the sutural ridge persists in valvogenic cells in the region of the suture; otherwise they become very thin. The sutural ridge and junction are reinforced by bundles of densely-packed fibrils. Cell organelles disappear during valvogenesis. An electron-dense, PAS-positive substance probably originating from Golgi-like bodies was deposited in the cortical region of the entire valve. The tight sutural junction became filled with fuzzy substance. This fuzzy substance had a different appearance than the periodically structured material seen in the suture of Zschokkella (Lom and dePuytorac, 1965b). Mature valves of Myxosoma contained no organelles and had a highly ordered, laminated appearance.

Capsulogenesis in Myxosoma is essentially the same as that described for other myxosporidan genera (Lom and Vavra, 1965; Lom and dePuytorac, 1965b) and for the Actinomyxida (Ormieres, 1970). The early capsulogenic cell is identified by its rich development of rough endoplasmic reticulum followed by the appearance of the capsular primordium and the external tube. The youngest stage of the capsule primordium is a flask-shaped body with a finely granular matrix. Soon after its appearance the external tube develops. A hollow bundle of microtubules appears to precede external tube formation. The distal end of the external tube abuts the anterior pole of the capsulogenic cell. The remainder of the tube winds in random fashion. Usually two or three loops of the tube can be seen in sections through the central part of the capsulogenic cell. The proximal end of the external tube is continuous with the capsule primordium. Coarse-granules, anlagen of the future polar filaments, appear in the central matrix of both the tube and primordium. These coalesce into a dense central core which ultimately forms the polar filament. The portion of the filament which forms in the external tube is subsequently forced into the capsule primordium as the external tube shortens. The series of events leading to formation of the mature capsule are shown in Figure 53.

The capsulogenic cell surface adjacent to the distal end of the external tube becomes thickened and dense. In addition, the valvogenic cell produces a thick dense plug in the future filament discharge channel.

Sections of mature polar filament provide no clues to either its chemical nature or function. The filament has a thin dense cortex with a homogeneous electron-lucent matrix similar to that described in *Henneguya* by Lom and dePuytorac (1965b). However, Lom and dePuytorac present photomicrographs of *Myxobolus* which show its matrix to have longitudinally-striated, periodic structure. If the core of the filament is solid rather than hollow, Lom's (1964) thesis that the polar filament everts during the process of extrusion is untenable.

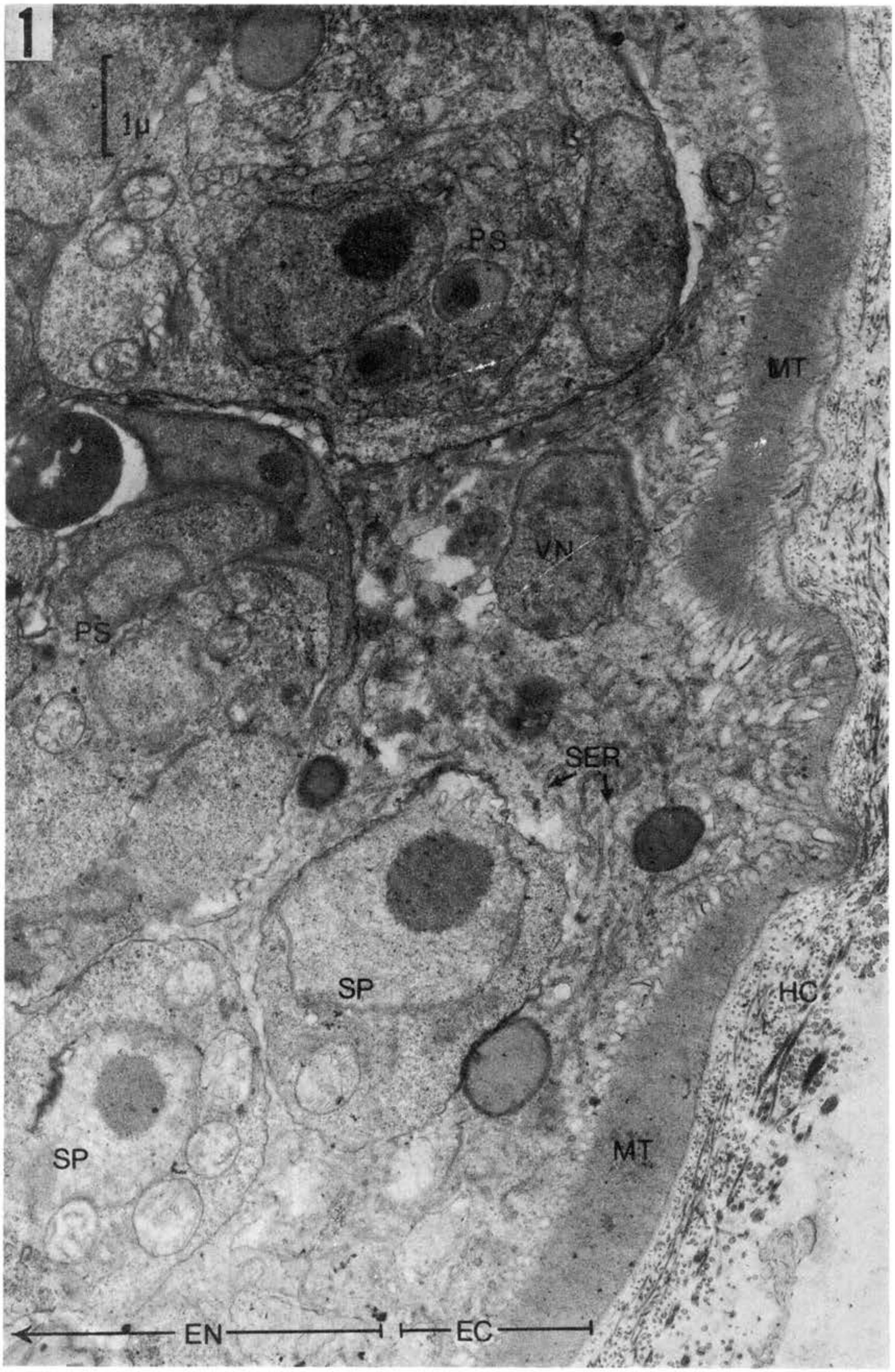
The sporoplasm of Myxosoma is diplokaryotic and contains two kinds of granules, the smaller of which resembles granules of glycogen-rich mammalian tissues. The large granules are similiar to the "kugelige Korper" seen in Henneguya by Schubert (1968). One nucleus lacks a nucleolus and therefore appears not to take part in cell metabolism.

The present findings, in conjunction with previous studies on other myxosporidan genera, allow certain generalizations about the Myxosporida:

1. The trophozoite develops as a syncytium into a multinucleate and multicellular plasmodium. Sporogenesis proceeds concomitantly with the vegetative growth of the plasmodium.
2. The surface of the plasmodium of Myxosoma is organized into dense clumps of narrow, tubular invaginations which effect as much as a 400-fold increase in the surface area of the plasmalemma.
3. The spore is formed from six cells which originate from a single generative cell, the sporont, after it unites with a second cell, the envelope cell.
4. No sexual process occurs either in developing plasmodia or during sporogenesis.
5. There are four chromosomes in M. pharyngeus.
6. Classes Myxosporida and Actinomyxida are mutually exclusive of the classes Microsporida and Haplosporida with which they are often aligned within the subphylum Cnidospora; nor are the former two classes closely related to any other present-day Protozoa.

7. Capsulogenesis in the Myxosporida and Actinomyxida closely parallels cnidocyst formation in the Coelenterata, indicating probable common ancestry. This points to close links between the protozoan classes Sarcodina and Mastigophora.

gure 1. Peripheral organization of dermal plasmodium of *Myxosoma pharyngeus* containing early sporogenic stages. HC, host collagen; EC, ectoplasm; EN, endoplasm; PS, pansporoblast; VN, vegetative nuclei; MT, microtubules; SP, sporont; SER, smooth-surfaced endoplasmic reticulum. X 16,000.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1µ
PS
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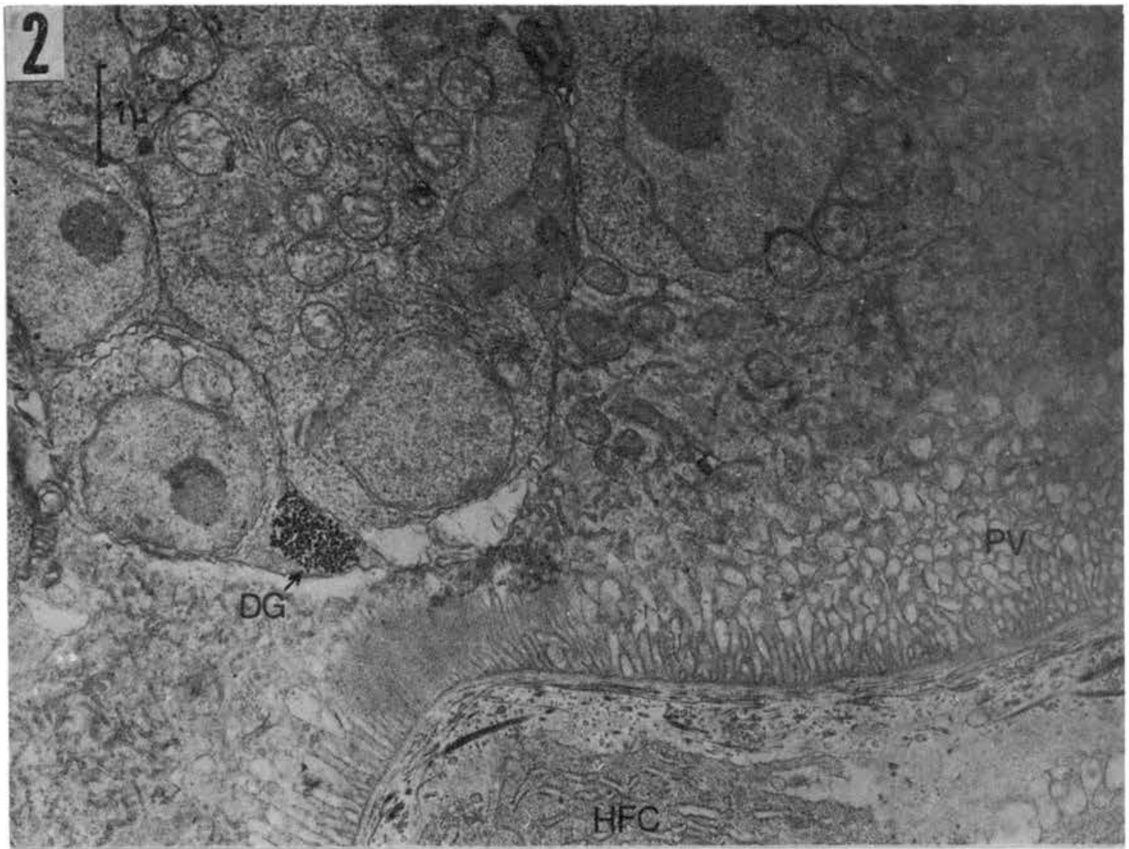
gure 2. Plasmodium of _M. pharyngeus_, showing diversity in shape and size of microtubules. DG, dense granules (glycogen); PV, pinocytotic vesicles; HFC, fibrocyte of host connective tissue. X 13,000.

gure 3. Clump of densely packed microtubules with pinocytotic vesicles (PV) on medial surface of _M. pharyngeus_ plasmodium. The microtubules are continuous with the plasmodial plasmalemma (arrow). X 57,200. Inset: clustered microtubules in cross section. X 80,000.

2



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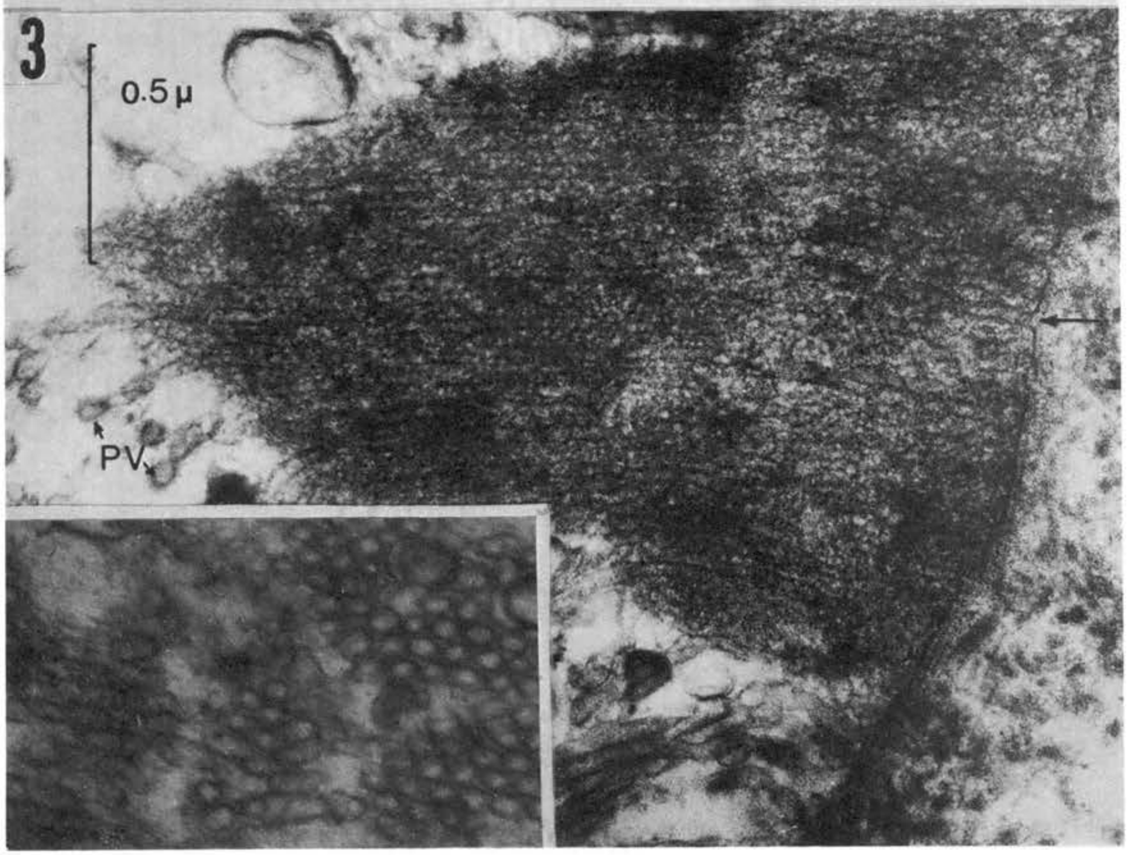
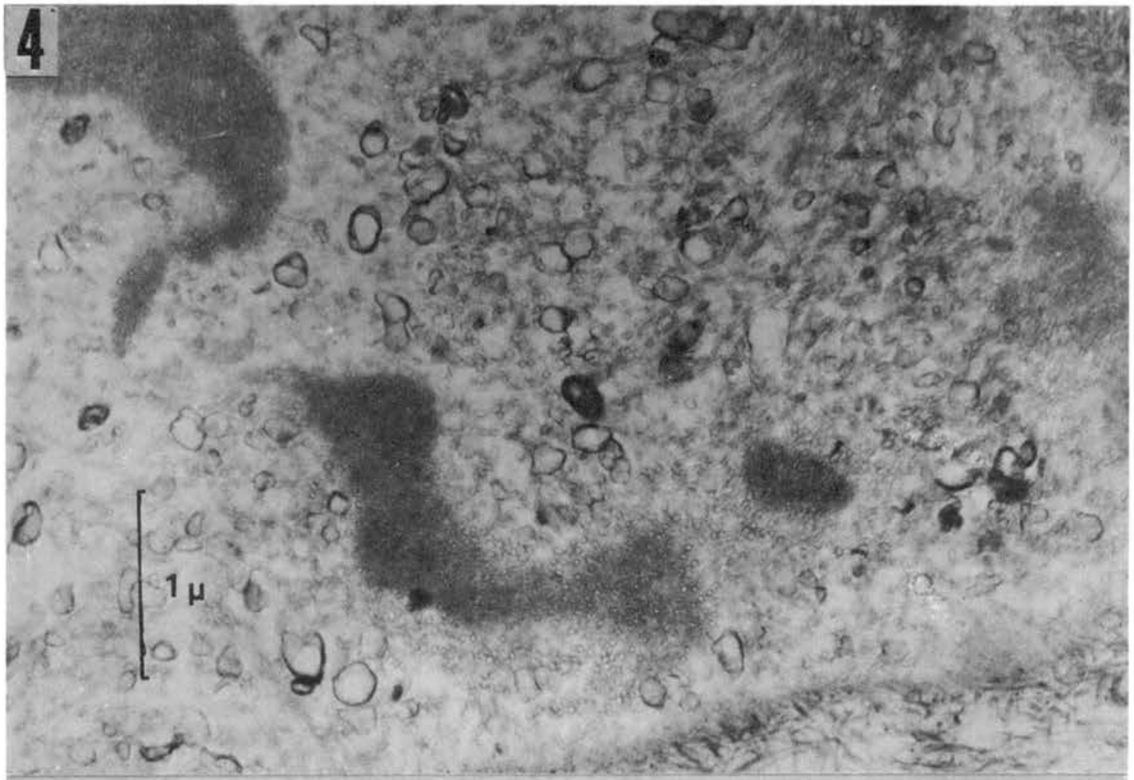


gure 4. Ectoplasm of *M. pharyngeus* plasmodium showing banded pattern of microtubules in cross section. The electron-dense cytoplasmic matrix which supports the tubules is similarily distributed. X 25,000.

gure 5. Cross-sectional view of diffuse microtubular system of *M. cyprini* sp. n. X 80,000. Inset: arrows point to bases of microtubules showing them to be invaginations of the plasma membrane. X 32,000.

4



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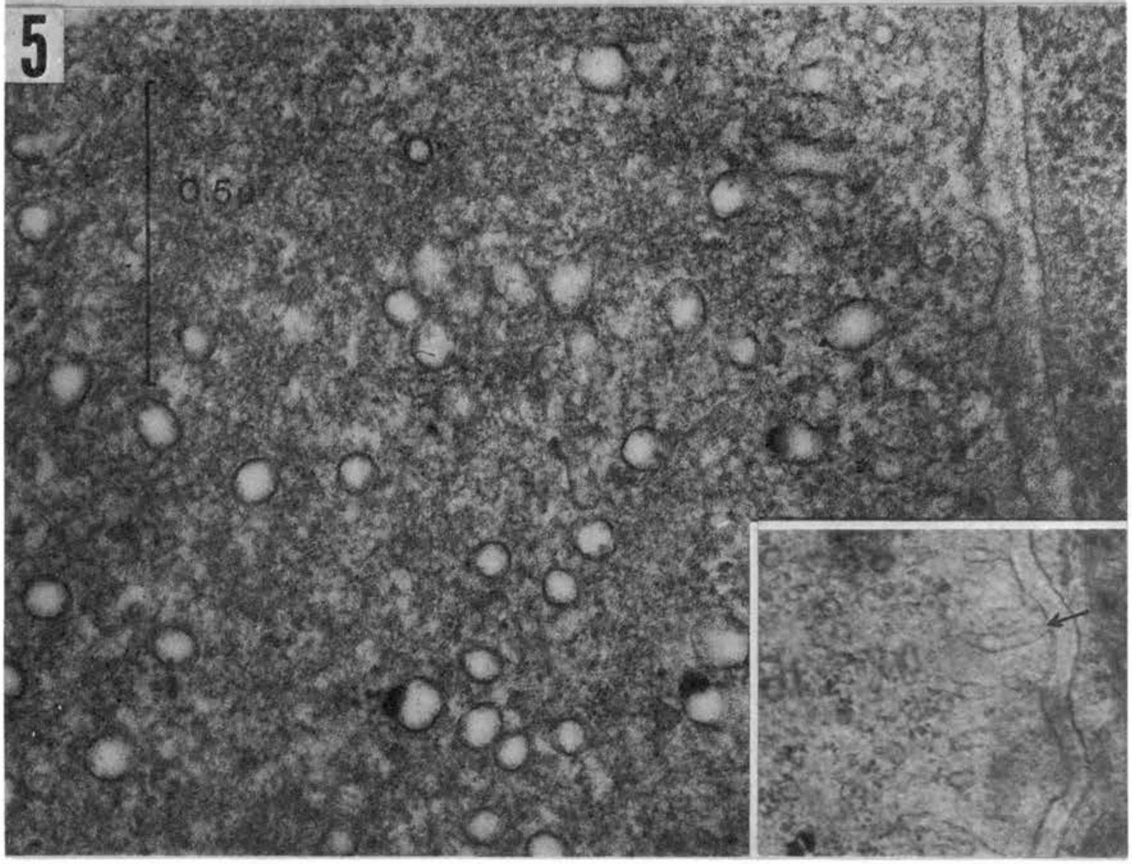
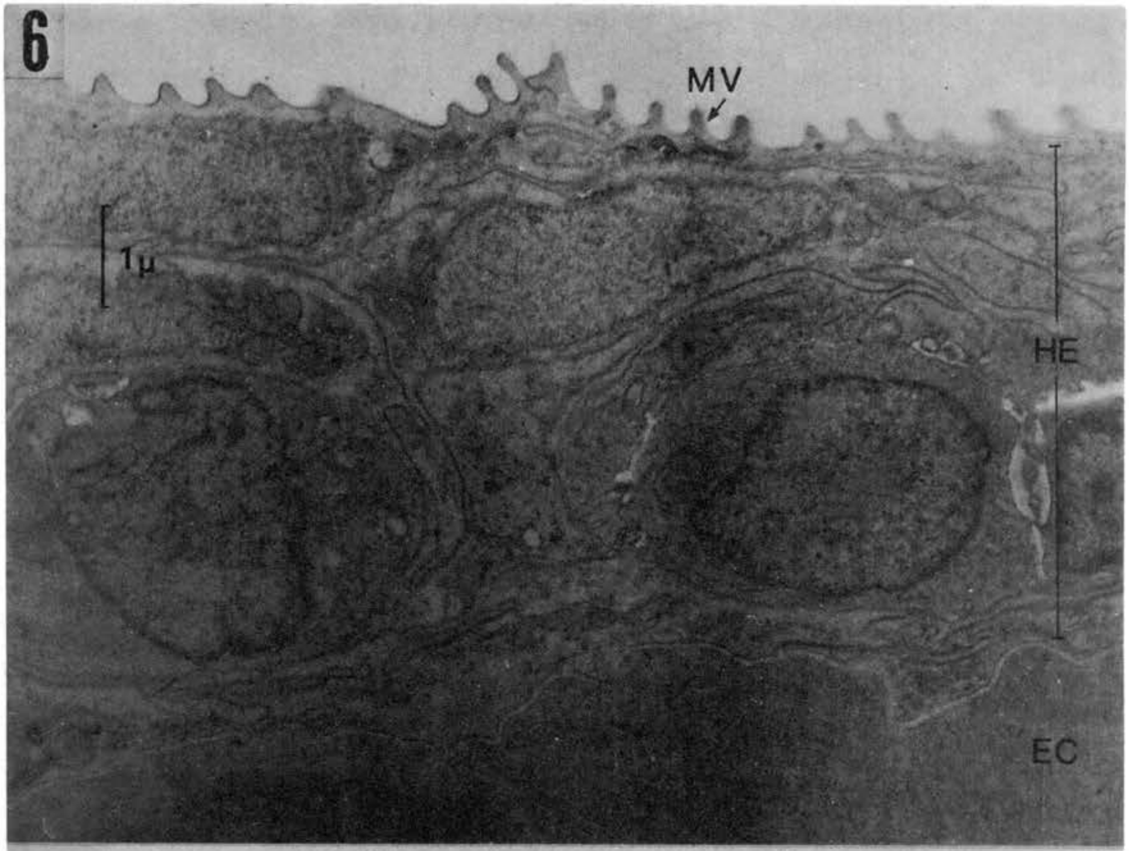


gure 6. Pharyngeal sac-like cyst of *M. pharyngeus* showing microvillosities (MV) on the free border of the epitheloid covering the plasmodium. HE, host-tissue epitheloid; EC, ectoplasm of plasmodium. X 13,300.

gure 7. Cyst of *M. cyprini* sp. n. adjacent to capillary in secondary gill lamella. Only the thin wall of capillary endothelium (CE) separates the plasmodium from the host's blood compartment. RBC, red blood cell; LC, lymphocyte; EC, ectoplasm of parasite. X 22,300.

6



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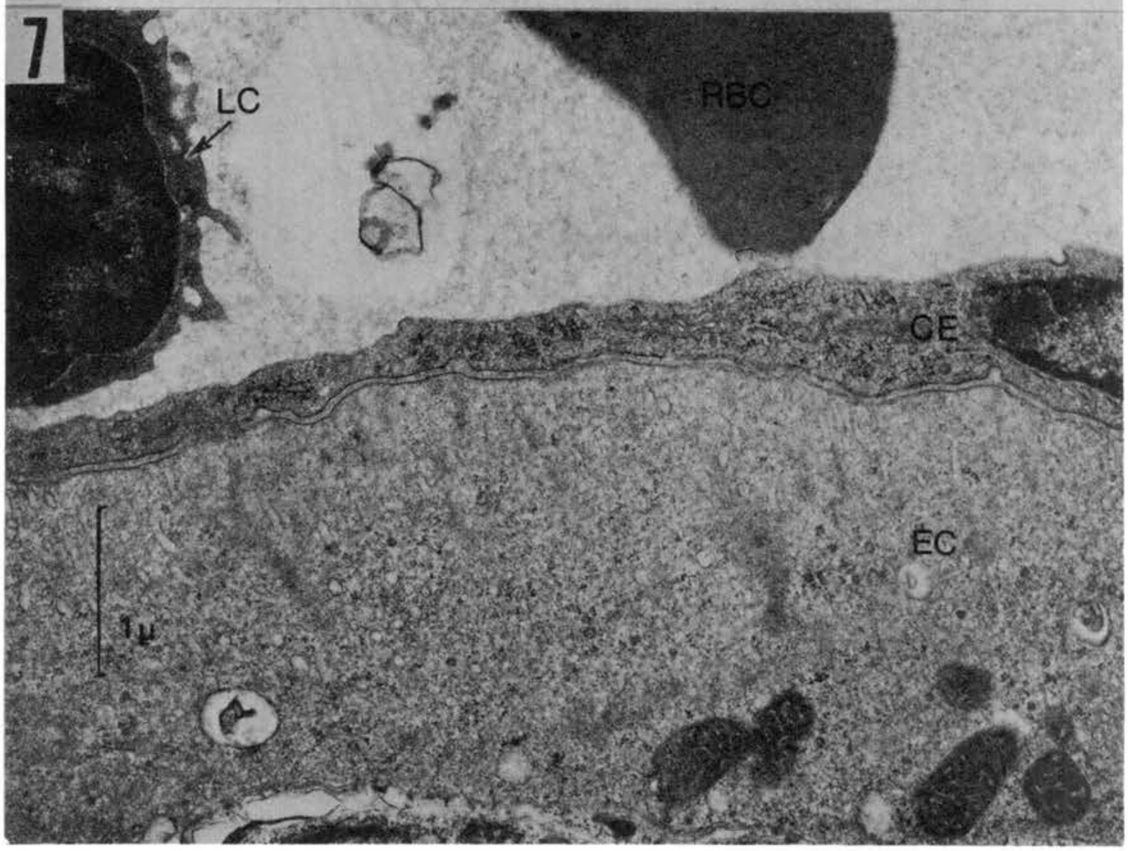
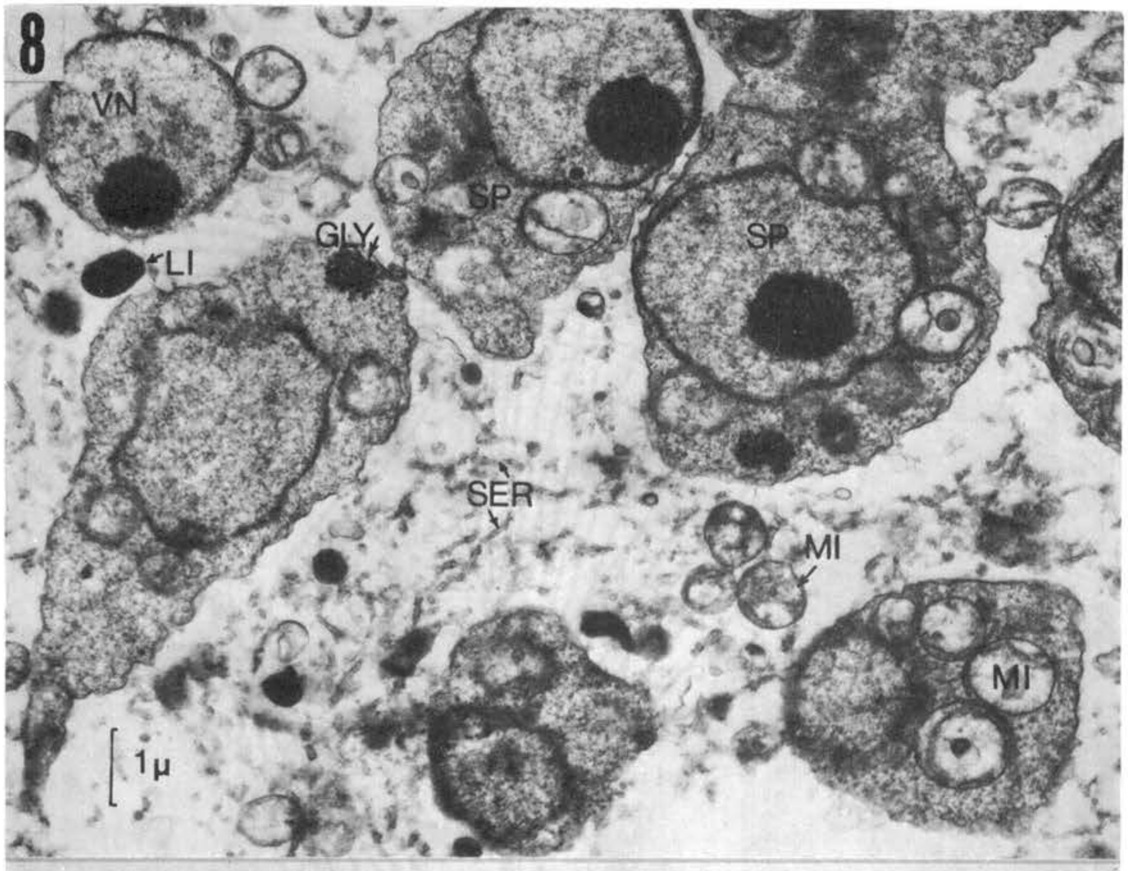

LC
RBC
CE
EC
1μ

gure 8. Peripheral endoplasm of *M. pharyngeus* plasmodium with vegetative cells (VN), sporonts (SP), poorly preserved mitochondria (MI), smooth endoplasmic reticulum (SER) and lipoid inclusions (LI); GLY, glycogen bodies. X 10,000.

gure 9. Endoplasm of *M. cyprini* sp. n. plasmodium with one and two cell sporogenic stages. Nuclei of these stages in this species contain more heterochromatin than comparable nuclei of *M. pharyngeus*. X 15,600.

8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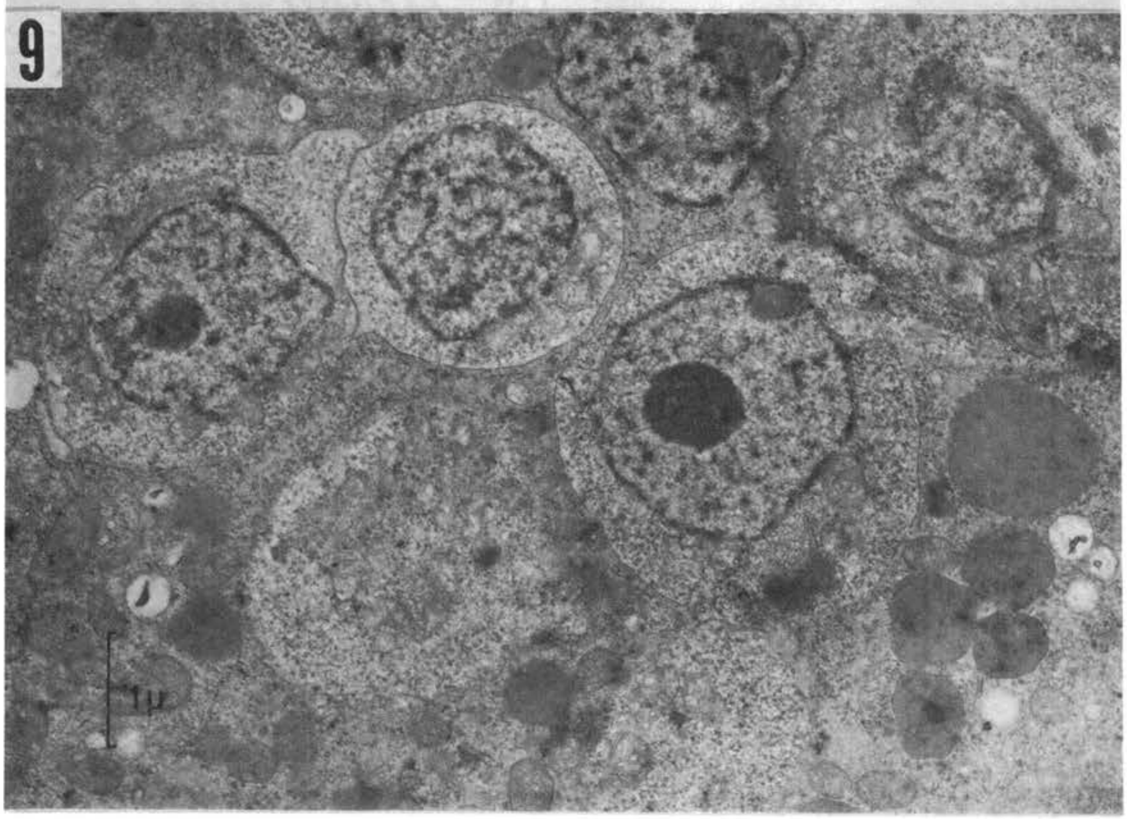

gure 10. Endoplasm of compact *M. pharyngeus* cyst containing numerous pansporoblasts (PS) in a variety of developmental stages. X 8,000.

gure 11. Portion of endoplasm of *M. cyprini* sp. n. plasmodium which has branching tubular cristae in mitochondria (MI). Vesicular and tubular smooth endoplasmic reticulum (SER) and small clusters of ribosomes (RI) both appear rather randomly dispersed. MBV, membrane-bound, granular vesicle. X 58,400.

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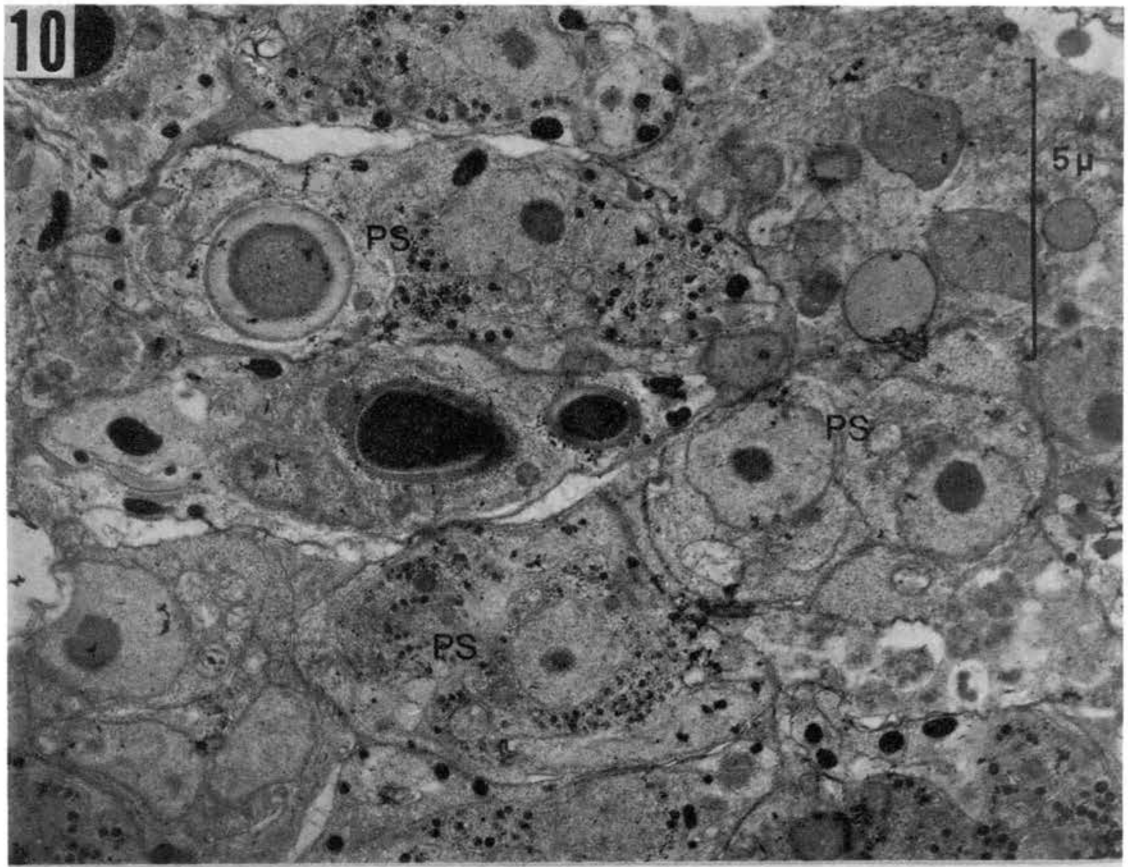

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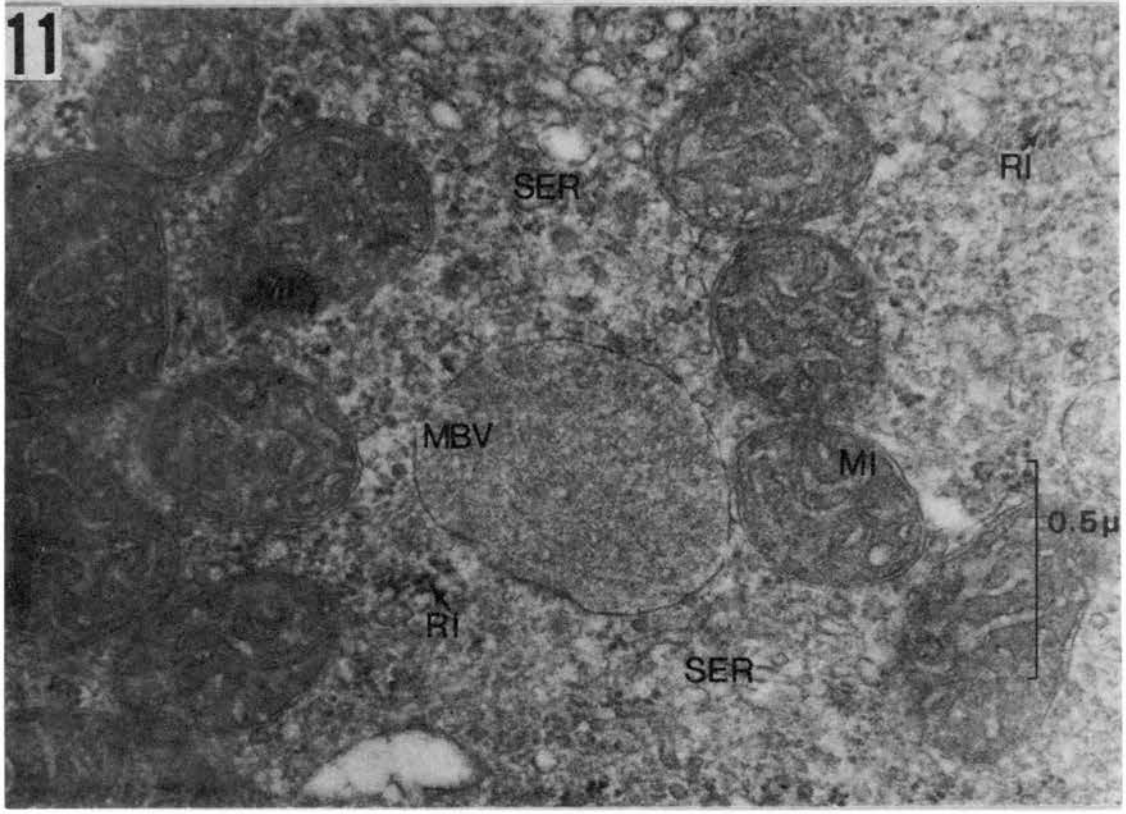

SER
RI
MI
MBV
MI
0.5µ
RI
SER

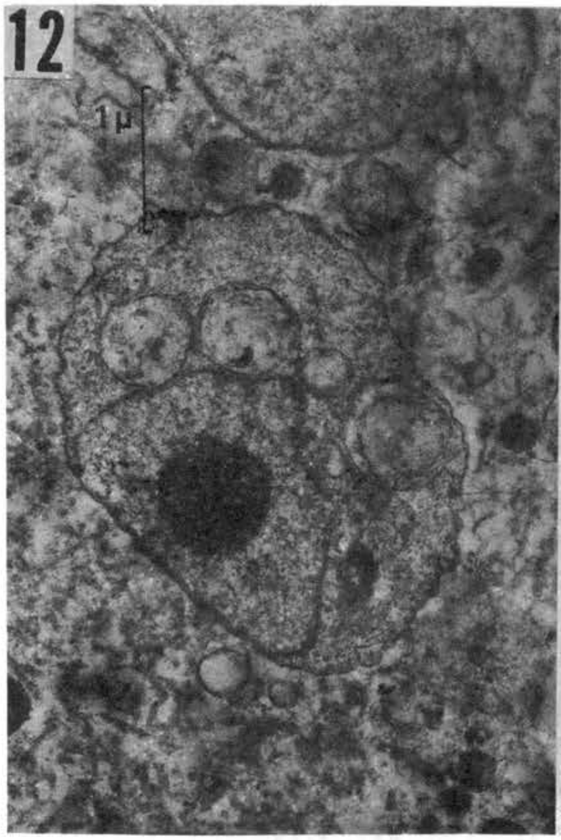
igure 12. One-cell stage of *M. pharyngeus*. The nucleus is usually acentric in this species (compare with Fig. 9). X 19,300.

gure 13. Elongate, two-cell stage of *M. pharyngeus* with pseudopodia (PS). X 19,300.

gure 14. Mitochondrion of undifferentiated cell in early *M. pharyngeus* pansporoblast. Generative cells of the sporoblast are enclosed within the closely adherent envelope cell plasmalemma (arrows). X 57,000.

gure 15. Similar to figure 14. X 57,000.

12



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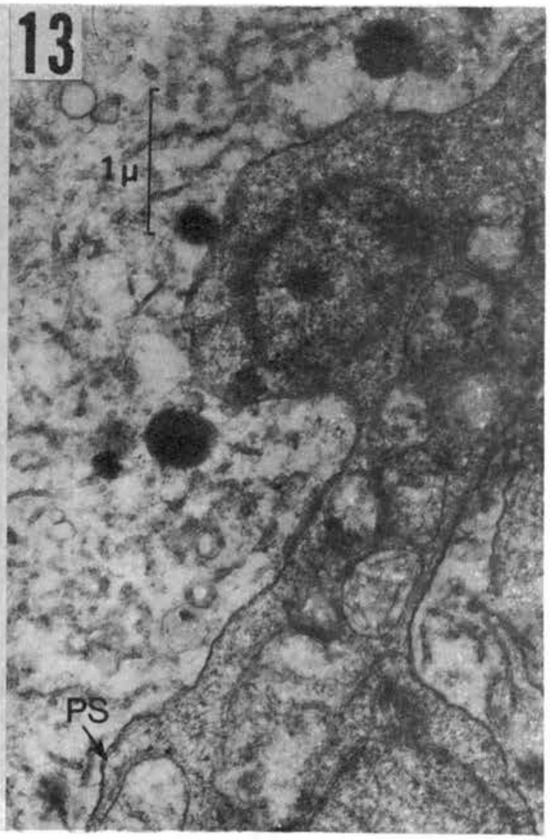

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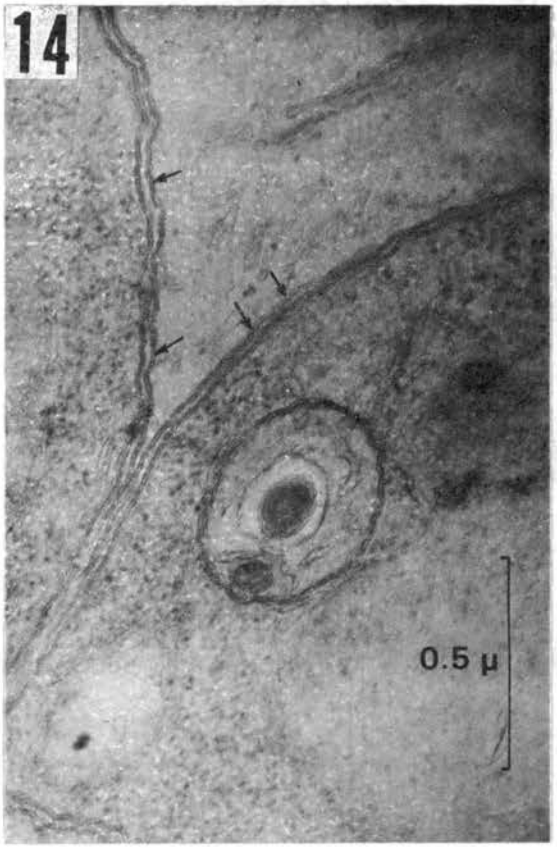

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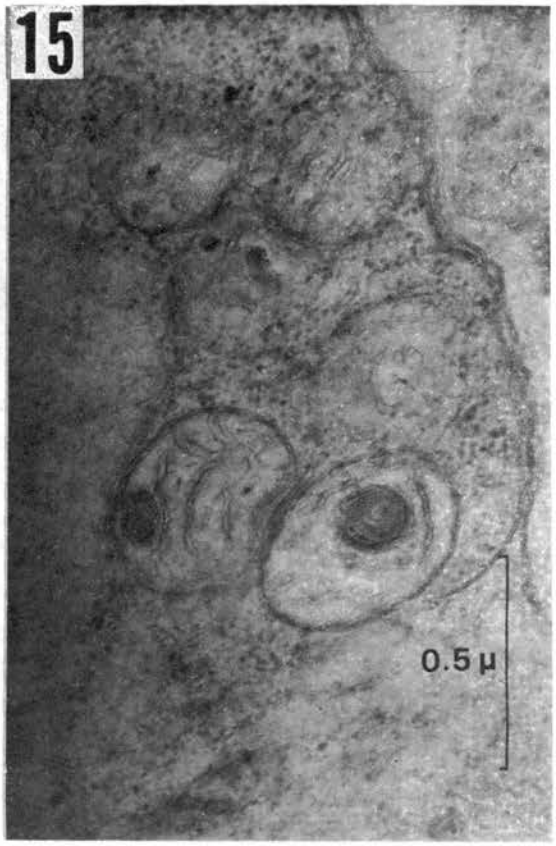
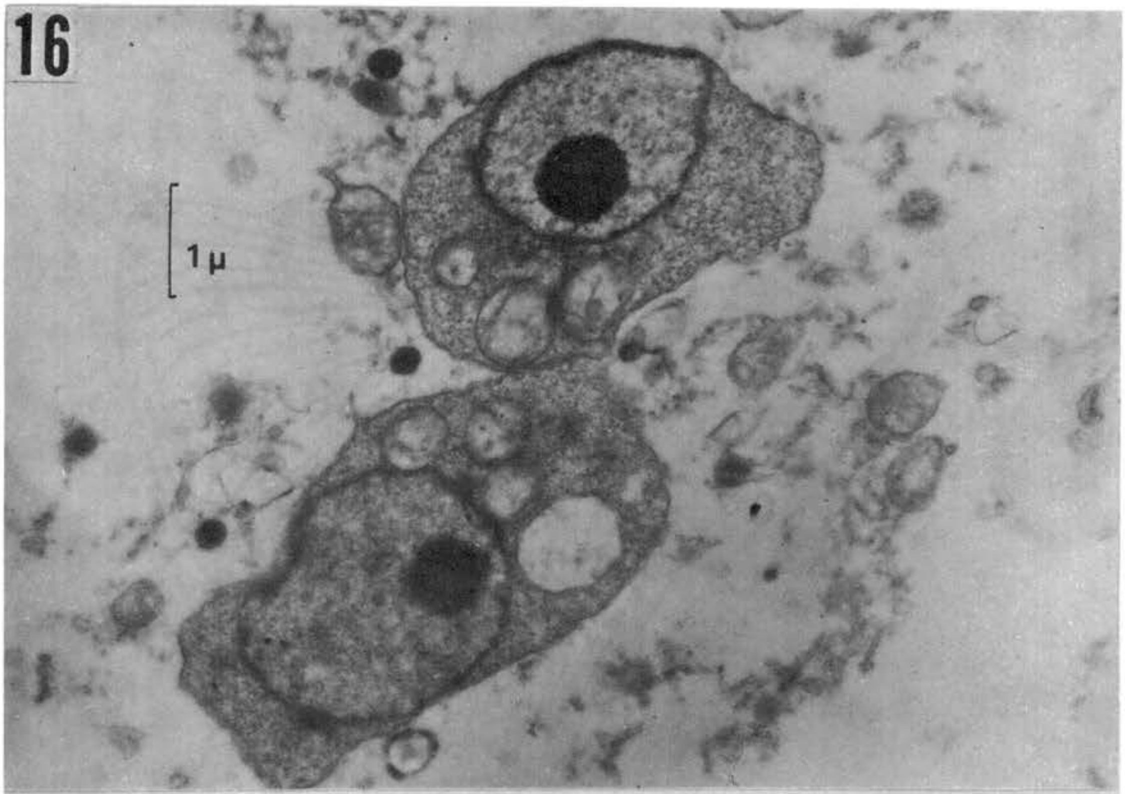


igure 16. Early association of two independent cells within plasmodium of *M. pharyngeus*. X 14,900.

igure 17. Similar to figure 16. Interdigitating pseudopodia appear between the neighboring cells during their early union. X 25,400.

igure 18. Two-celled stage of *M. pharyngeus*. The upper cell has enveloped the lower. ENV, envelope cell; SP, sporont. X 21,200.

16



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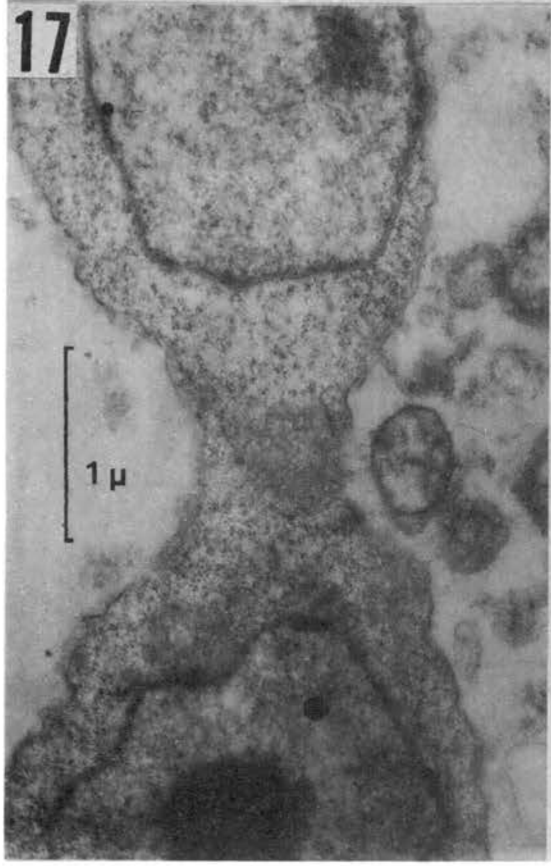

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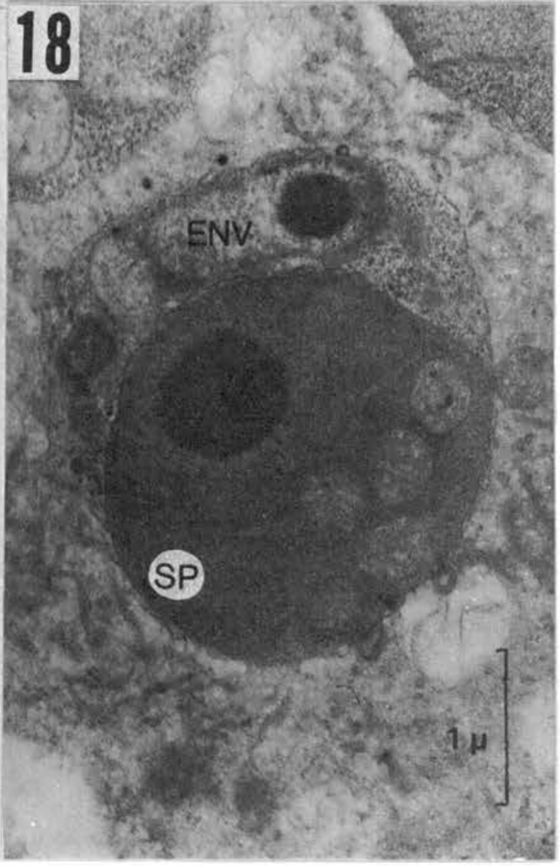
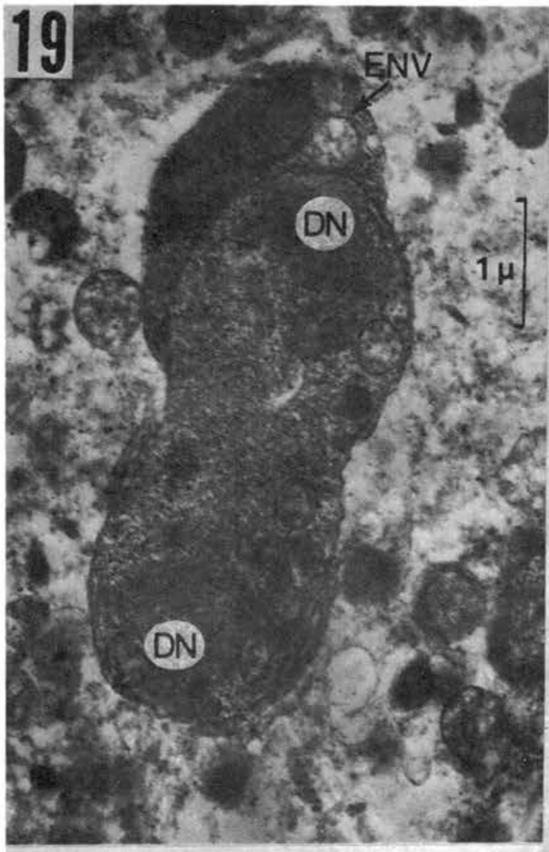


igure 19. Mitosis (telophase) of the sporont of *M. pharyngeus*. ENV, envelope cell; DN, daughter nucleus. X 17,000.

gure 20. Diplokaryotic sporont of *M. pharyngeus*. Four chromosomes (arrow) are seen in one nucleus. X 15,100.

gure 21. Multicellular sporoblast of *M. pharyngeus* during early cytodifferentiation. X 16,100.

19



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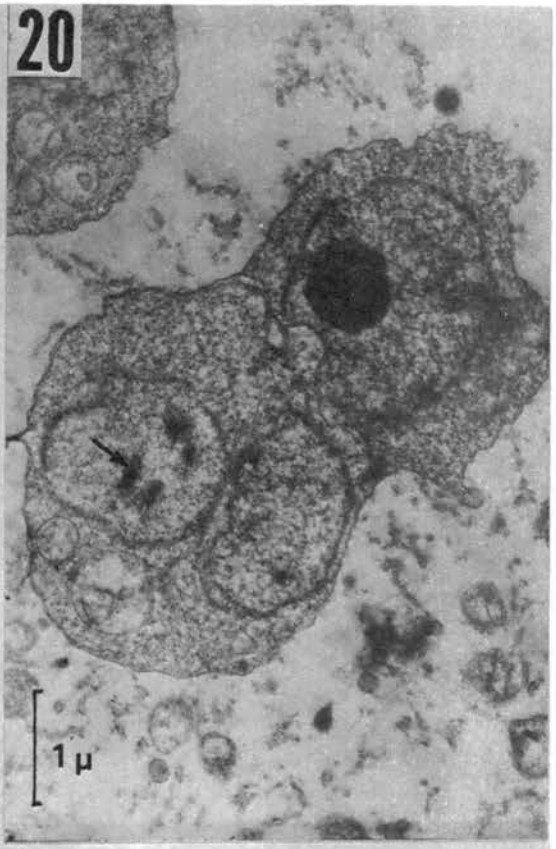
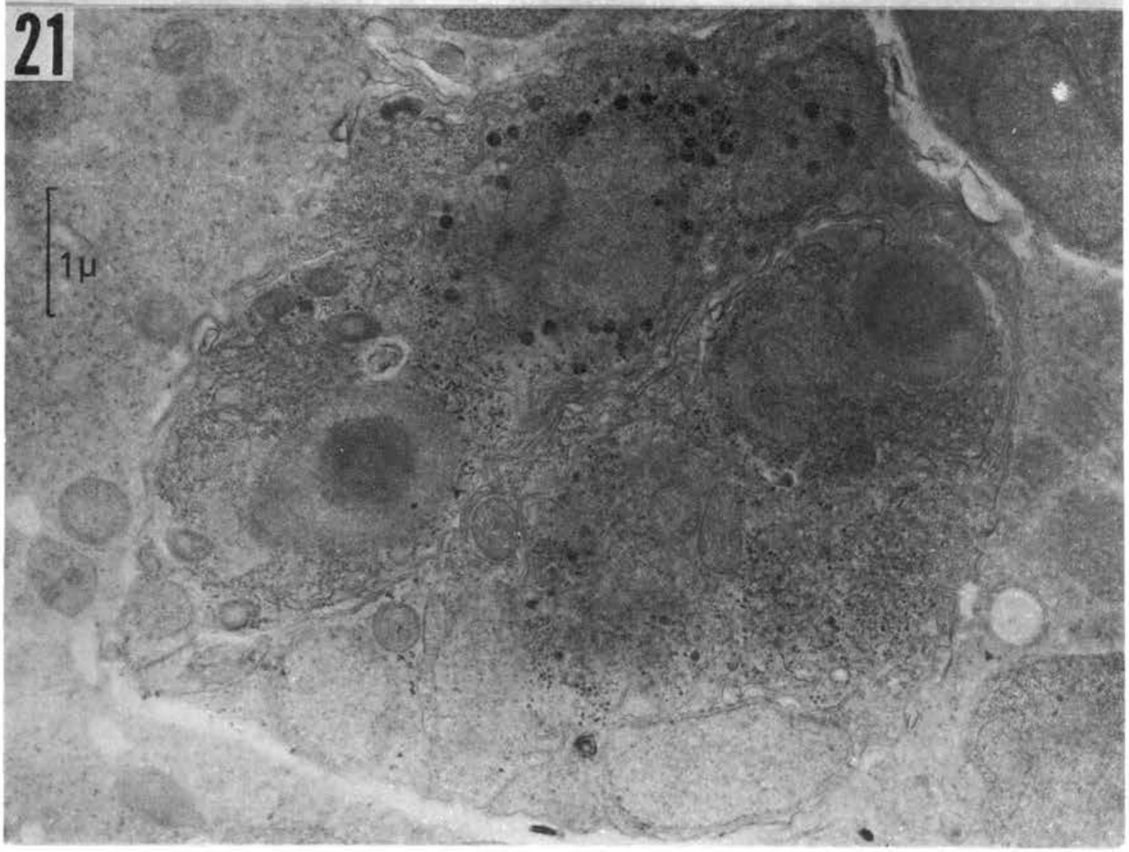
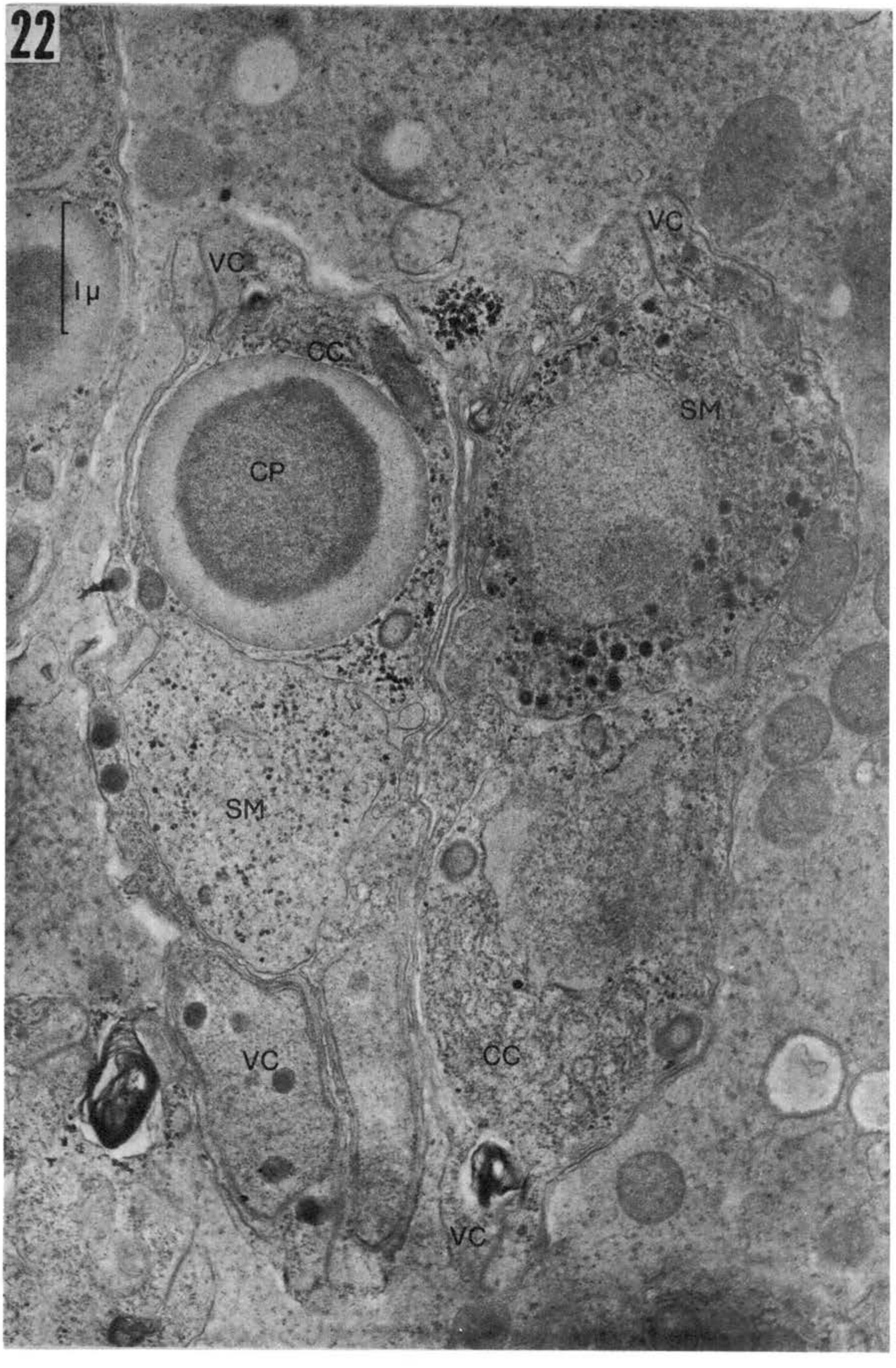


21



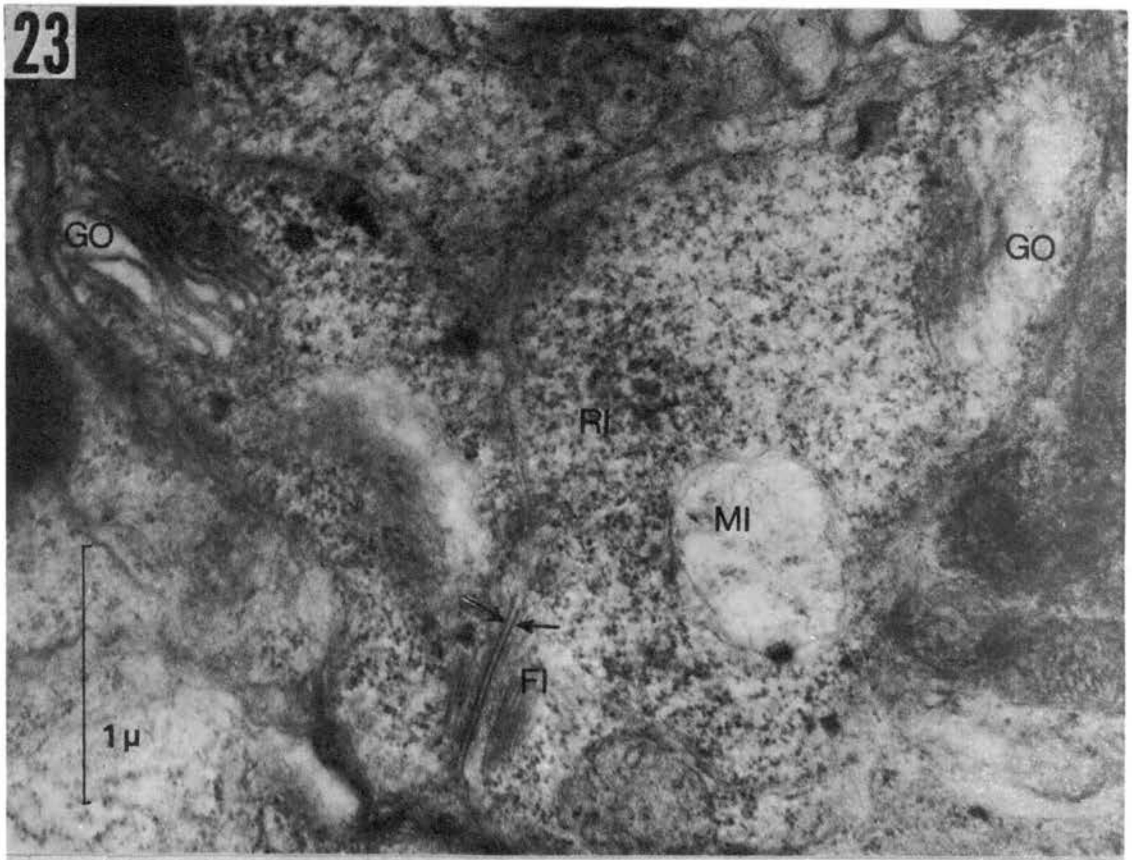
gure 22. Pansporoblast of *M. pharyngeus* containing two partially differentiated spores. VC, valvogenic cell; CC, capsulogenic cell; SM, sporoplasm; CP, capsule primordium. X 22,400.

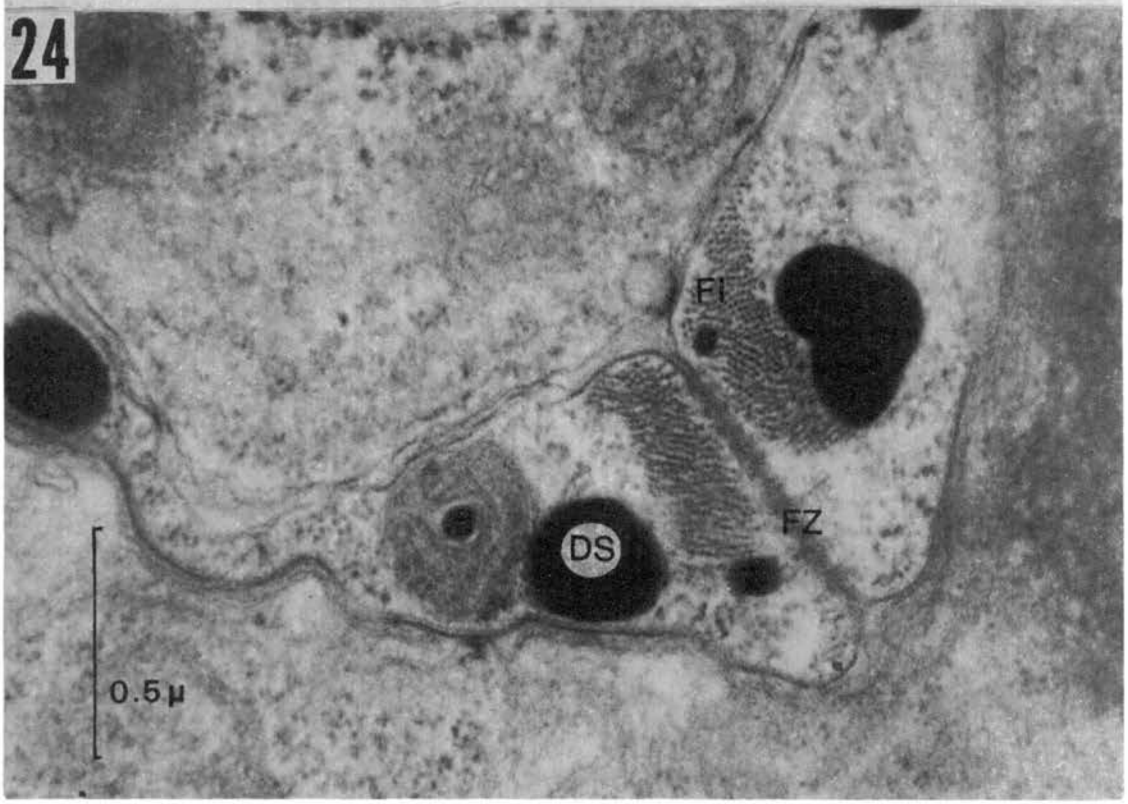
22


1 μ
VC
CC
CP
SM
VC
SM
VC
CC
VC

igure 23. Valvogenic cells of *M. pharyngeus* during early morphogenesis. Cellular features include a Golgi-like apparatus of flattened cisternae (GO), many free ribosomes (RI), poorly preserved mitochondria (MI) and a bundle of fibrils (FI) which support the thickened plasmalemma in the region of the valve suture (arrows). X 34,000.

gure 24. Valvogenic cells of *M. pharyngeus* during later development. Deposits of electron dense substance (DS) are seen in the thickened portion of the cell which supports the suture. A fuzzy precipitate (FZ) is seen in the suture. FI, supporting fibrils. X 61,500.


23
GO
GO
RI
MI
FI
1 µ

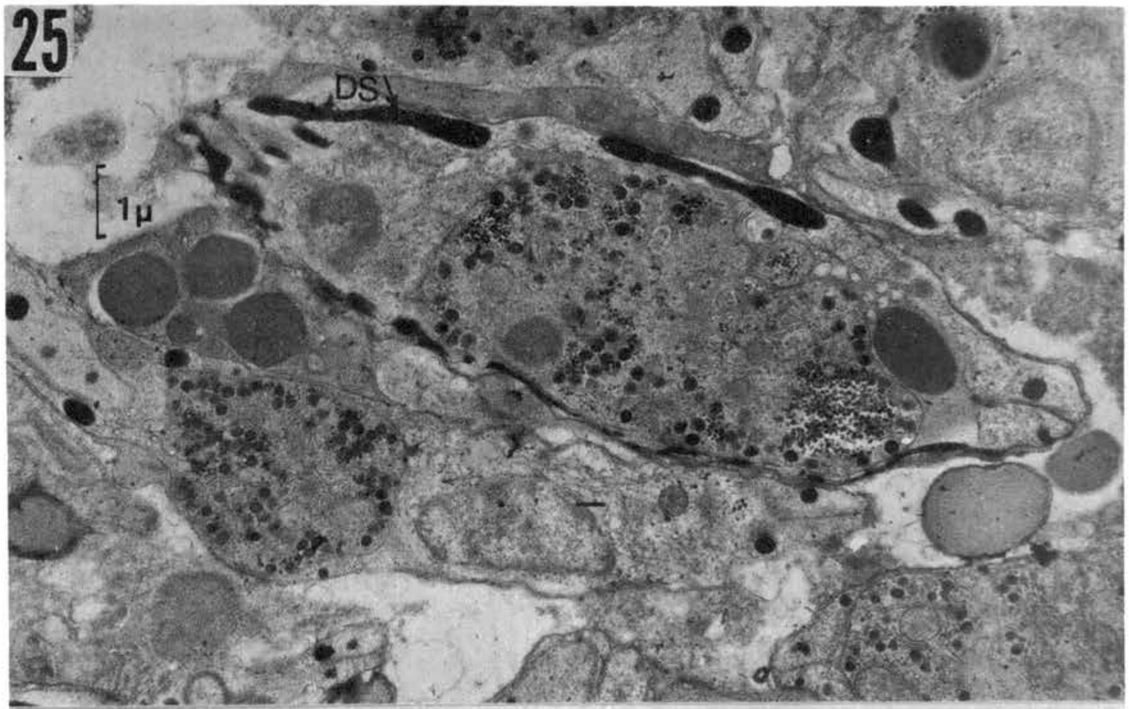

24
FI
FZ
DS
0.5 µ

igure 25. Sporoblast of *M. pharyngeus* in advanced stage of development. Electron dense substance (DS) of valvogenic cells becoming distributed throughout the thinning cytoplasm. X 9,600.

igure 26. Sporoblast of *M. pharyngeus* in advanced stage of development. Dense substance (arrows) occurs throughout both the inner and outer cortical zone of the entire valve cell. X 13,000.

igure 27. Sporoblast of *M. cyprini* sp. n. CC, capsulogenic cell; VC, valvogenic cell; MT, microtubules; ET, external tube; CS, cap or stopper substance of the valvogenic cell. Apposing plasmalemmae (TP) of the capsulogenic and valvogenic cells are much thicker where they adjoin near the cap substance. X 61,400.

25



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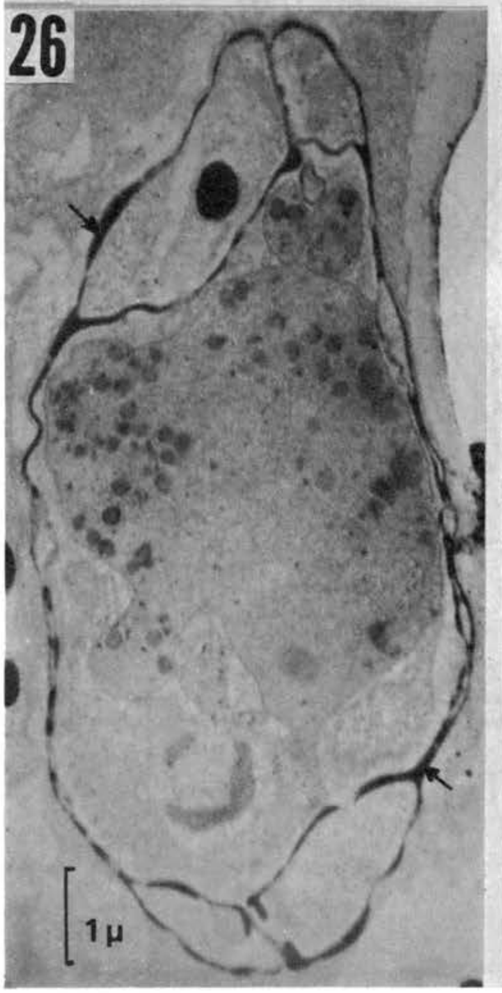
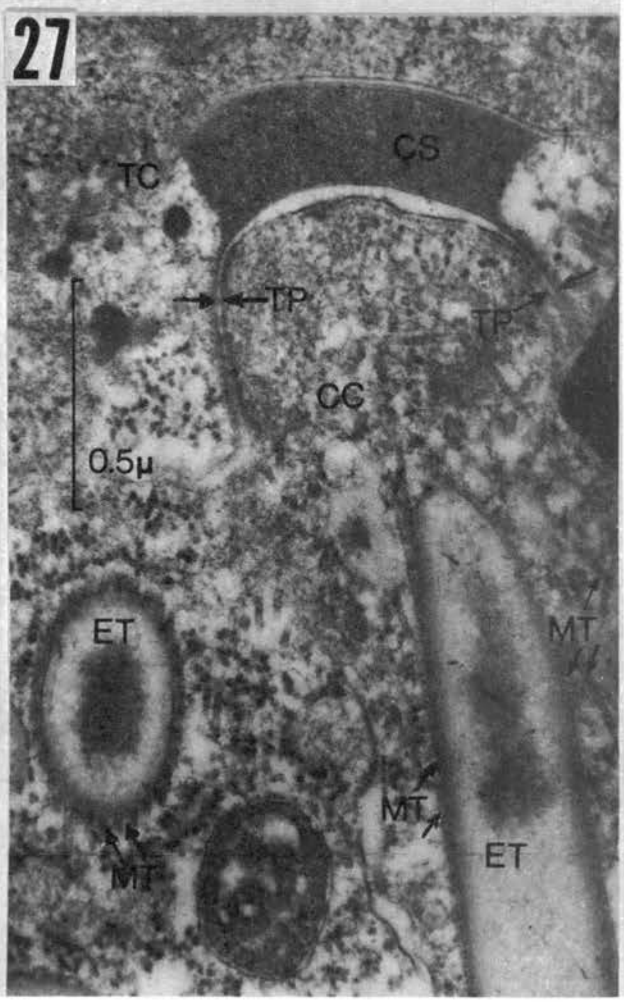


27


CS
TC
TP
TP
CC
0.5μ
ET
MT
MT
MT
ET

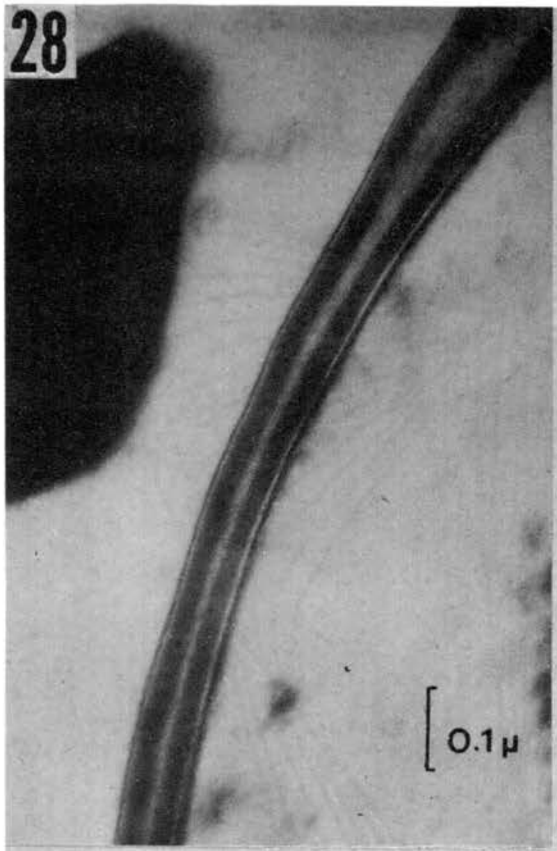
igure 28. Mature valve of *M. pharyngeus*. Freeze-substituted preparation, stained with uranyl acetate only. X 107,100.

igure 29. Mature valve of *M. pharyngeus*, prepared same as for figure 28. This is a longitudinal view and shows poorly preserved capsular material extruding through an anterior channel in each valve, the discharge channel (DC). X 25,600.

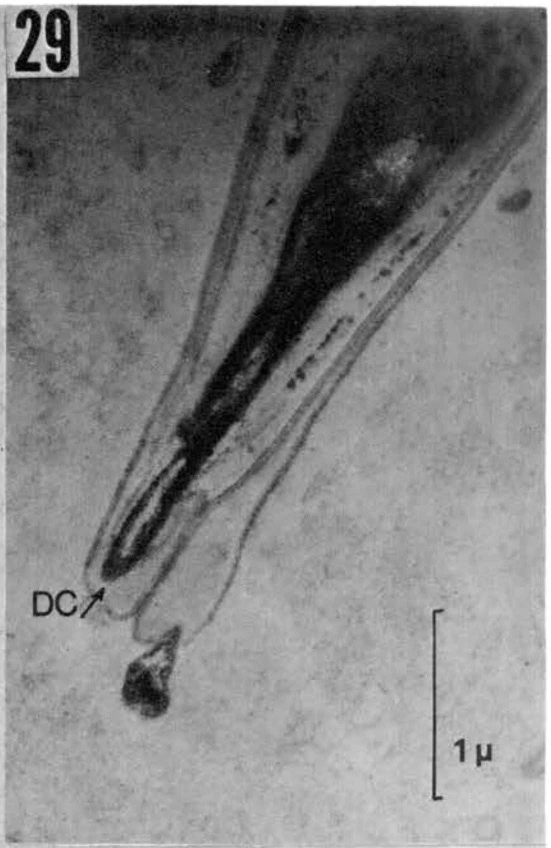
gure 30. Mature valves of *M. pharyngeus*, cross-section near posterior pole of spore. Freeze-substituted, stained with uranyl acetate and lead citrate. SM, sporoplasm; V, valve. X 19,000.

gure 31. Cross-section through anterior end of mature *M. pharyngeus* spore showing polar filament discharge channel in each valve (DC). Glutaraldehyde-fixed, postfixed in osmium tetroxide and stained with uranyl acetate and lead citrate ("routine" preparation). X 31,000.

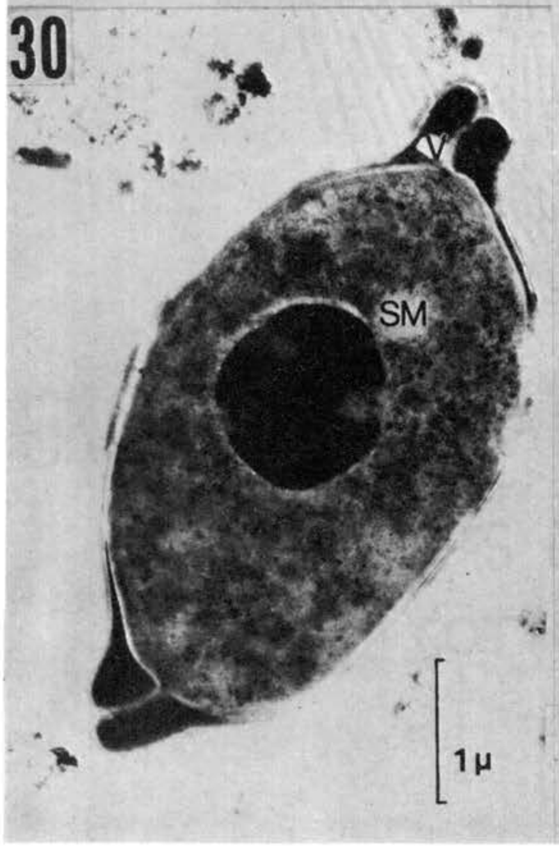
28



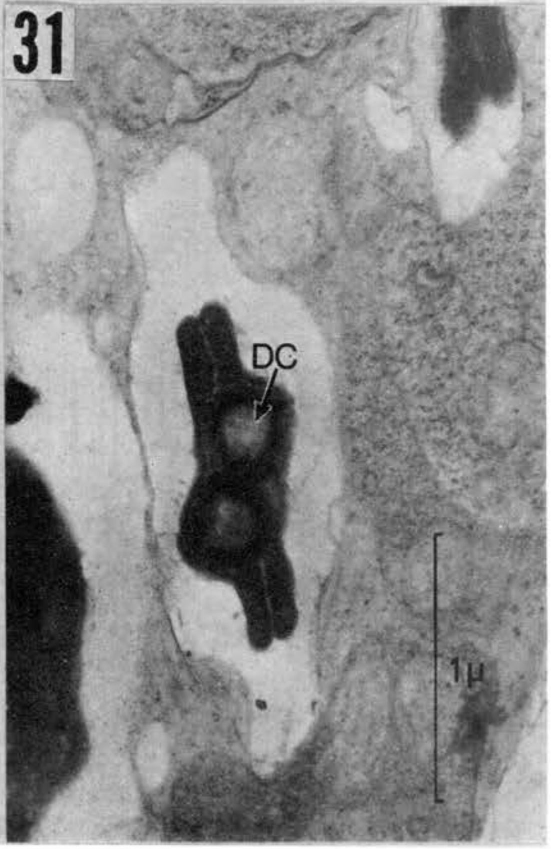
29



30



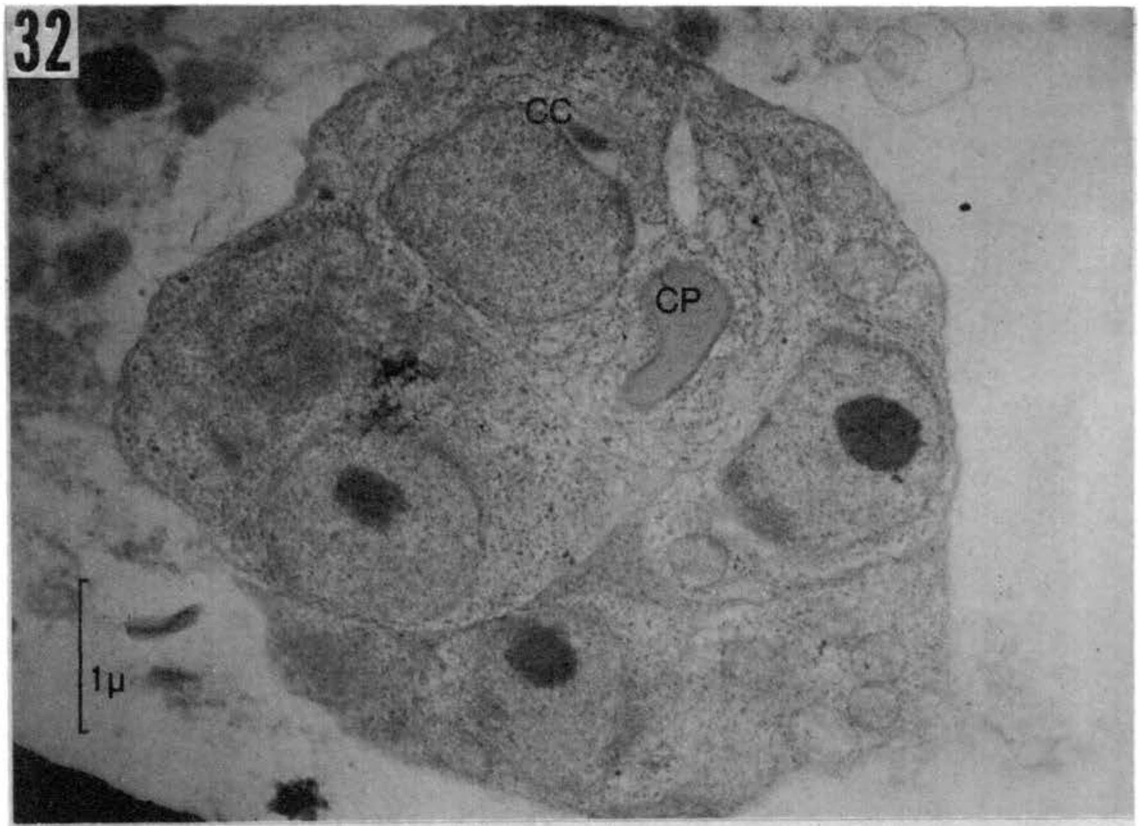
31



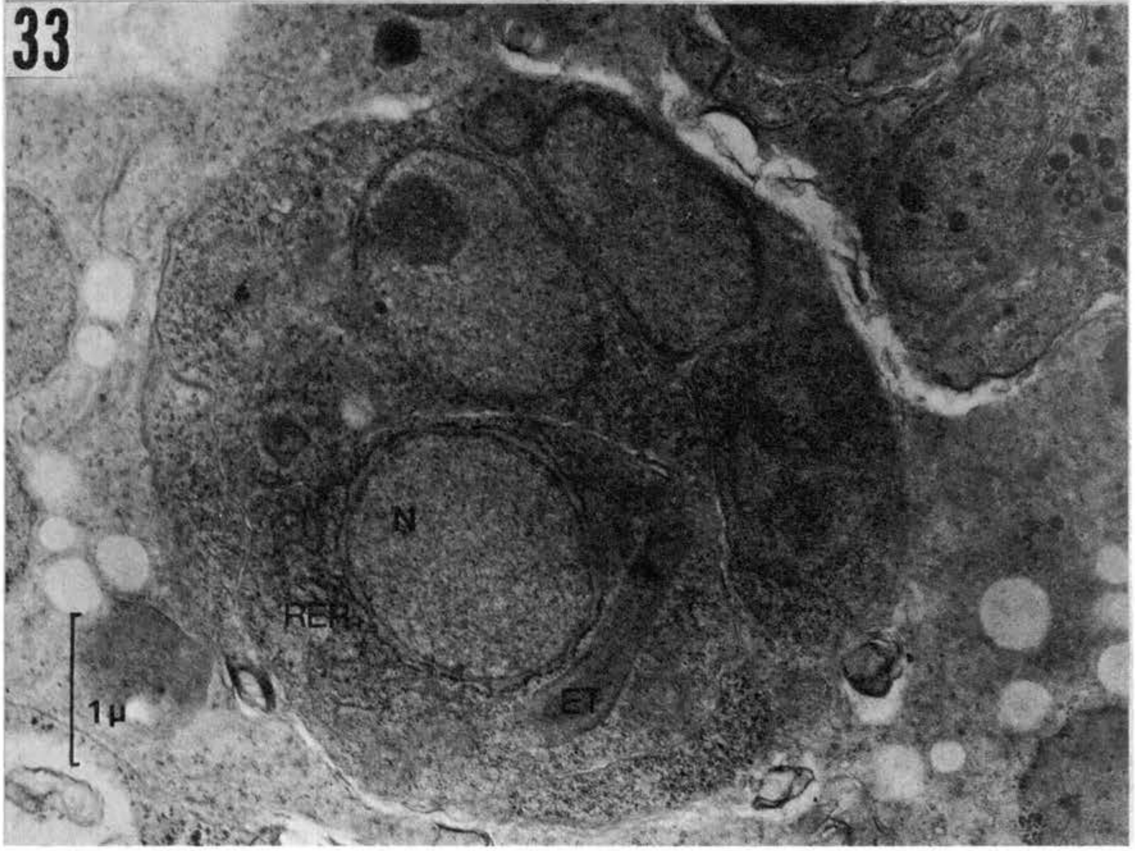
gure 32. Early differentiating pansporoblast of *M. pharyngeus* showing initial form of polar capsule primordium (CP) in capsulogenic cell (CC). X 20,000.

gure 33. Pansporoblast of *M. pharyngeus*. Early capsulogenic cell can be identified by nucleus (N) without nucleolus, abundant rough surfaced endoplasmic reticulum (RER) and external tube (ET). X 20,000.

32



33



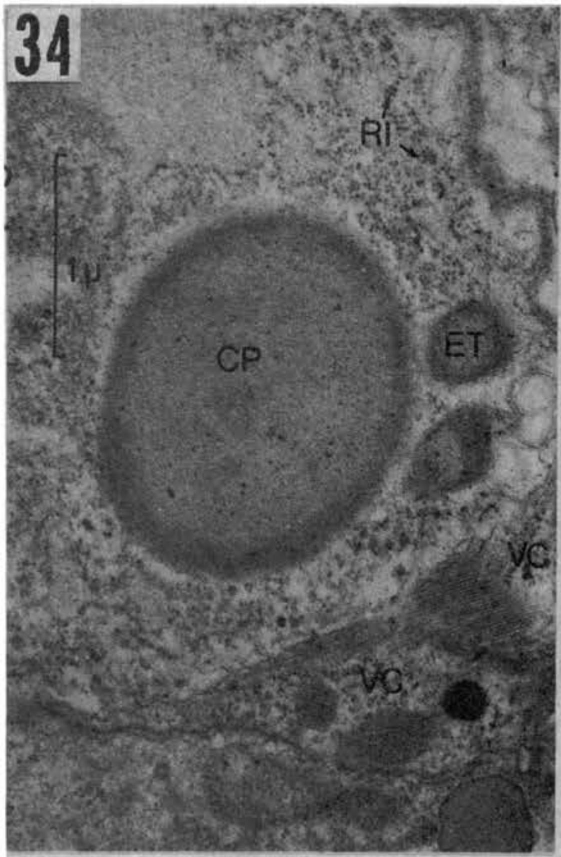
gure 34. Early capsulogenic cell of *M. pharyngeus*. The matrix in both the capsule primordium (CP) and external tube (ET) is non-granular. Numerous free and bound ribosomes (RI) are observed in the pericapsular cytoplasm. VC, valvogenic cell. X 26,900.

gure 35. Capsulogenic cell of *M. pharyngeus* after appearance of dense granular substance (arrows) within the precapsular matrix. X 26,900.

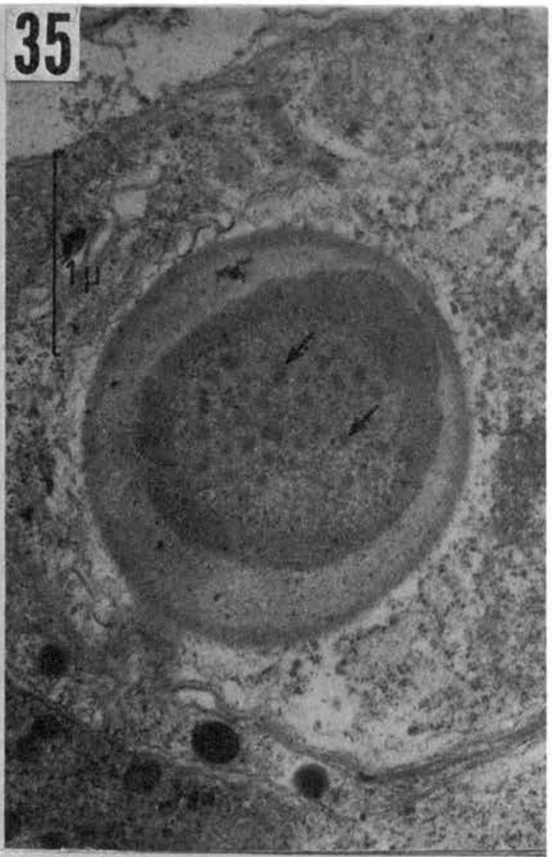
gure 36. Capsulogenic cell of *M. pharyngeus* showing relationship of external tube (ET) with the capsule primordium (CP). X 27,700.

gure 37. Advanced polar capsule of *M. pharyngeus* after polar filament anlage has formed by precipitation of granules into a small dense central zone. Nucleus (N) is degenerate at this stage. X 14,700.

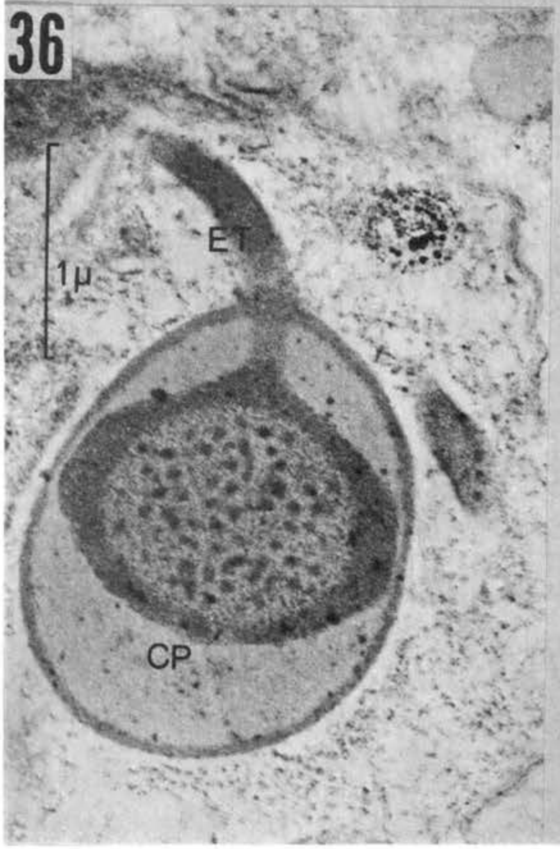
34


RI
1µ
CP
ET
VC
VC

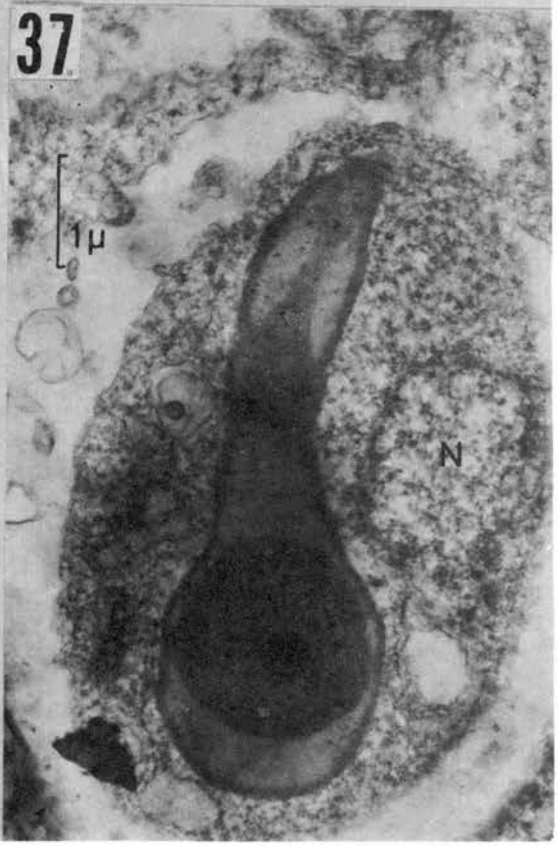
35



36



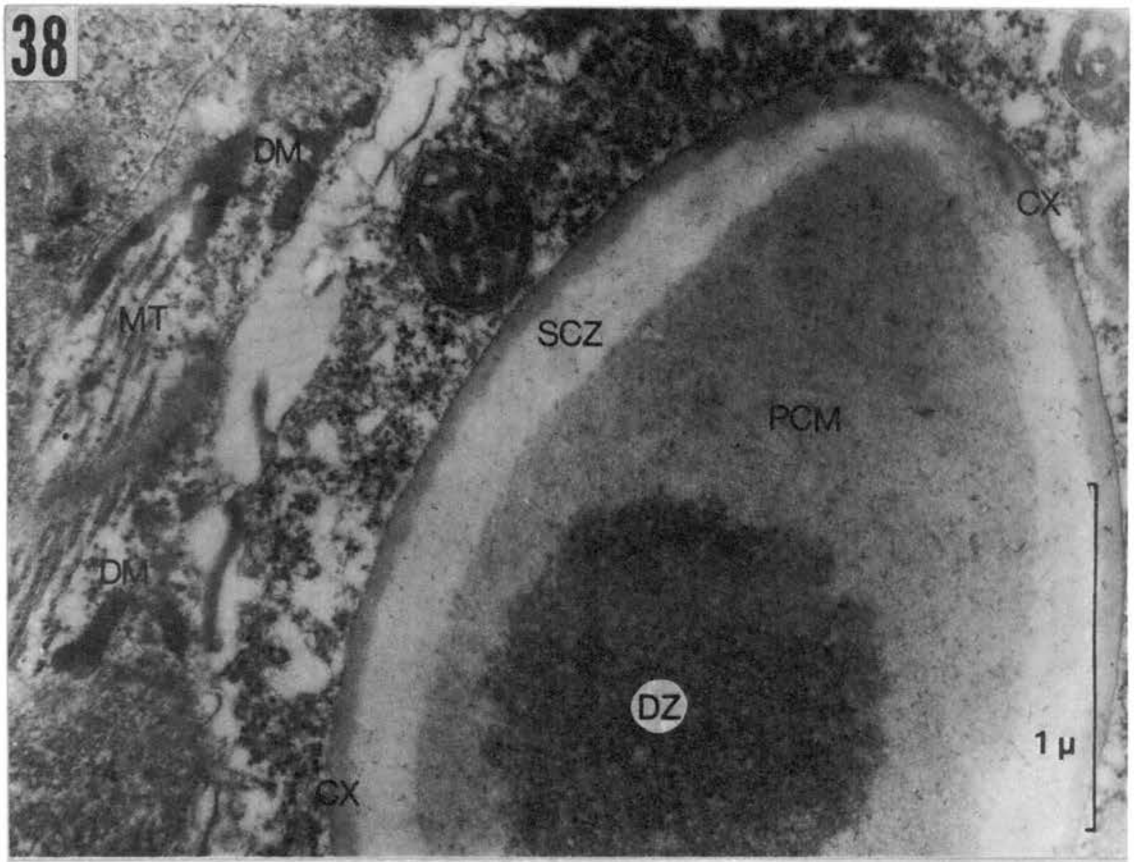
37



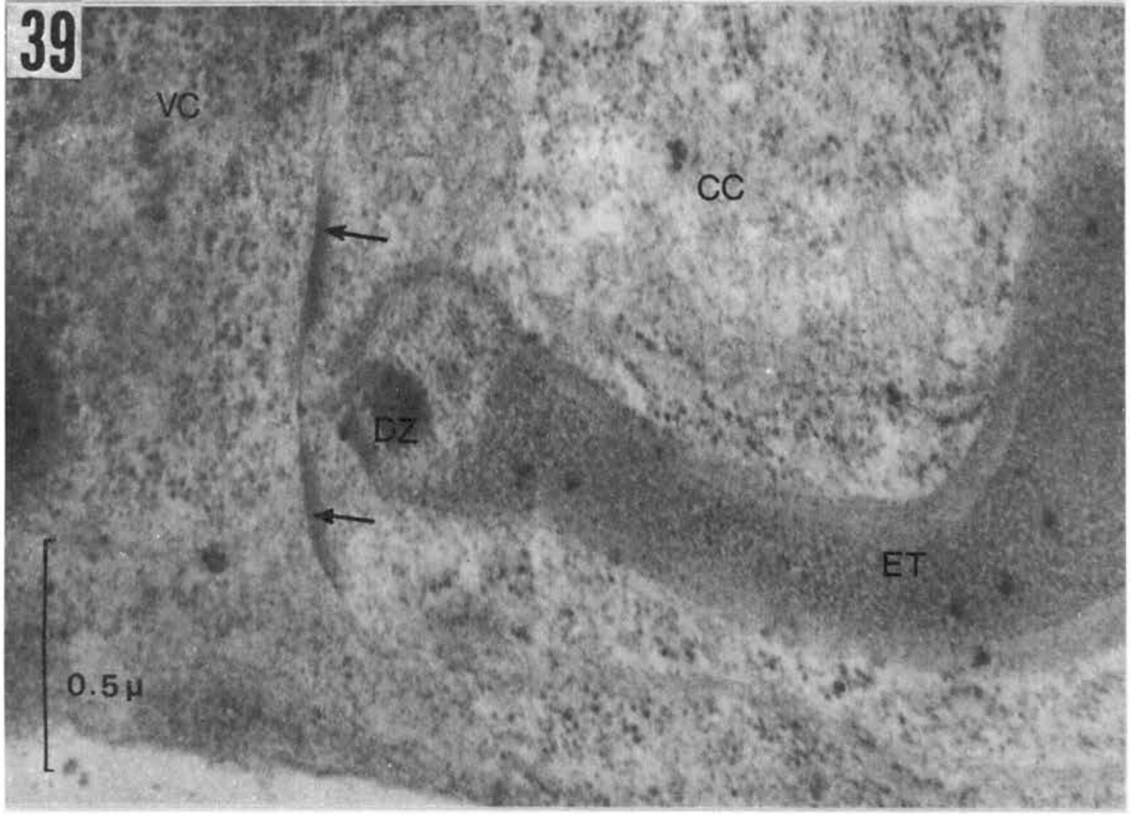
igure 38. Precapsule of M. cyprini sp. n. MT, microtubules which precede and possibly contribute to the formation of the external tube; DM, dense material, possible anlage of external tube associated with cluster of microtubules; CX, electron dense cortex of primordial capsule; SCZ, electron lucent subcortical zone; PCM, finely granular precapsular matrix; DZ, dense flocculated central zone which possibly gives rise to polar filament. X 46,000.

gure 39. Tangential section across free end of external tube (ET) of M. pharyngeus capsulogenic cell (CC). DZ, dense central zone; VC, valvogenic cell. The arrows point to the fuzzy, thickened plasmalemma of the capsulogenic cell where it adjoins the cell membrane of the valvogenic cells (the"stopper" mechanism of Lom; see also figure 27). X 61,500.

38


DM
CX
MT
SCZ
PCM
DM
DZ
1 μ
CX

39

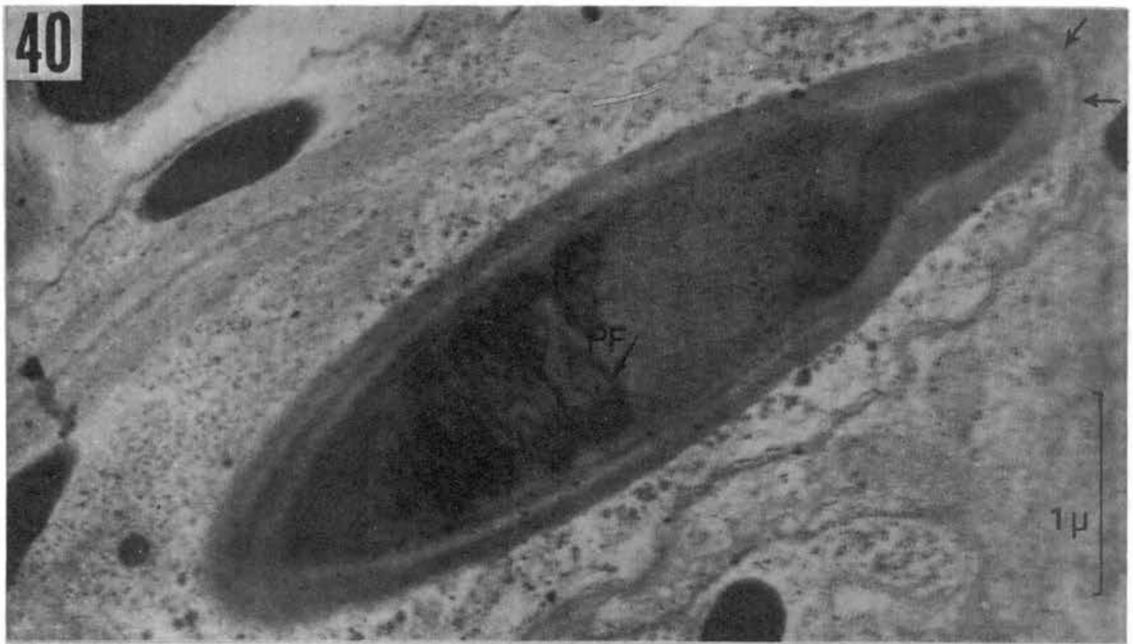

VC
CC
DZ
ET
0.5 μ

igure 40. Nearly ripe polar capsule of M. pharyngeus. The extracapsular cytoplasm including the external tube and nucleus of the capsulogenic cell have largely disappeared. Note the persistance of the thickened, dense zone of the anterior plasmalemma (arrows). PF, polar filament. X 26,900.

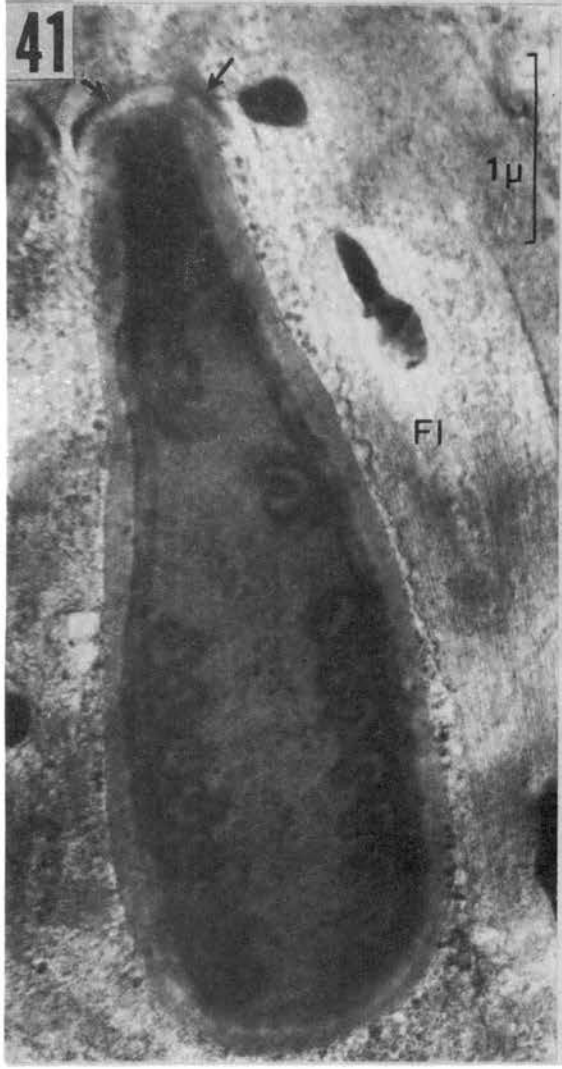
.gure 41. Longitudinal section of M. pharyngeus mature polar capsule. Arrows same as in figure 40. FI, fibrils of valve cell which parallel long axis of polar capsule. X 25,000.

.gure 42. Transverse section of M. pharyngeus ripe polar capsule. The coiled polar filament (PF) also is additionally highly twisted. FI, fibrils of valve cells cut in cross section. X 25,000.

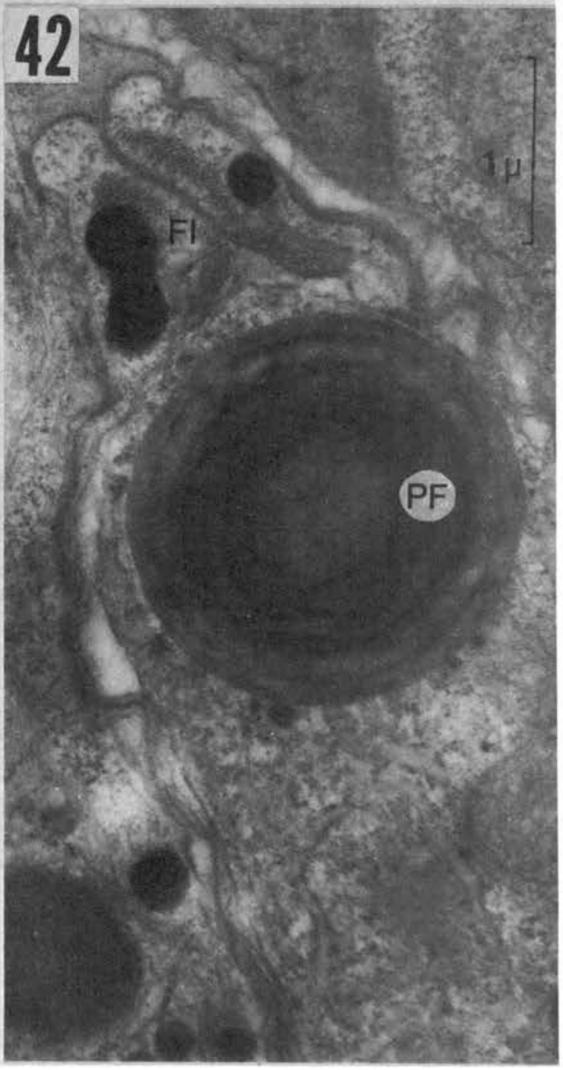
40



41



42

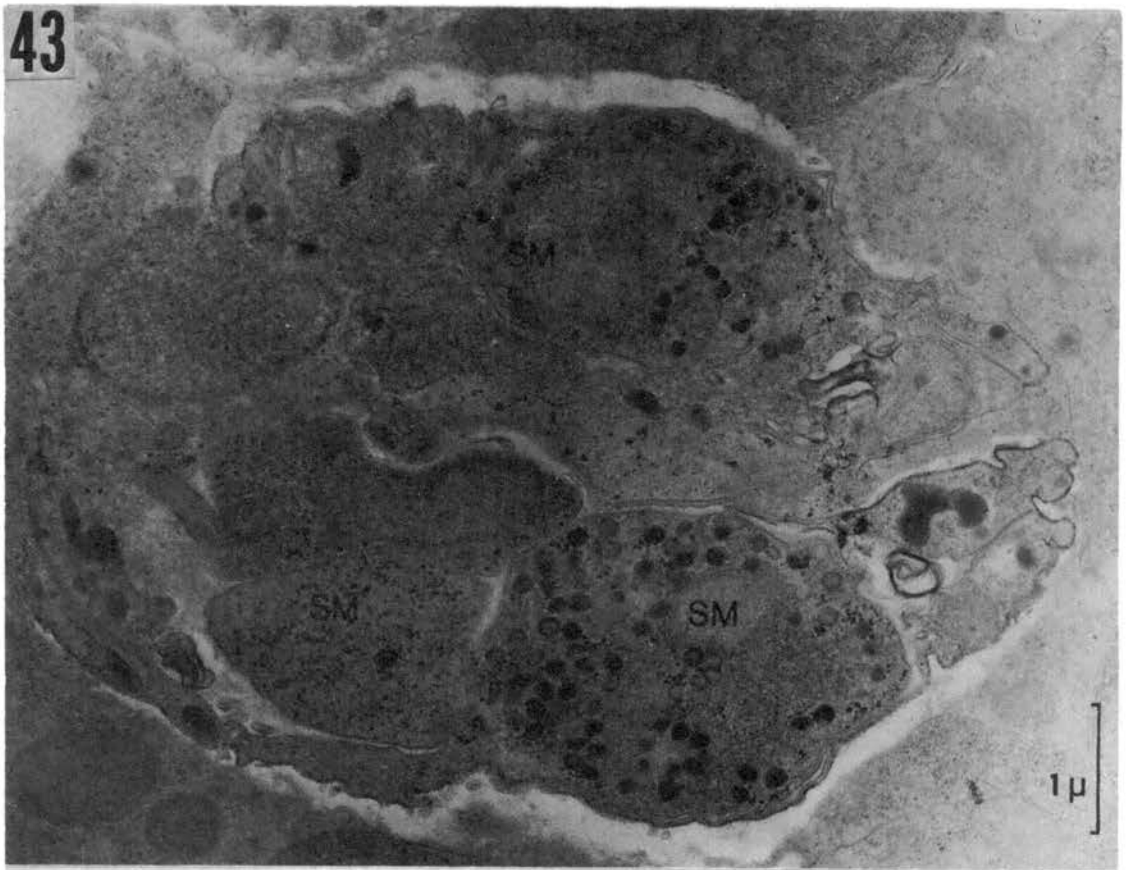


igure 43. Young pansporoblast of *M. pharyngeus*. Note the bilobed nature of the lower cell. SM, sporoplasm; SM, secondary lobe of sporoplasm. X 16,500.

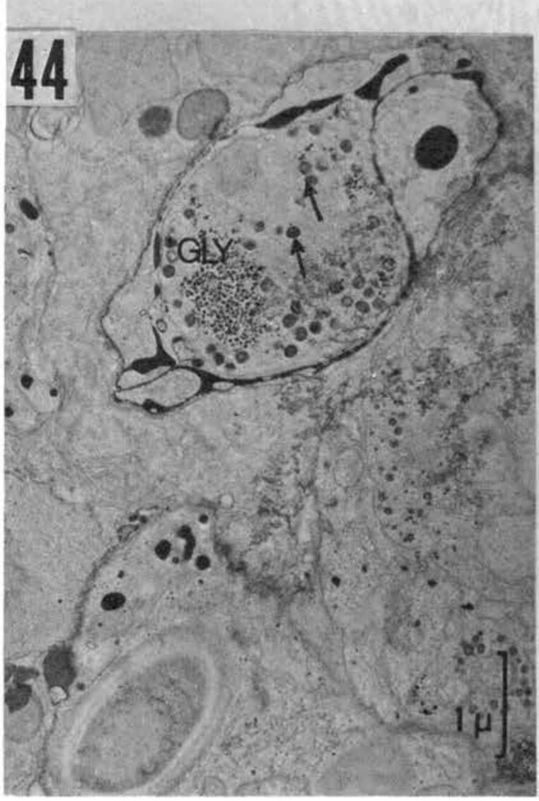
igure 44. Low magnification micrograph of developing sporoblast of *M. pharyngeus*. GLY, aggregations of small, dense glycogen bodies in sporoplasm. Arrows point to the larger, spherical dense granules ("kugelige Korper" or reserve bodies of Schubert, 1968) which are more randomly dispersed. X 14,600.

igure 45. Similiar to figure 44. X 14,600.

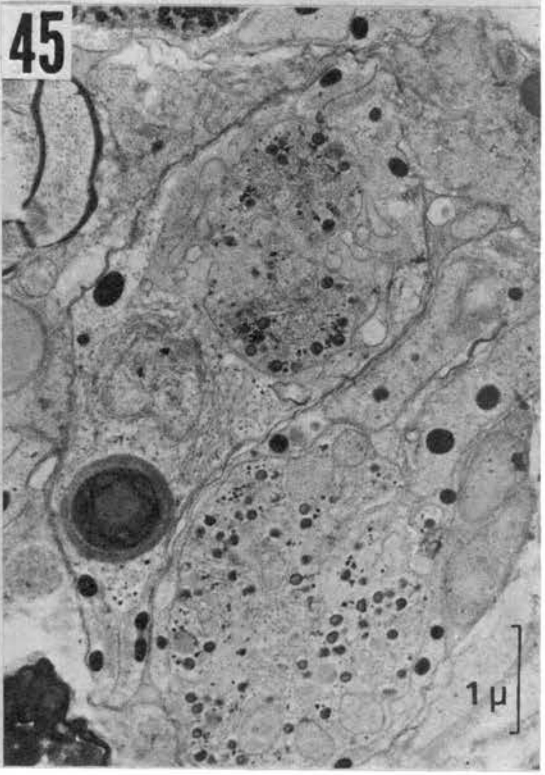
43



44



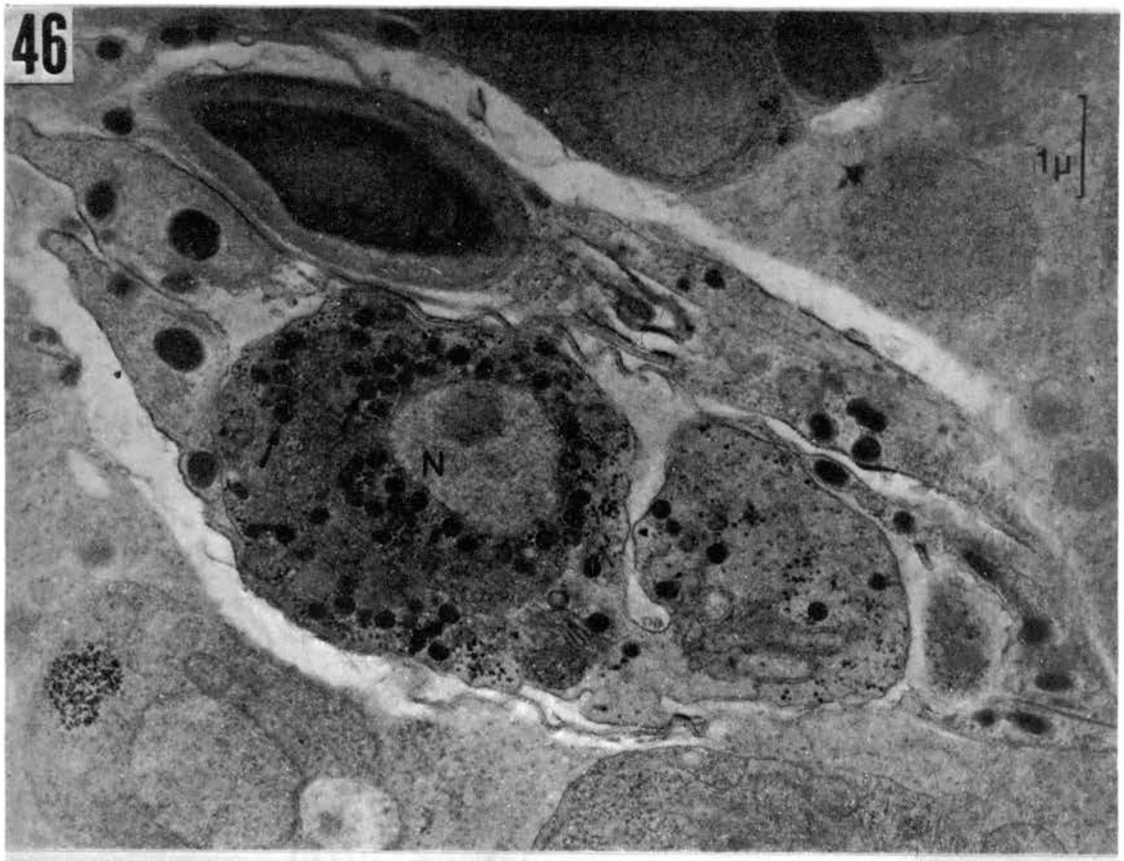
45



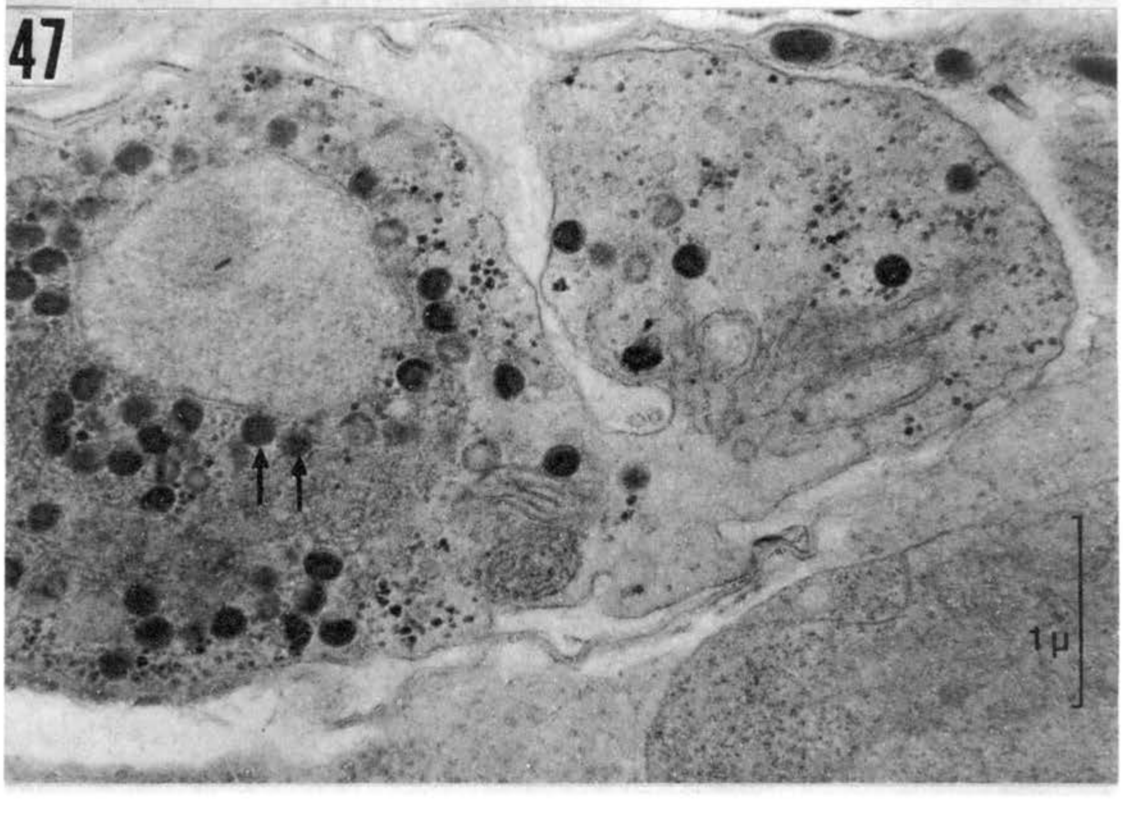
igure 46. Pansporoblast of M. pharyngeus showing bilobed sporoplasm. The reserve bodies (arrows) are most abundant in the same lobe which contains the nucleus (N). X 13,400.

gure 47. Enlargement of sporoplasm of previous figure. Arrows point to reserve bodies. X 25,100.

46



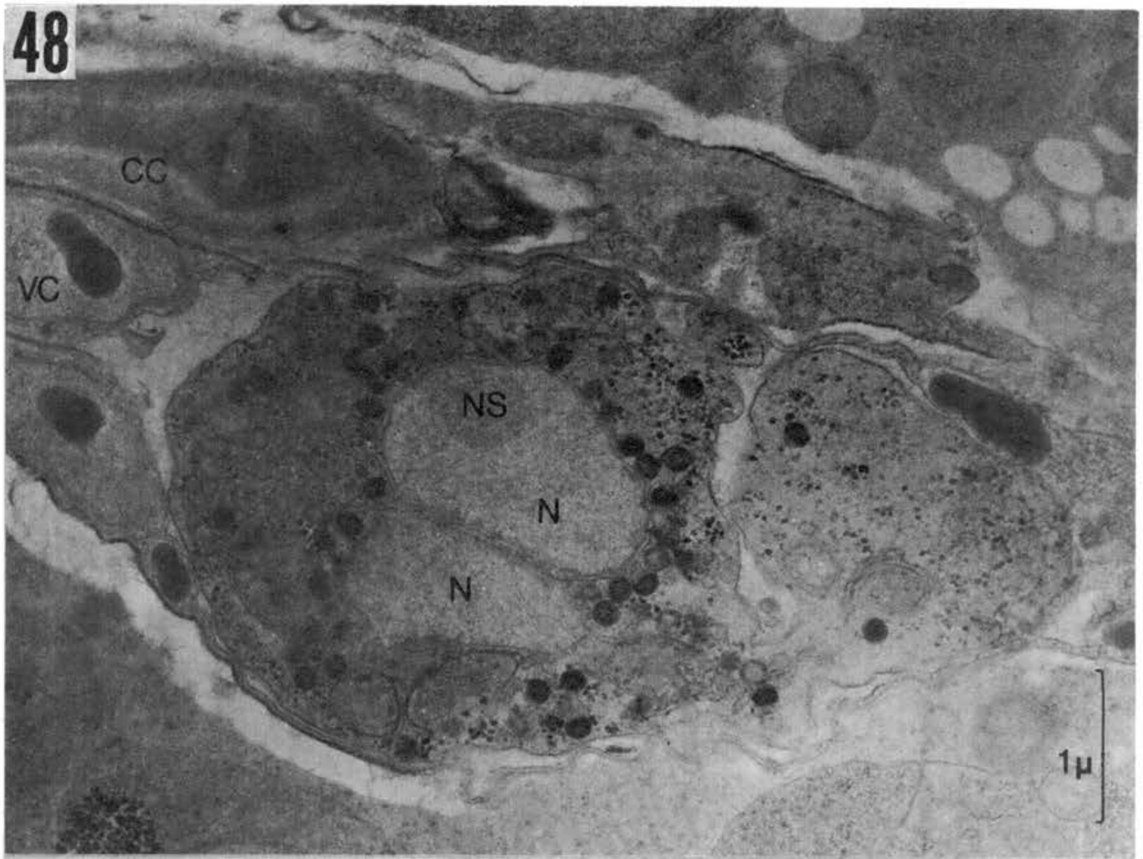
47



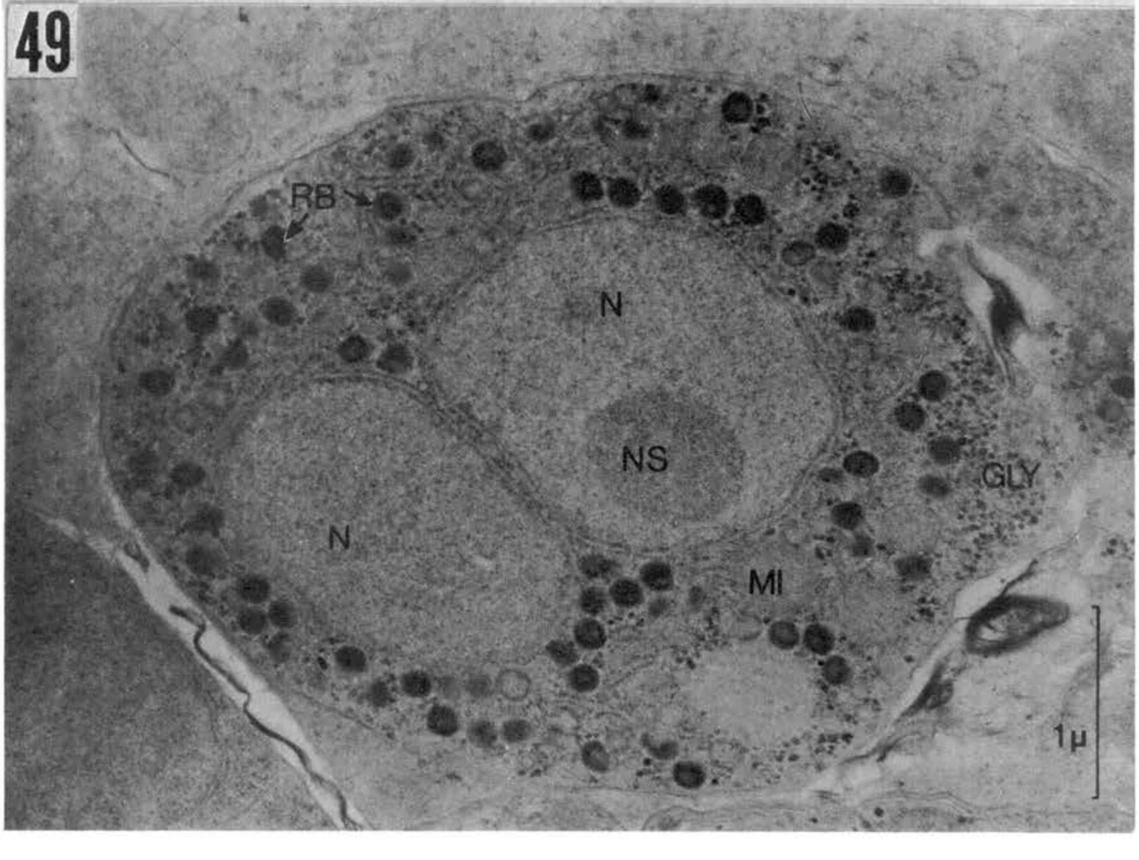
igure 48. Parallel section of same pansporoblast as figures 46 and 47 showing diplokaryotic sporoplasm. Nuclei (N) are closely associated; one lacks a nucleolus (NS). VC, valvogenic cell; CC, capsulogenic cell of second sporoblast. X 20,500.

igure 49. Diplokaryotic sporoplasm of ripening *M. pharyngeus* spore. GLY, glycogen granules; MI, mitochondrion; N, nucleus; NS, nucleolus; RB, reserve bodies. X 25,200.

48


CC
VC
NS
N
N
1µ

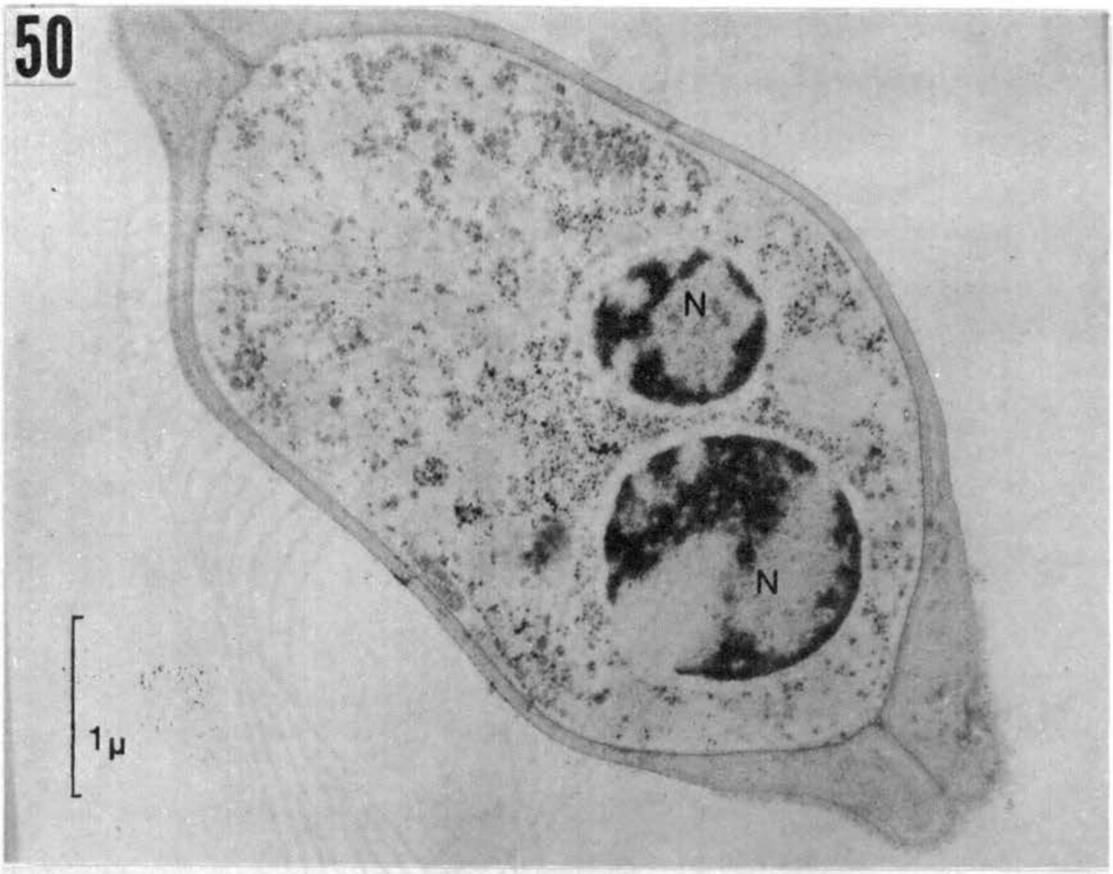
49


RB
N
NS
GLY
N
MI
1µ

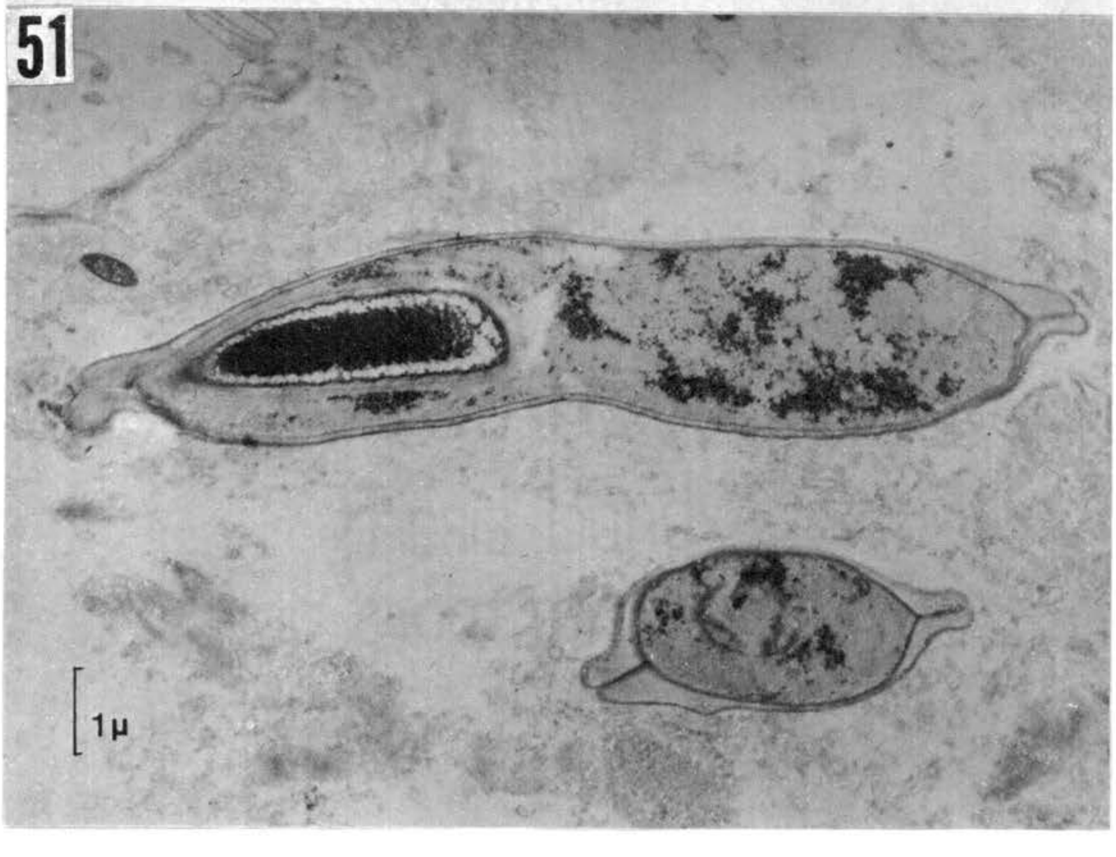
igure 50. Freeze-substituted preparation of ripe *M. pharyngeus* spore. Uranyl acetate only. Transverse section. N, nucleus. X 24,000.

igure 51. Mature spore of *M. pharyngeus* prepared the same as for figure 50. Top spore longitudinally sectioned showing poorly preserved polar capsule. Lower spore cross-sectioned through posterior region. X 11,700.

50



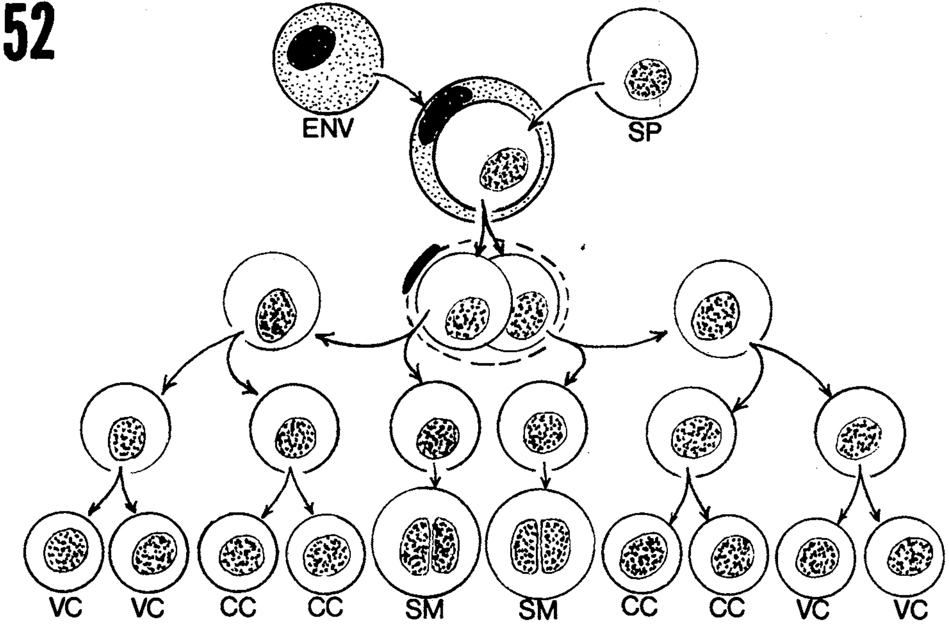
51



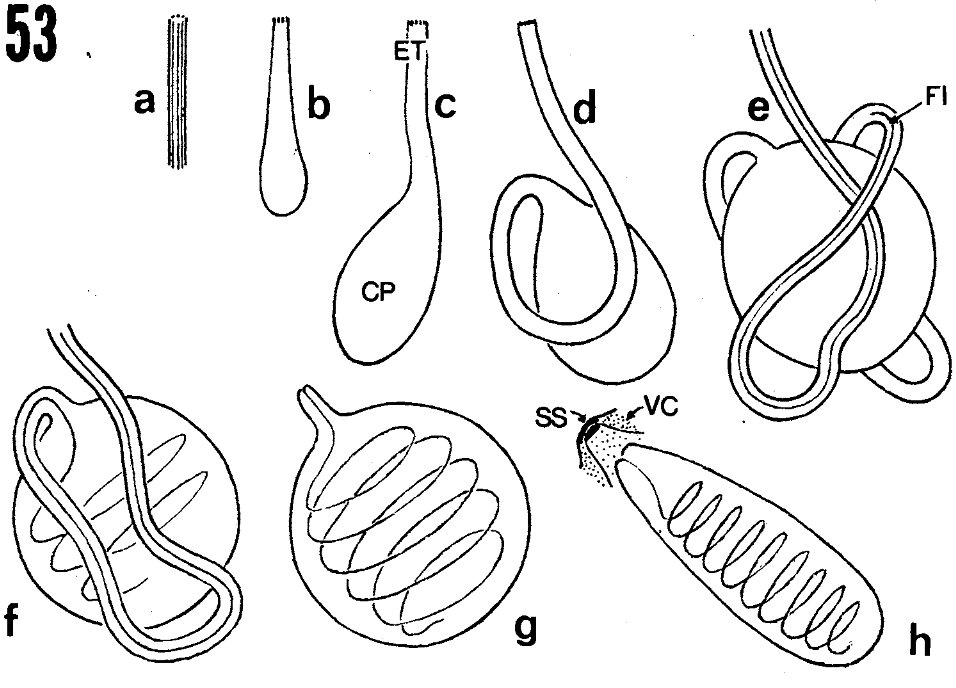
igure 52. Schematic representation of cell division and differentiation during sporogenesis in *Myxosoma*. VC, valvogenic cell; CC, capsulogenic cell; SM, sporoplasm; ENV, envelope cell; SP, sporont.

gure 53. Hypothetical schema for polar capsule development (adapted from Lom and dePuytorac, 1965b). a, cylindrical cluster of microtubules which possibly precedes formation of the external tube (ET) and/or capsule primordium (CP); b, flask-shaped CP with short ET; c and d, growth of CP and ET; e, polar filament (FI) forming within ET and CP; f and g, filament being forced into CP as a result of shortening of the ET; h, ripe capsule with discharge channel of the valve (VC) plugged by the stopper substance (SS).

52


ENV
SP
VC
VC
CC
CC
SM
SM
CC
CC
VC
VC

53


a
b
ET
c
d
e
FI
CP
SS
VC
f
g
h

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Thesis: STUDIES ON THE ULTRASTRUCTURE, ONTOGENY AND TRANSMISSION OF *MYXOSOMA PHARYNGEUS* AND *M. CYPRINI* (PROTOZOA: MYXOSPORIDA)

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