PROTOZOAN PARASITES OF FISH

by

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A B S T R A C T

Studies have been carried out on protozoan parasites of freshwater fish from the Amazon region of Brazil, and the British Isles. Special emphasis has been given to aspects of the taxonomy and life - history of species of the order Myxosporida.

In section 1, full descriptions are given of Myxosporida from Brazilian and British fish. The Brazilian species include three of the genus Myxobolus from piranha (Serrasalmus sp. and S. rhombeus) and tambaqui (Colossoma bidens), and Agarella gracilis from lungfish (Lepidosiren paradoxa). In British fish, Myxobolus species are described from roach (Rutilus rutilus), gudgeon (Gobio gobio) and dace (Leuciscus leuciscus); Myxidium species are described from roach, gudgeon and salmon (Salmo salar).

The similarity of the genera Myxobolus and Myxosoma is noted, and the difficulty of distinguishing them is pointed out. From the result of histochemical tests for glycogen in spores of selected species, it is proposed that the two genera should be synonymised as Myxobolus.

Accounts are given of attempts to establish infections of Myxidium in Myxidium - free roach and salmon. The negative results of these experiments suggests that myxosporidan spores may need to undergo some form of further development outside the host before becoming infective to a new host.

Check - lists are given of species of Myxobolus, Myxidium and recently described Myxosoma species.

In section 2, other protozoa, including two species of microsporida, one coccidian species, and Rhabdospora thelohani are described and discussed.

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CONTENTS

A C K N O W L E D G E M E N T S

This work was carried out in the Department of Parasitology, London School of Hygiene and Tropical Medicine, and at Winches Farm Field Station, St. Albans, during the tenure of a Medical Research Council Scholarship for Training in Research Methods between October, 1964 and September, 1966.

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INTRODUCTION

Until recently, the study of parasites of fish has remained largely of academic interest only. With the growing importance of the sea as a source of food, and with developments of hatcheries for freshwater fish such as salmon and trout, the economic importance of this study is now being realized. A large research programme in the U.S.S.R. has resulted in the publication of the first book providing a general treatment of the subject (Dogiel et al., 1961) and the first key to parasites of freshwater fish (Bykhovskaya - Pavlovskaya et al., 1962).

Protozoa are responsible for a wide variety of fish diseases, both in aquaria and under natural conditions. In aquaria, ectoparasitic ciliates and flagellates are of particular importance, descriptions of some of these being given in a short review by the present author (Walliker, 1966a). Under natural conditions, ectoparasitic organisms and endoparasites such as trypanosomes, trypanoplasms, myxosporida, microsporida and coccidia cause many diseases of importance to the fish industry.

In the present study, emphasis has been given to the myxosporida, which are possibly the most common of all fish protozoa. In Section 1, myxosporidan species found in various freshwater fish collected both in Brazil, during the Guy's Hospital Expedition to the Lower Amazon, 1964, and in the British Isles between 1964 and 1966 are described and identified. Experimental work on the taxonomy and life-history of selected species is then described. In Section 2, some other protozoa from fish collected in the London area are described and discussed.

MATERIAL AND METHODS

1. Collection and maintenance of fish

(i) Brazilian fish.

Fish were collected in Brazil between July and September, 1964, during the Guy's Hospital Expedition to the Lower Amazon. Adult specimens of piranha, Serrasalmus spp., were caught with a baited rod and line in the Rio Negro, approximately 100 miles above Manaus, and in the Rio Prêto, a small tributary of the Amazon, approximately 50 miles from Manaus. An adult specimen of tambaqui, Colossoma bidens (Agassiz) was caught in a similar way in the Rio Solimoes (Amazon) approximately 100 miles above Manaus. Specimens of lungfish, Lepidosiren paradoxa Fitzinger were netted in a small brook in the city of Belem by a local fisherman.

Smears of blood, liver, spleen and kidney of the Serrasalmus species and C. bidens were dried in air, fixed in methanol and stained with Giemsa's stain while in Brazil. Portions of liver, spleen and kidney were fixed in 10% formol saline and brought back to this country for sectioning and staining. Intact fish were fixed in formalin for subsequent identification by the British Museum (Natural History). Specimens of L. paradoxa were brought to this country alive and kept in aquaria at Guy's Hospital, London.

(ii) British fish

Species of the family Cyprinidae were collected in four localities in the London area either by netting or with the aid of traps.

Each trap (Fig. 1) was made of $\frac{3}{4}$" wire mesh and was cylindrical in

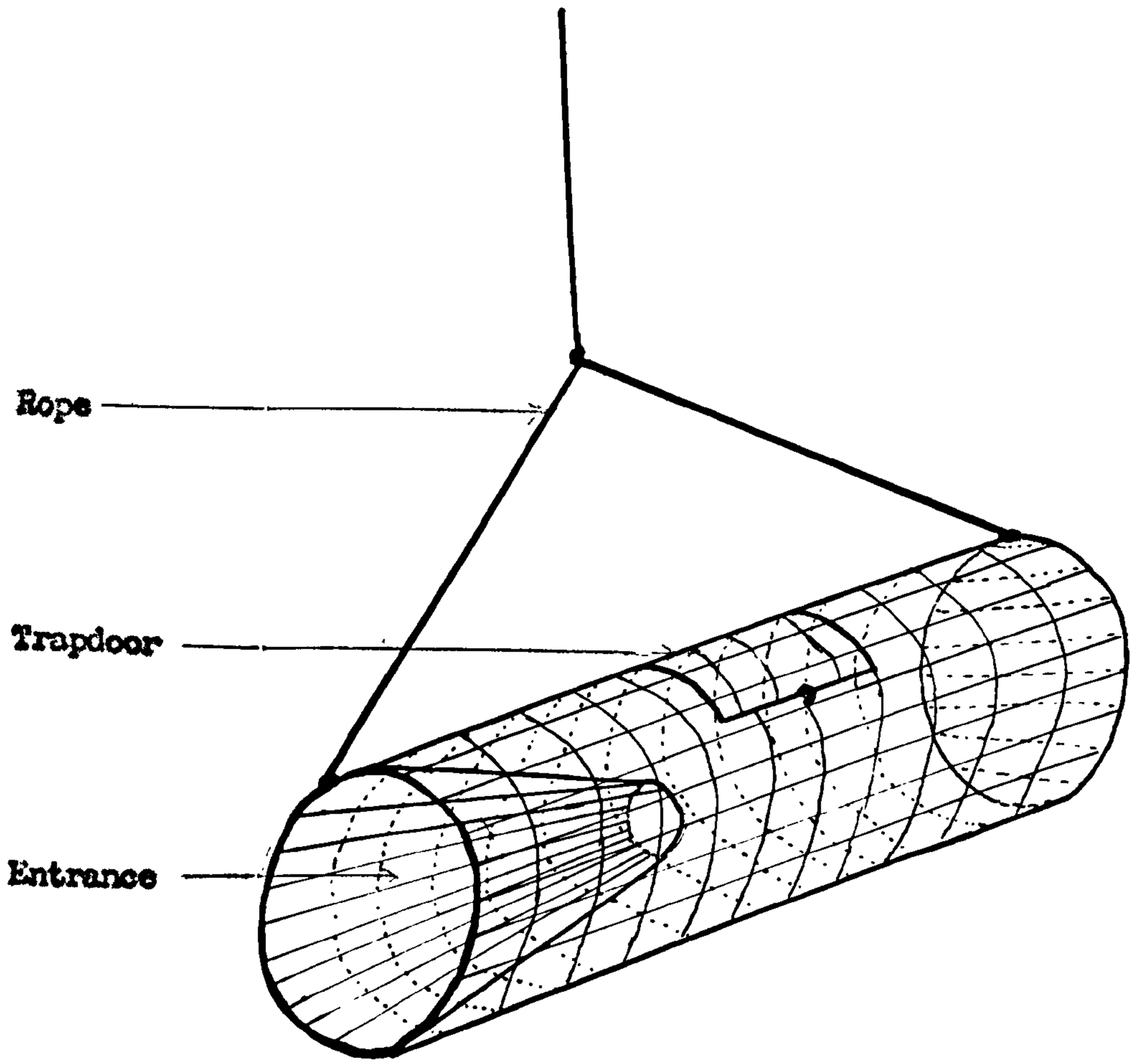


FIGURE 1.

FISH TRAP.

shape, measuring 3 ft. in length by 1 ft. in diameter. The entrance, of conical shape, was at one end. A small trap door in one side, closed while the trap was in use, was used to extract the fish caught. The trap was usually set overnight, with bread as a bait.

The localities and times at which fish were collected were as follows:-

a. St. James's Park, London. Specimens of roach, Rutilus rutilus (L.) were trapped at various intervals between November, 1964 and November, 1966.

b. The River Colne, near Rickmansworth, Hertfordshire. In June and July, 1965, a large number of R. rutilus and gudgeon, Gobio gobio (L.) were netted by a local Angling Society. Living specimens were taken to Winches Farm for subsequent examination.

c. The Brocket Estate, near Welwyn, Hertfordshire. Specimens of R. rutilus were trapped at intervals between January and August, 1966 in a lake formed by the river Lea.

d. The Lea Navigation Canal, near Hertford. Specimens of dace, Leuciscus leuciscus (L.) were caught by a local angler in July, 1966, and kept alive at Winches Farm for further examination.

At Winches Farm, the above-mentioned fish were kept indoors in containers made of galvanized iron, or of vitreous enamel; others were kept temporarily in polythene bins. All containers were provided with electrically operated aeration pumps which ran continuously. The fish were fed twice a week on "Tetramin", a commercially prepared food. The water was changed once every two weeks under normal conditions; in the course of certain experiments, however, it was not changed at all.

In addition to the above fish, specimens of 1-year old salmon, Salmo salar L., were obtained from the Ministry of Agriculture and Fisheries. These were originally brought from a hatchery near Axminster, Devon, to a laboratory at the Ministry in London early in 1966. In June, 1966, 24 specimens were taken from this laboratory to Winches Farm, where they were kept in two outdoor ponds. Each pond was rectangular and of the same size, measuring 9 ft. long by 5 ft. wide and 3 ft. deep. They were constructed of concrete and lined with terylene netting, the latter being used for hauling out fish when required. A continuous supply of tap-water ran through each pond. The fish were fed daily on a salmon feed marketed by Astra-Hewlett Ltd.

2. Examination of Infected Material

Fish were examined for Protozoa by the techniques suggested by Markevich (1951). As most internal organs of fish are of softer texture than those of higher vertebrates, the most convenient method of examining fresh material was to prepare organ squashes. These were mounted between a coverslip and slide in distilled water or physiological saline. The organs and tissues examined included blood, liver, spleen, kidney, gall bladder, urinary bladder, alimentary canal, musculature, gonads and brain, as well as the external surface, fins and gills. Infected material was also mounted in Lugol's or Gram's iodine solution for the detection of Myxosporidan species of the family Myxobolidae.

Smears were prepared from infected organs, and dried in air, fixed in methanol and stained with Giemsa's stain. Pieces of infected tissue were fixed in 10% formol saline or in Carnoy's fluid. Sections, 5 μ

and 8μ thick, were cut and stained with Ehrlich's haematoxylin and eosin, Gram's stain, the Giemsa-colophonium technique (Shortt and Cooper, 1948), the Feulgen technique (as given by Casselman, 1959) and the P.A.S. method (McManus, 1948). The presence of glycogen in sectioned material was tested with the modified Langhans iodine method (Carleton and Drury, 1957), Best's carmine (the technique given by Casselman, 1959) and the Bauer modification of the Feulgen technique (Bensley, 1939).

Drawings were made with the aid of a camera lucida from both fresh material mounted in distilled water, and material fixed as smears or in sections. Myxosporidan spores were measured both from camera lucida drawings and with the aid of an eyepiece micrometer.

SECTION 1.

STUDIES ON MYXOSPORIDA

1. Introduction

The Myxosporida are almost exclusively parasites of lower vertebrates, particularly of fish. They are found in all organs and tissues, some species being apparently organ and host specific, while others have been reported in many sites and hosts. While the majority of species appear to be non-pathogenic, many are of undoubted economic importance. Among these may be mentioned Myxosoma cerebralis (Hofer, 1903) which attacks the cartilage and semi-circular canals of many salmonid fish, causing "twist" disease; the parasite is of particular importance in hatcheries, where outbreaks can cause fatal epizootics (Dogiel et al., 1961). Unicapsula muscularis Davis, 1924 invades the musculature of halibut, causing a condition known as "wormy" halibut (Davis, 1924). Hennegnya salminicola Ward, 1919 produces cysts in the musculature of species of Pacific salmon, causing "tapioca" disease (Fish, 1939). Myxidium oviforme Parisi, 1912 can cause inflammation of the liver in many salmonids, when present in the hepatic ducts in large numbers (Bykhovskaya-Pavlovskaya et al. 1962). Kudoa thyrsites (Gilchrist, 1924) causes liquefaction of the musculature of barracouta, the condition being known as "milky barracouta" (Willis, 1949).

(i) Life-cycle

According to the accounts of Wenyon (1926), Noble (1944), Grassé (1953), Bykhovskaya-Pavlovskaya et al. (1962) and Kudo (1966), the life-cycle of a typical myxosporidan is briefly as follows.

An infection starts when spores are swallowed by the host. Individual spores are composed of an outer shell, consisting of one or more valves,

enclosing an amoeboid sporoplasm and polar capsules containing coiled polar filaments. In the digestive tract, the polar filaments are extruded, probably serving to attach the spore to the intestinal wall, and the spore valves open to release the sporoplasm. The nuclei of the sporoplasm unite either just before or immediately after its release. The sporoplasm is then thought to penetrate the intestinal epithelium and migrate either through the blood-stream, the lymphatic system or the coelomic fluid to reach its specific organ. Here, the sporoplasm grows into a multinucleate trophozoite. Trophozoites take on various forms, according to the species and the organ parasitized. Those inhabiting cavities such as the gall-bladder or urinary bladder are described as coelozoic, and generally take the form of amoeboid plasmodia, moving by means of pseudopodia. When parasitic in organs and tissues (histozoic) they may form cysts covered by a connective tissue capsule of the host, or diffuse infiltrations into host tissue with no apparent host reaction. Within the trophozoite, a series of nuclear divisions followed by cellular differentiation results in the formation of pansporoblasts which give rise to spores. Naville (1931) and Noble (1944) give full accounts of the theories concerning nuclear changes leading to spore maturation.

If the site of infection is near the external surface, mature spores are probably shed directly into the water. In the case of species inhabiting the gall-bladder and swim-bladder, spores may pass out with the faeces: in the urinary bladder and uriniferous tubules they probably leave with the urine. In the case of species inhabiting internal organs, spores are probably liberated on the death of the host.

(ii) Systematic position

Following the recommendations of the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists (1964) the Myxosporida are given the rank of order in the subphylum Cnidospora Doflein, 1901. The Cnidospora are unique among Protozoa in their possession of spores containing a sporoplasm and polar capsules with coiled polar filaments. The structure of polar capsules is closely similar to that of coelenterate nematocysts (Lom and Vavra, 1965), a feature which has led various authors (Grasse, 1953, Grell, 1956) to raise the status of Cnidospora to Mesozoa.

In the orders Myxosporida and Actinomyxida, several cells participate in spore formation, while in the Microsporida each spore is derived from a single cell. This difference led Lom and Vavra (1962) to subdivide the Cnidospora into two classes, the Heteronucleida containing the Myxosporida and Actinomyxida, and the Isonucleida containing the Microsporida. The Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists suggest the names Myxosporidea and Microsporidea for the Heteronucleida and Isonucleida respectively. Schulman (1965) suggested removing the Microsporida from Cnidospora altogether.

(iii) Classification

The Myxosporida have been classified in a variety of ways, most systems relying entirely on features of the spore. Kudo (1919) considered that "at the present time, the charactors concerning the vegetative form do not appear to afford a better and more natural basis for the classification than those of the spore. Thus, from the taxonomic point

of view, the present situation does not seem to be much improved as compared with that at the end of the last century". The same statement can be re-iterated today. While knowledge of the life-cycles and host-specificity of the group has advanced little since 1919, the number of species described has grown enormously. For example, Kudo (1919) describes 26 species of the genus Myxidium Bütschli, 1882 and 63 of the genus Myxoholus Bütschli, 1882. At the present time (1967) the numbers have risen to 98 of Myxidium and 209 of Myxoholus. Many new species have been described from spores only. Some experimental evidence, reviewed in part 4, indicates some degree of host-specificity, but until infection experiments can be carried out, any system of classification based solely on spore morphology will be unsatisfactory. Further, Kudo (1921b) points out that spore dimensions vary according to the conditions under which they are measured, fixed or fresh. Thus even spore morphology may not be a reliable guide to identification if the conditions under which measurements are taken are not specified.

Most authors (e.g. Awerinzew, 1907, Davis, 1918, Kudo, 1919, Tripathi, 1948, Lom, 1961) have acknowledged the disadvantages of systems of classification based only on spore features. Thélohan (1892) was the first to attempt such a classification, and his system was expanded by Gurley (1893) and Labbé (1899). Doflein (1899), however, attempted to use features of the trophozoite. He divided the group into two "legions", the Disporea and the Polysporea, according to the number of spores formed by each trophozoite. Auerbach (1910) followed Doflein's

system and later (1910a, 1911) subdivided the group further by adding two new legions, the Monosporea and the Miktosporea. Davis (1918) and Kudo (1919), however, pointed out that the number of spores formed by each trophozoite varied even within one species, and thus rejected this feature as a valid taxonomic criterion.

Davis (1918) used the spore form in his division of Myxosporida into two sub-orders, the Cystosporea in which spores were laterally compressed, and the Myxosporea in which spores were not laterally compressed. He also considered that trophozoites of the Myxosporea were, with few exceptions, free-living in the body-cavity while the Cystosporea were mainly tissue parasites. Kudo (1919) considered that this was not a reliable feature on which to base a classification and replaced the Myxosporea and Cystosporea with three new sub-orders, the Eurysporea, Sphaerosporea and Platysporea, all being distinguished by spore features alone.

The system of Kudo (1919) has provided a basis for all systems of classification since that date. These include proposals by Kudo (1933), Tripathi (1948), Schulman (1959), Weglitsch (1960) and Kudo (1966). The sub-orders Unipolarina and Bipolarina, named by Tripathi (1948), have been adopted by the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists (1964).

(iv) Scope of the present work

The work done during the present investigations is described in parts 2, 3, 4 and 5.

In part 2, Myxosporida found in fish collected in the Amazon region

of Brazil, during the Guy's Hospital Expedition to the Lower Amazon, and in fish collected in the London area are described. A species of the genus Myxidium from Irish salmon is also described.

In part 3, a study is made of the two genera Myxobolus Bütschli, 1882 and Myxosoma Thélohan, 1892. A review of work by previous authors is followed by an account of histochemical tests relating to the distinction between these genera.

In part 4, attempted experimental infections of uninfected roach and salmon are described. This account is preceded by a review of work by previous authors.

In part 5, check-lists are given of the genera Myxobolus and Myxidium Bütschli, 1882, together with a list of recently described species of Myxosoma.

Unless stated otherwise, information on species described before 1919 has been taken from Kudo's monograph (1919), and information on species from freshwater fish in the U.S.S.R. is that given by Bykhovskaya - Pavlovskaya et al. (1962).

2. Descriptions of species

(1) Myxosporida of Brazilian fish.

a. Myxobolus sp. 1 from Serrasalmus rhombeus (L.) collected in the Rio Negro.

Five specimens of Serrasalmus rhombeus (family Serrasalmidae) from the Rio Negro were examined for Myxosporida. Giemsa - stained smears revealed spores in the liver, spleen and kidney of each fish. All spores possessed two valves, with two polar capsules lying in the sutural place and opening to the anterior. In some, a round vacuole could be seen in the sporoplasm. In sectioned preparations stained with Best's carmine, the vacuole appeared bright red. For these reasons, the spores could be allocated either to the genus Myxobolus Butschli, 1882 (sub-order Unipolarina, super - family Myxoboloidea, family Myxobolidae, according to Tripathi's classification, 1948) or to the genus Myxosoma Thélohan, 1892 (sub-order Unipolarina, super - family Ceratomyxoidea family Ceratomyxidae, sub - family Myxosomatinae), these two genera differing only by the presence of a glycogenous vacuole in Myxobolus spores and the absence of such a vacuole in Myxosoma spores. As there is evidence, to be reviewed in part 3, that Myxosoma should be synonymised with Myxobolus, the species is here allocated to the genus Myxobolus.

Spores of two distinctly different shapes and sizes were seen in smears, and it was considered at first that two species might be present. Sectioned preparations, however, revealed the presence of both spore types in the same cyst (Fig. 3). T- tests were used to compare the dimensions

of each type of spore from one fish with those from the other fishes, and also to compare spores from one organ with those from other organs. No significant differences were found, and it was therefore concluded that each organ of each fish was infected with one and the same species.

Spores; In Giemsa's stain, the sporoplasm and valves appeared light blue, while the polar capsules stained deep red; this staining reaction was characteristic for spores of all myxosporidan species examined in the present study. Spores were of two distinct types, which are described here as macro- and micro - spores.

Macrospores (Fig. 2b) :- Spores symmetrically oval in front view with two polar capsules opening at the anterior end. Polar capsules equal in size and lying in the sutural plane. Spore valves thin. Sutural line distinct in sectioned preparations.

In sections stained with Ehrlich's haematoxylin and eosin, two nuclei were visible in the sporoplasm, and, in immature spores, the two capsulogenous nuclei were visible (Fig. 2c). In sections stained with Best's carmine, the sporoplasm of some spores took up a red coloration while a few others showed a bright red vacuole. The majority of spores, however, remained unstained. Spores were measured from camera - lucida drawings of methanol - fixed and Giemsa - stained smears. Measurements, in μ, were as follows (69 spores measured); -

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	14·84	8·61	7·71	3·16
Range	12·50 - 18·00	7·00 - 10·00	6·00 - 9·00	2.50 - 4·00

Microspores :- (Fig. 2a) Spores pyriform in front view with a pointed anterior end. Two polar capsules of equal size lying in the sutural plane and opening to the anterior. Spore valves thin. Sutural line distinct in sectioned preparations.

In some spores, the polar capsules extended for most of the length of the spore, the sporoplasm being small. Two nuclei were seen in the sporoplasm in sectioned preparations stained with Ehrlich's haematoxylin and eosin, (Fig: 2d). In Best's carmine, the majority of spores remained unstained, although a red coloration was taken up by the sporoplasm in a few cases. Spore measurements, in μ, taken from camera - lucida drawings of methanol-fixed and Giemsa - stained smears were as follows (53 spores measured):-

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	8·32	4·08	5·82	1·58
Range	7·00 - 9·50	3·50 - 5·00	5·00 - 7·50	1·00 - 2·00

Trophozoites ; - These were studied in sections of spleen and kidney, infections being particularly heavy in these organs. In all cases, both macro- and micro - spores were found in the same cyst.

In the spleen, trophozoites were surrounded by a sheath of pigmented fibrous material (Figs: 3, 4a,b,c, 5a,c,) forming a cyst wall. Cysts measured up to 100 μ in diameter. All cysts contained dense patches of a dark brown pigmented material; similar pigment was also seen to be scattered throughout the spleen, but less densely.

Two types of cyst could be distinguished in the spleen. One type, presumed to be in an advanced state of development, contained few inclusions other than mature spores. In the second (Fig: 5c,d.), developing pansporoblasts were seen as groups of nuclei; immature spores were often associated in pairs (Fig: 5e.), indicating that each pansporoblast produced two spores (disporous). In a third type of cyst (Fig: 4a,b,c.), schizont - like inclusions of varying size were found, containing from a few to several hundred small nucleated bodies. In the fourth (Fig: 5a,b) the contents were mainly regions of non-nucleated tissue, appearing grey in Giemsa - stained sections; a similar type of material was found to occur in most of the myxosporida examined during the present study.

In the kidney, trophozoites were of similar appearance to those in the spleen, but lacked the fibrous cyst wall. In all sections examined they were closely associated with tubules (Fig:6); macro- and micro - spores were normally found with lumps of pigment and the non - nucleated material common in trophozoites in the spleen.

Identification

As mentioned above, the species described here can be allocated either to the genus Myxobolus or to the genus Myxosoma. For this reason, comparisons are made in this section with previously described species of both genera.

Eleven species of Myxobolus have been described from South America,

and these are listed in Table 1. No species of Myxosoma has been recorded, although an iodinophilous vacuole is neither described in the text, nor illustrated in spore diagrams of Myxobolus associatus Nemeczek, 1926, M. chondrophilus Nemeczek, 1926, M. cunhai Penido, 1927, M. inaequalis Gurley, 1893 or M. pyrocentris Penido, 1927.

The most striking feature of the present material is the occurrence of macro- and micro - spores in the same cyst. The possibility of a double infection cannot be ruled out, as trophozoites of two species in the same site may have developed beside each other and become surrounded by a common cyst wall. This is considered unlikely, however, as the two spore types were invariably found together; in no cysts were either macro- or micro - spores found alone.

None of the species listed in table 1 have both macro- and micro - spores. The spores of M. associatus have a variable shape, being either rounded or pointed at the anterior end, but have similar dimensions. The spores of M. pyrocentris and M. cunhai correspond fairly closely, on dimensional grounds, to the macro- and micro - spores respectively of the present material. It is also noteworthy that these two species were described from spores only, from the intestinal contents of the same host - the piranha Serrasalmus piraya Cuvier. As it is possible that spores of histozoic myxosporida may leave their host via the alimentary canal, M. pyrocentris and M. cunhai may represent spores of the species described here. If so, the species could be identified as M. cunhai, as this was described before M. pyrocentris in Penido's paper (1927). M. pyrocentris would then be synonymised with M. cunhai.

Until the trophozoites of M. cunhai and M. pygocentris are described, however, this identification must remain provisional.

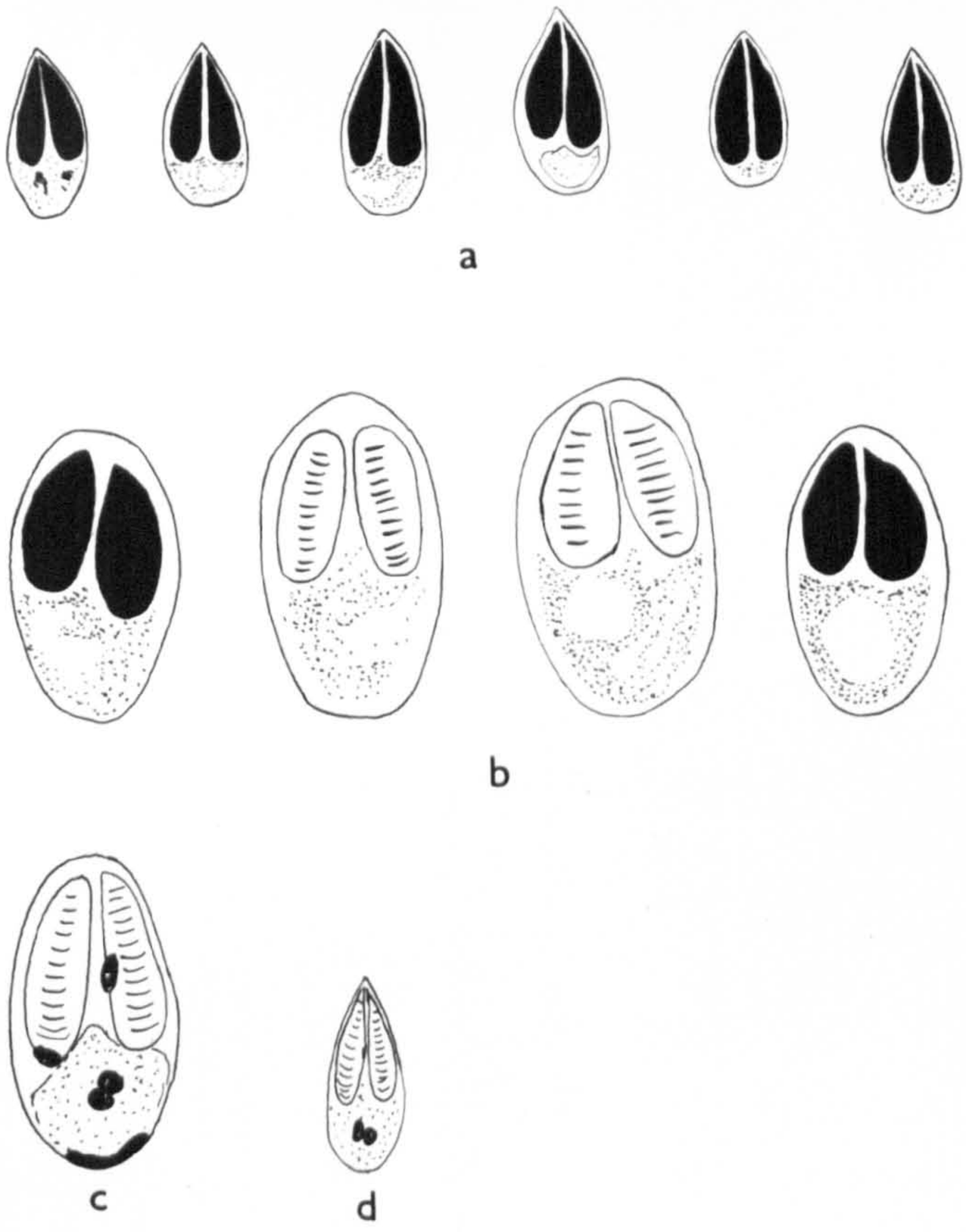
Among species of Myxobolus and Myxosoma from other parts of the world, a few exhibit spore variability. Lom (1961) described a species of Myxosoma with two forms of spores, large and small. In Myxosoma sphaerica (Fujita, 1924), M. hudsonis Bond, 1938 and Myxobolus sp. Wegener, 1910, spores of two different shapes are found, while in Myxobolus cycloides Gurley, 1893 and Myxosoma heterospora Baker, 1963 three types of spore occur. In cysts of Myxobolus mutabilis Kudo, 1934 there is "great variation among spores in one and the same cyst" (Kudo, 1934). According to Bykhovskaya - Pavlovskaya et al. (1962), spores of Myxobolus muelleri Bütschli, 1882 exhibit considerable variability. In Myxobolus hungaricus Jaczo, 1940 and M. variabilis Jaczo, 1940 two types of spore, tailed and untailed, are illustrated. In none of these species, however, are spore dimensions and features comparable with those of the present material.

If the macro- and micro - spores of the species described here represent two distinct species occupying the same site, there are several existing species of Myxobolus and Myxosoma which may be considered for comparison. In the case of the microspores, only Myxobolus cunhai has similar spore characteristics,. In the case of the macrospores, Myxobolus noguchii Pinto, 1928, M. carassii Klokačeva, 1914, M. cyprini Doflein, 1898, M. ellipsoides Thélohan, 1892, M. lussi Akhmerov, 1960, M. ovatus Kudo, 1934 and Myxosoma commersonii Fantham, Porter and Richardson, 1939 all have similar spores.

The only species in this list described from South America is Myxobolus noguchii. Reference to table 1 shows that the spores of this species were described from (?) the gills of the piranha Serrasalmus spilopleura Fner. in Brazil; trophozoites were not described. Spore dimensions compare rather more favourably with the present material than do those of M. pygocentris, discussed above, although the conditions under which spores of M. noguchii were measured were not stated by Pinto.

Of the remaining six species, spores of Myxobolus carassii and M. ovatus have folds or thickening on the valves, and can thus be eliminated from further comparison. Trophozoites of Myxobolus lussi form white cysts on the gills of fish of the family Cyprinidae in the U.S.S.R., and those of Myxosoma commersonii form cysts on the skin of Catostomus commersoni (Lacépède) (family Catostomidae) in Canada; cysts of this type were not seen in the present material. Myxobolus ellipsoides and M. cyprini are both common in cyprinid fishes in Europe and Asia, and occur in several organs, including liver, spleen and kidney, as diffuse infiltrations. Of these two species, M. ellipsoides is the less similar to the present material, as its polar capsules usually extend for less than half of the spore length.

In summary, therefore, it can be seen that the species described here as Myxobolus sp. 1 cannot be identified with certainty as a species described previously. The similarity of the macro- and micro - spores respectively to Myxobolus pygocentris and M. cunhai suggests that it may be M. cunhai, but this cannot be confirmed until trophozoites of M. pygocentris and M. cunhai are found. The macrospores are similar to those of several other species, notably Myxobolus noguchii, M. cyprini and M. ellipsoides.



F I G U R E 2.

Myxobolus sp. 1 from Serrasalmus rhombeus

a. Microspores (Giemsa's stain)

b. Macrospores (Giemsa's stain)

c. Macrospore showing sporoplasm and capsulogenous nuclei (Ehrlich's haematoxylin and eosin stain)

d. Microspore showing sporoplasm nuclei (Ehrlich's haematoxylin and eosin stain).

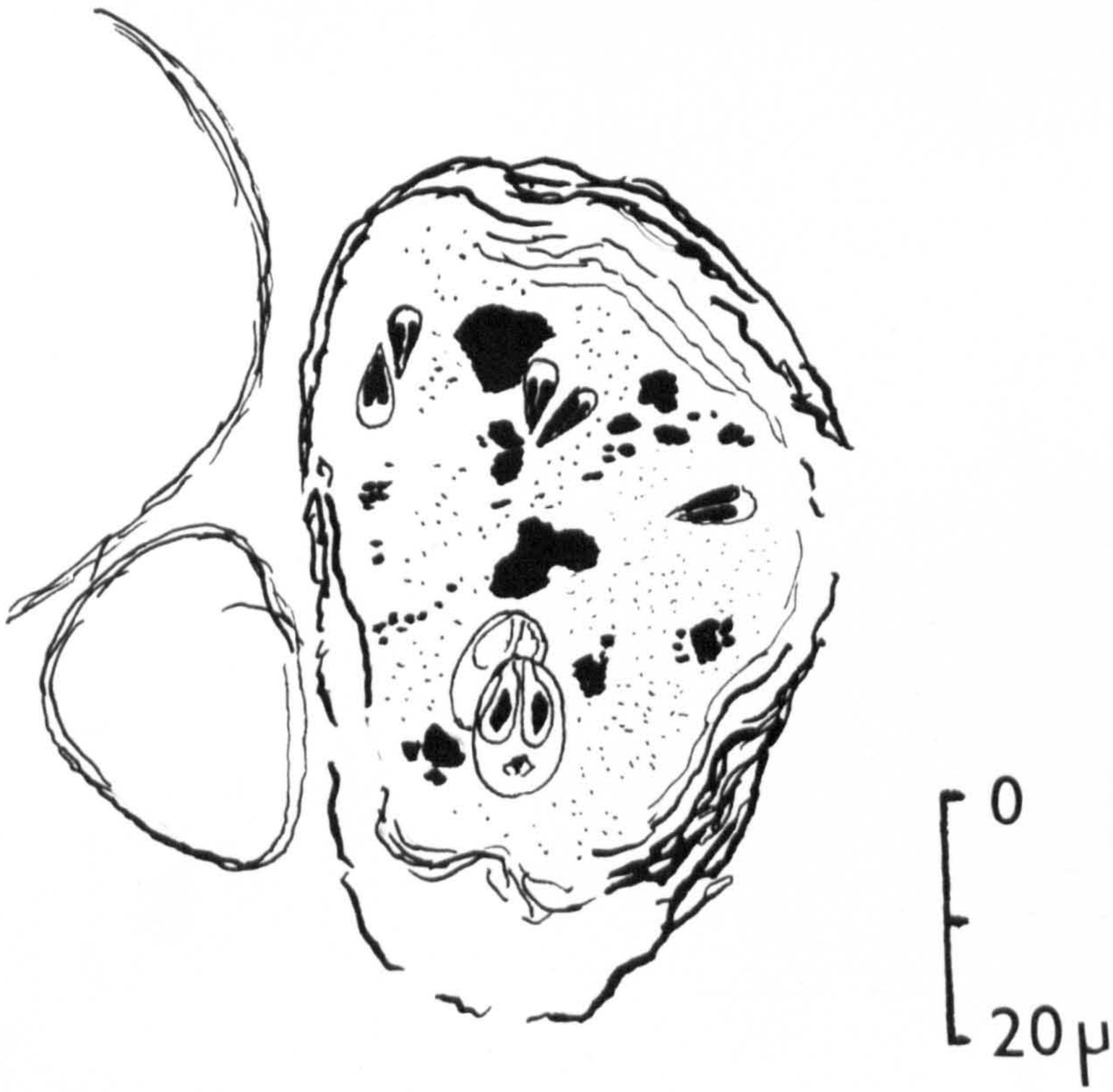
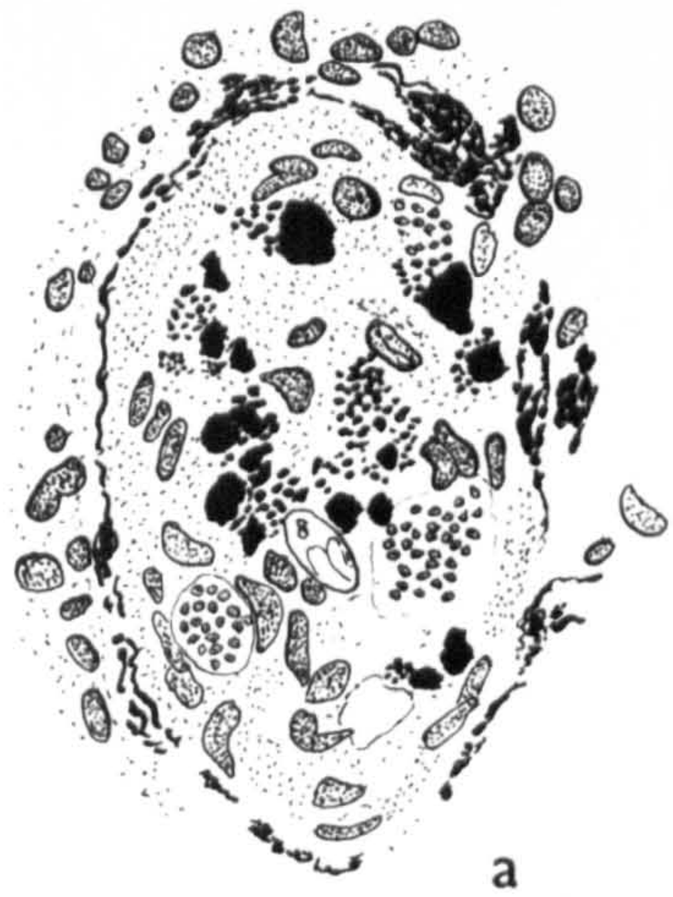


FIGURE 3.

Myxobolus sp. 1 from Serrasalmus rhombeus.

Section of trophozoite in spleen, showing fibrous 'cyst wall', and microspores and macrospores within the same cyst.



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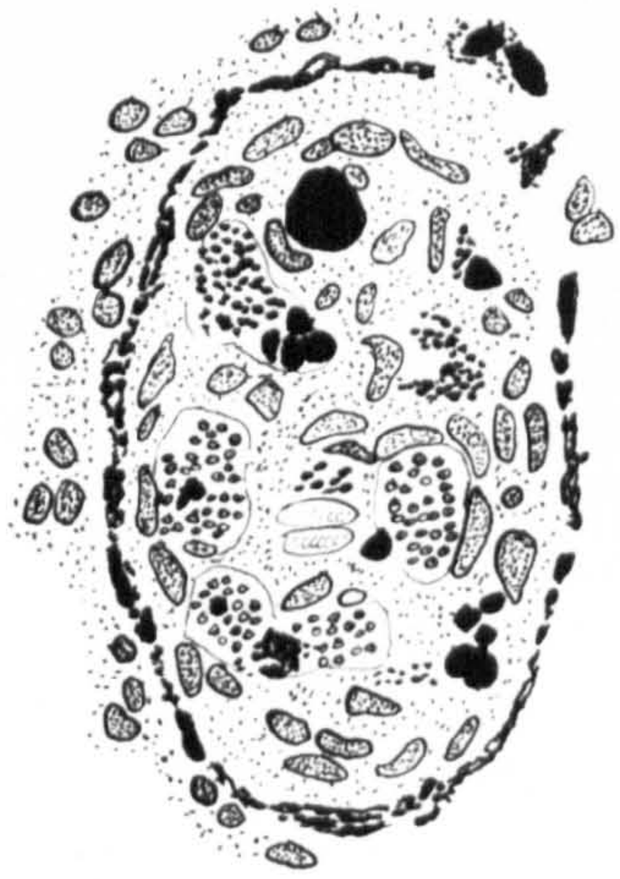

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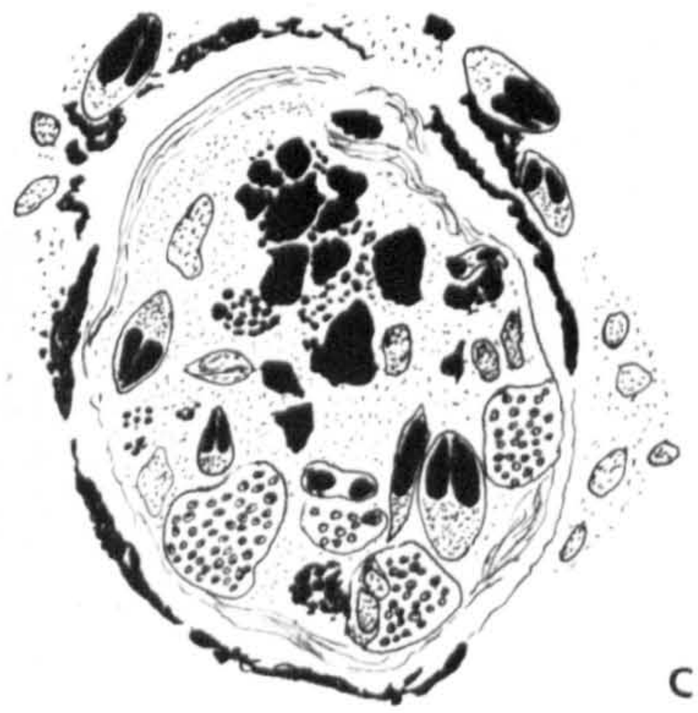

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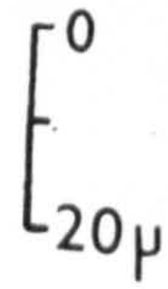


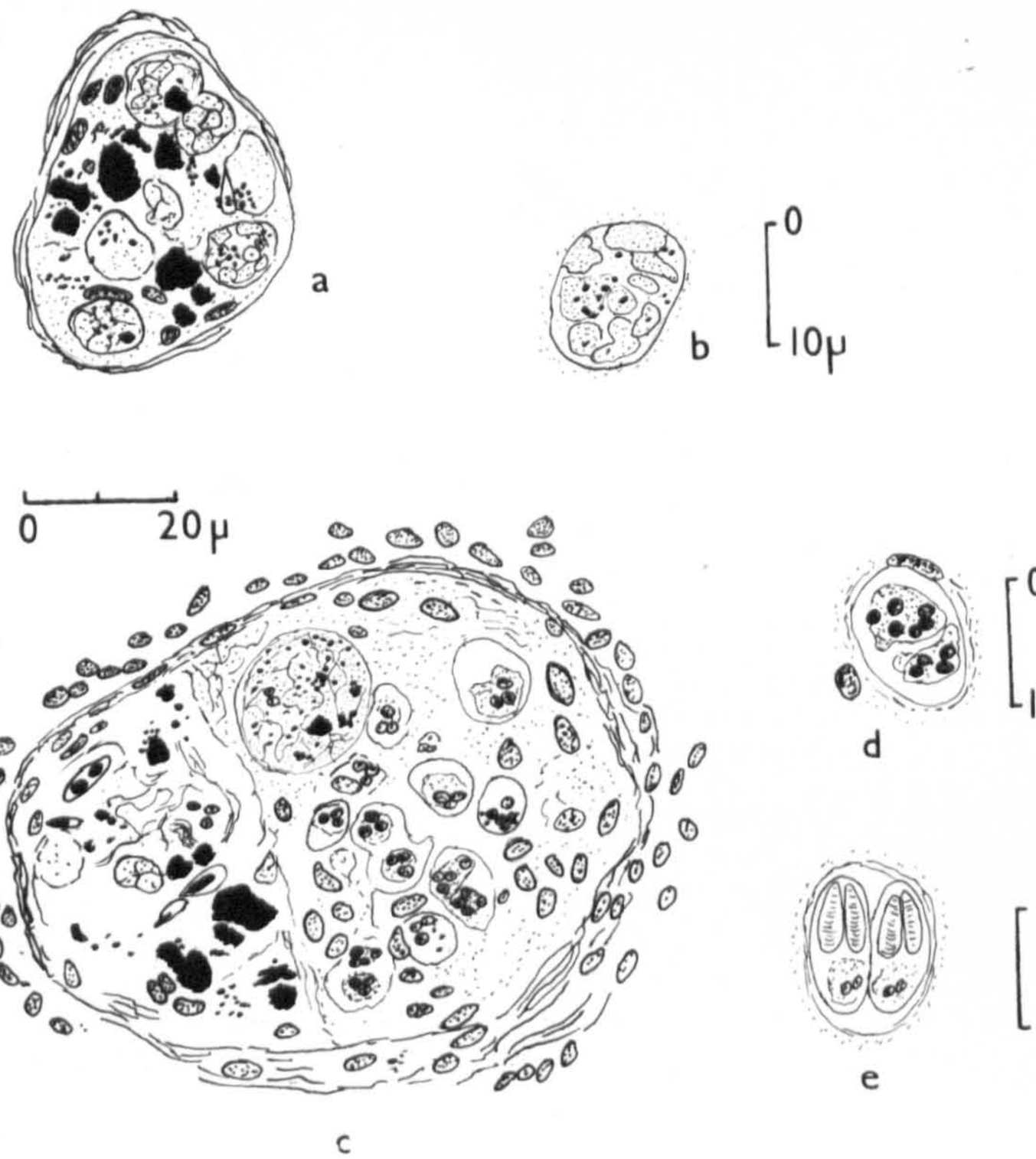
F I G U R E 4.

Myxobolus sp. 1 from Serrasalmus rhombeus.

Sections of trophozoites in the spleen, showing schizont - like inclusions.

a, b. Ehrlich's haematoxylin and eosin stain.

c. Giemsa's stain.



F I G U R E 5.

Myxobolus sp. 1 from Serrasalmus rhombeus.

Sections of trophozoites in spleen. Giemsa's stain.

a. Cyst enclosing regions of non - nucleated tissue and lumps of pigment.

b. Cyst inclusion showing non - nucleated tissue with scattered pigment.

c. Cyst showing developing pansporoblasts.

d. Pansporoblast.

e. Immature spores.

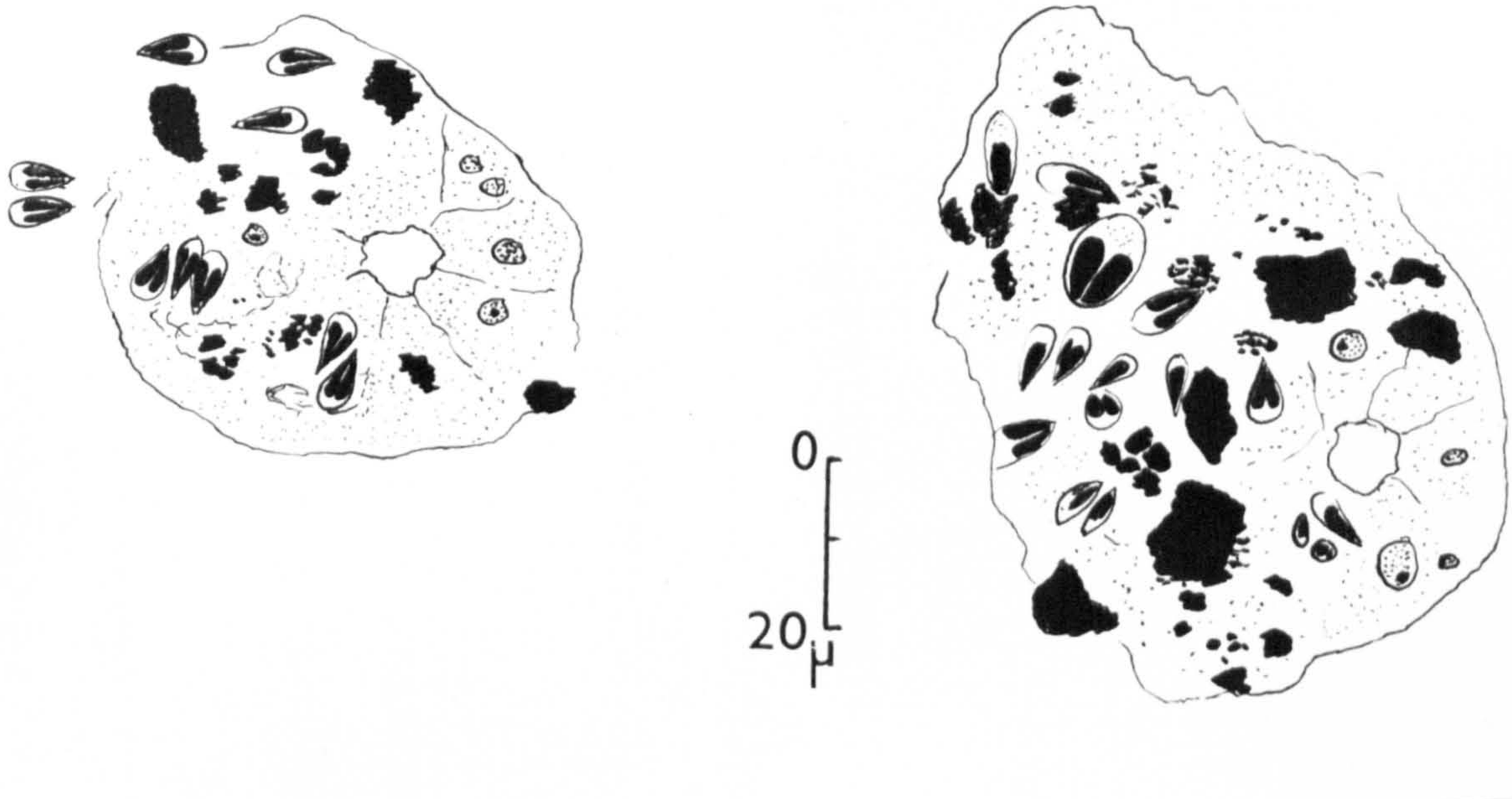


FIGURE 6.

Myxobolus sp. 1 from Serrasalmus rhombeus.

Trophozoites in kidney. Giemsa's stain.

T A B L E 1.

Species of Myxobolus described from South America

Species	Author	Host	Site of infection	Locality
M. associatus	Nemeczek, 1926	Leporinus mormyrops	Kidney	Minas Gerais, Brazil
M. chondrophilus	Nemeczek, 1926	Sardinella enchovia	Gills	Rio de Janeiro, Brazil
M. cunhai	Penido, 1927	Serrasalmus piraya Pimelodus clarias	Intestinal contents Cloaca	Mato Grosso, Brazil
M. galaxii	Szidat, 1953	Galaxias maculatus	Musculature, kidney, "other organs"	Argentina
M. inaequalis	Gurley, 1893	Pimelodus clarias * Synodontis schall	Skin of head	Guiana, Surinam
M. kudoi	Guimaraes and Bergamin, 1938	Nematognatha (catfish)	Sub - dermal	Sao Paulo, Brazil
M. lutzi	Aragao, 1919 (see Pinto, 1928a)	Poecilia vivipara	Testis	Rio de Janeiro, Brazil
M. magellanicus	Szidat, 1953	Galaxias maculatus	Gills	Argentina
M. noguchii	Pinto, 1928	Serrasalmus spilopleura	(?) Gills	Sao Paulo, Brazil
M. pygocentris	Penido, 1927	Serrasalmus piraya	Intestinal contents	Mato Grosso, Brazil
M. stokesi	Pinto, 1928	Pimelodus sp.	Sub - cutaneous	Sao Paulo, Brazil

* According to the British Museum (Natural History), Synodontis schall occurs only in Africa.

TABLE 1. (Continued)

Species	Spore measurements (in μ)						Trophozoite
	Length	Breadth	Thickness	Polar capsule length	Polar capsule breadth	Condition of spores when measured	
M. associatus	15	10	-	7	-	Not stated	Cyst, 80 x 60 - 70 μ
M. chondrophilus	6	4.5	3.5	3	-	Absolute alcohol	Spherical white cysts, 125μ-1 mm
M. cunhai	9 - 11	4 - 6	-	-	-	Not stated	Not described
M. galaxii	13.7 - 15	8.8 - 10	-	-	-	Not stated	White cysts
M. inaequalis	5.2	3.3	-	-	-	Not stated	Small pustules
M. kudoi	6.5 - 8.9	-	-	3.5 - 4.2	1.3 - 2.0	Not stated	Cysts, 0.5 - 1 mm
M. lutzi	10	7	-	-	-	Not stated	Large areas of testis occupied
M. magellanicus	10 - 13	8.1 - 8.8	-	3	-	Not stated	White cysts, up to 0.6mm.
M. noguchii	13.6	8.5	-	6.8	2.2	Not stated	Not described
M. pygocentris	15 - 16	9 - 11	-	9 - 11	3 - 4	Not stated	Not described
M. stokesi	8.5	5.3	-	3.4	1.7	10% formalin	Tumour, 1 mm. diameter

b. Myxobolus sp. 2 from Serrasalmus sp., collected in the Rio Prêto.

One specimen of an unidentified species of piranha, Serrasalmus sp., collected in the Rio Prêto, was examined for Myxosporida. Giemsa - stained smears revealed spores in the kidney. For the reasons given under Myxobolus sp. 1, the spores could be allocated either to the genus Myxobolus or to the genus Myxosoma. Spores were drawn and measured from the smears, and trophozoites were examined in sectioned preparations.

Spores (Fig. 7a); - Ovoid in front view with a bluntly pointed anterior end. Two polar capsules of equal size lying in the sutural plane and opening to the anterior. Two nuclei visible in the sporoplasm in sections stained with Ehrlich's haematoxylin and eosin (Fig. 7b). No vacuole visible in the sporoplasm. Spore measurements (in μ) were as follows (20 spores measured):-

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	10.25	5.53	5.60	1.78
Range	9.00-11.00	5.00 - 6.50	5.00 - 6.00	1.50 - 2.00

Trophozoites (Fig. 7 c,d): - Trophozoites were found in sections of kidney, but not in the liver or spleen. In appearance, they were similar to those of Myxobolus sp. 1 from the kidney of S. rhombeus. In all sections, trophozoites were associated with tubules, spores being found both between tubule epithelial cells and occasionally in the tubule lumen. No cyst wall or fibrous tissue surrounded the trophozoites. Apart from the presence of spores, little structural detail could be seen

other than lumps of brown pigment and regions of the non - nucleated material common in Myxobolus sp.1.

Identification; While the trophozoites of this species are similar to those of Myxobolus sp.1, only one type of spore was found, the dimensions of which differed from both the macro - and micro - spores of Myxobolus sp.1.

Of the South American species of Myxobolus listed in table 1, the spores of M. cunhai and M. lutzi are comparable to those of the present material. M. cunhai differs slightly in the length of the polar capsules, which, in Penido's illustrations (1927), appear to extend for most of the length of the spore. M. lutzi has almost identical dimensions to those of the present species; measurements of the polar capsules were not given by Arogao, according to Pinto (1928a), but in his illustrations they appear to extend approximately half the spore length. M. lutzi was described from the testis of its host Poecilia vivipara Bloch and Schneider (family Cyprinodontidae).

Among species of Myxobolus from other parts of the world, three have spores with comparable dimensions. These are M. exiguus Thélohan, 1895, M. koi Kudo, 1919 (Nakai's description, 1926) and M. macrocapsularis Reuss, 1906. Of these, M. exiguus can be excluded from further comparison as its spores are characterised by a series of folds in the shell. The spores of M. koi are very similar to those of the present species (Measurements; 10 - 13 μ long by 6-7 μ broad; polar capsules 5-7 μ by 2-2.5 μ), but the

trophozoites were described by Nakai (1926) as cysts on the gills of Cyprinus carpio L. in Japan. M. macrocapsularis, found as cysts in various organs, including the kidney, of several cyprinid fishes, possesses spores having a slightly greater range of size; the spore breadth (6.00 - 9.00 μ) and polar capsule breadth (2.40 - 3.60 μ) differ slightly from those of the present material.

Among existing species of Myxosoma, only M. dujardini Thélohan, 1892 has spores of comparable shape and size, although the spore length (11.00 - 13.00 μ) is slightly greater than in the species described here. The trophozoites of M. dujardini form cysts on the gills of many species of freshwater fish.

In summary, therefore, Myxobolus sp.2 resembles Myxobolus lutzi most closely. As,however,M. lutzi was described only from the testis of a host belonging to a different family, this identification remains a tentative one.

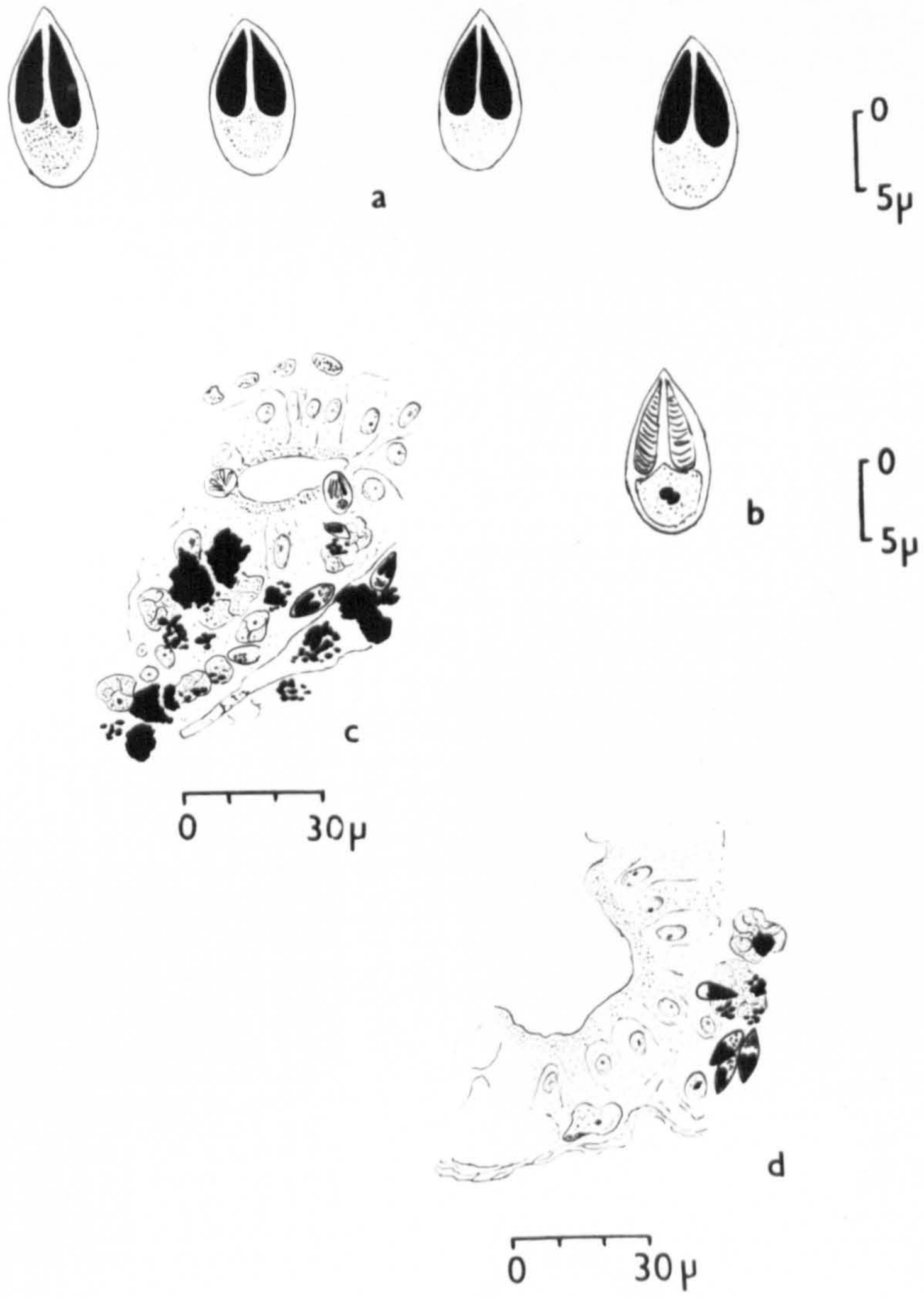


FIGURE 7.

Myxobolus sp. 2 from Serrasalmus sp.

a. Spores. Giemsa's stain.

b. Spore. Ehrlich's haematoxylin and eosin stain.

c, d. Trophozoites in kidney. Giemsa's stain.

c. Myxobolus sp. 3 from Colossoma bidens collected in the Rio Solimoes (Amazon).

An adult specimen of tambaqui, Colossoma bidens (family Characidae), a common food fish of the Amazon region, was caught in the Rio Solimoes and examined for Myxosporida. Giemsa - stained smears of liver and spleen revealed spores in both organs. For the reasons given under Myxobolus sp. 1, these could be allocated either to the genus Myxobolus or to the genus Myxosoma. Spores were drawn and measured from smears, and trophozoites from sectioned preparations.

Spores (Fig: 8a): Variable in shape, ranging from symmetrically oval to ovoid with a pointed anterior end. Two equal-sized polar capsules lying in the sutural plane and opening to the anterior. A round vacuole visible in the sporoplasm of many spores. Measurements (in μ), from camera-lucida drawings, were as follows: -

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	9.16	4.75	4.27	1.57
Range	8.00-10.00	4.00-7.00	3.50-5.00	1.00-2.50

Trophozoites (Fig: 8b): Examination of liver and spleen sections failed to reveal spores or trophozoites in the substance of either organ. Sections of connective tissue adjacent to the spleen, however, showed trophozoites lying within fibrous tissue. In all sections, the trophozoites were elongated bodies, measuring up to 120 μ in length by 60 μ in breadth, and lay along the longitudinal axis of the fibres.

No cyst wall was visible. All trophozoites examined appeared to be

mature, as few inclusions other than mature spores were visible. Small amounts of pigmented material, similar to that noted in Myxobolus spp. 1 and 2, and nuclei were seen in a few trophozoites.

Identification: The spores of this species resemble those of Myxobolus sp. 2, although their shape is more variable and they are slightly smaller. A further difference is the site of infection: trophozoites of Myxobolus sp. 2 were found in the kidney only.

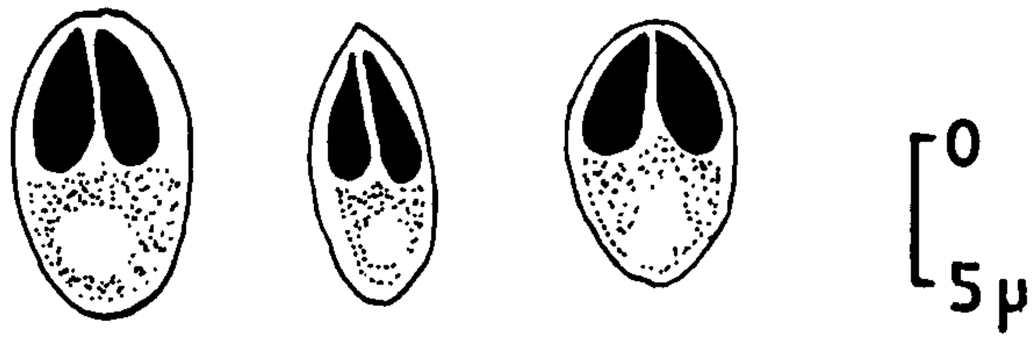
Among the South American species of Myxobolus listed in table 1, M. lutzi and M. stokesi Pinto, 1928 have spores with dimensions comparable to the present material. The spores of M. lutzi, however, compare only to the largest spores examined in the present species, and trophozoites were described only from the testis of Poecilia vivipara. M. stokesi has slightly smaller spores than those of the present species; the trophozoites form a sub-cutaneous tumour in a species of Pimelodus (family Pimelodidae).

Among species of Myxobolus from other countries, M. rhinichthidis Fantham, Porter and Richardson, 1939, M. percae Fantham, Porter and Richardson, 1939 and M. destruens Schuurmans-Stekhoven, 1920 have similar spore dimensions. Of these, spores of M. rhinichthidis have distinct polar capsule ducts and occasional striations on the valves, features not shared by the present material. M. percae spores correspond almost exactly to the present species, with the exception of a thickened sutural ridge. M. percae forms small cysts at the base of the pectoral fins of Perca flavescens (Mitchill) (family Percidae) in Canada. M. destruens has slightly longer spores (9.1 - 12.0 μ) than the present species. The

trophozoites form cysts in the body musculature of Scardinius erythrophthalmus L. (family Cyprinidae) in Holland.

No previously described species of Myxosoma have spores with features and dimensions comparable to those of the present material.

Myxobolus sp. 3, therefore, compares more favourably with Myxobolus lutzi and M. stokesi than with other South American species of Myxobolus. It also compares well with Myxobolus percae and M. lestrigens.



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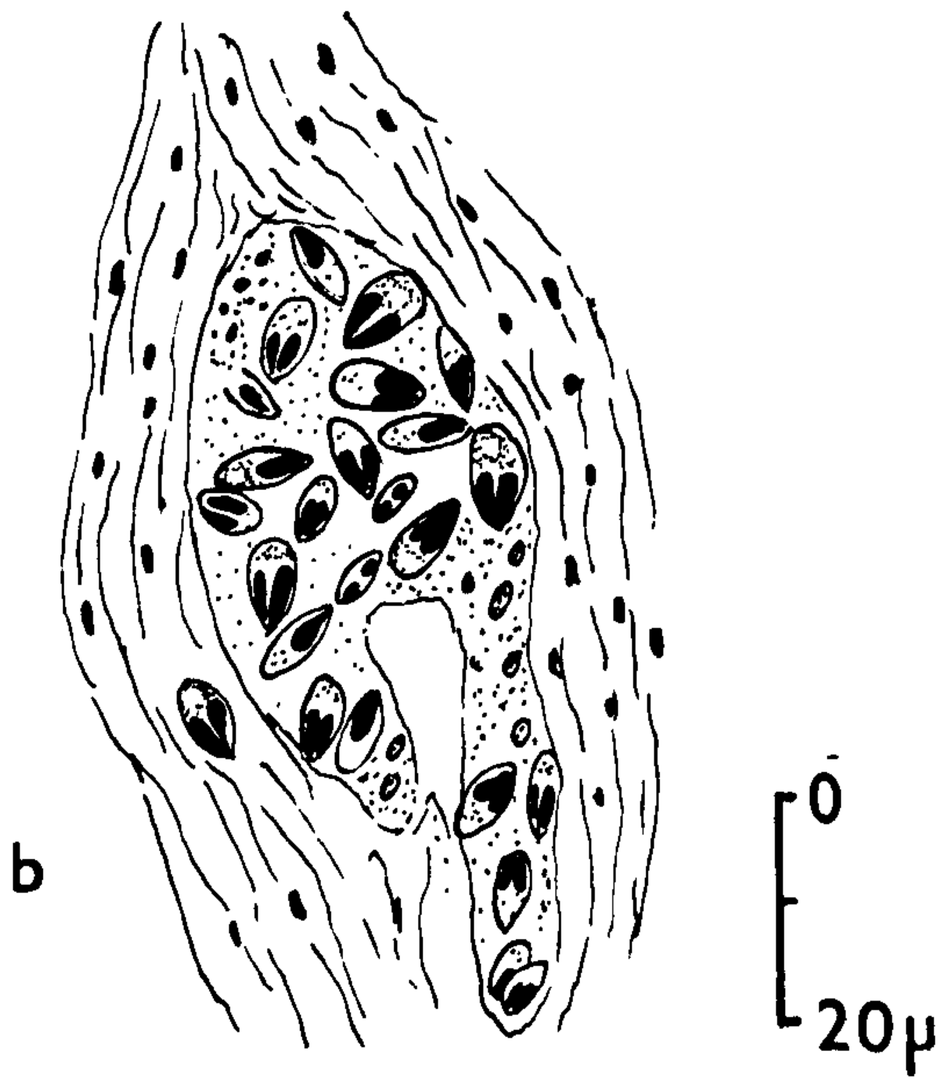


b

F I G U R E 8.

Lyrobolus sp. 3 from Colossoma bidens.

a. Spores. Giemsa's stain.

b. Trophozoite in fibrous connective tissue. Giemsa's stain.

d. Agarella gracilis Dunkerly, 1915 from Lepidosiren paradoxa collected in Belém

Specimens of lungfish, Lepidosiren paradoxa, were netted during September, 1964 in a small brook in the city of Belem. They were then brought to this country alive and kept in aquaria at Guy's Hospital in London. In July, 1965, one specimen died, and portions of liver, gall-bladder, kidney and testis were examined for Myxosporida. Giemsa - stained smears revealed spores in the kidney and testis which, for the reasons given below, could be identified as Agarella gracilis. Spores were drawn and measured from smears, and trophozoites from sectioned preparations.

Spores (Fig: 9a): Elongated shape with two valves extended posteriorly in the form of a tail. Anterior end pointed. Four polar capsules, one pair usually longer than the other, opening to the anterior. Polar capsules usually unstained in Giemsa - stained preparations, although appearing deep-red in a few spores. Suture line thin. Capsulogenous nuclei and sporoplasm nuclei visible in Giemsa - stained sections. No vacuole in the sporoplasm. In sections stained with Best's carmine, small red globules visible in some spores. Measurements in μ, were as follows (25 spores measured): -

	Total spore length including tail	Length of spore cavity	Breadth of spore cavity	Thickness of spore
Average	31.41	14.94	4.72	4.12
Range	28.00-35.00	13.25-16.50	4.00-5.50	3.75-4.50

	Length of larger polar capsules	Length of smaller polar capsules	Polar capsule breadth
Average	6.36	4.88	1.25
Range	5.00-7.75	4.50-5.50	1.00-1.50

Trophozoites; Sections of testis and kidney, which lie adjacent to each other in Lepidosiren paradoxa, showed trophozoites to be concentrated in the testis, while spores were present in both organs. Trophozoites lay between seminiferous tubules, causing some damage to the organ where present in large numbers. Three types of trophozoite could be distinguished. In one type (Fig: 9b), lumps of dark brown pigment were embedded in regions of non-nucleated grey material, similar to that noted in Myxobolus sp. 1; outlines of developing spores could also be distinguished. In the second (Fig: 9c), spore outlines were clearer, although mature spores with fully developed polar capsules were not seen. In the third type of trophozoite (Fig: 9d) which was presumably mature, clusters of mature spores were present; up to 20 spores were visible in each cluster.

No trophozoites or spores were detected in the sections of liver and gall-bladder examined.

Systematic position; Dunkerly (1915) placed Agarella gracilis in the family Chloromyxidae Thelohan, 1892 (sub-order Unipolarina, superfamily Ceratomyxoidea according to Tripathi's classification, 1948), a family distinguished chiefly by the presence of four polar capsules opening at the anterior end of the spore. Kudo (1933) placed it in the family Myxosomatidae Poche, 1913, because of its lack of an

iodinophilous vacuole. This position was also suggested by Schulman (1959). Tripathi (1948) and Weglitsch (1960) replaced it in the Chloromyxidae, and this classification was recently adopted by Kudo (1966).

Discussion: Lepidosiren paradoxa has a wide distribution in swampy regions of South America, ranging from Paraguay in the south to the Amazon region in the north. A. gracilis was first described by Dunkerly (1915) from specimens collected in rivers of Paraguay. Pinto (1928a) reported its presence in L. paradoxa of the Amazon region. The presence of the parasite in Belém indicates that it probably occurs throughout the range of the host. Although populations of L. paradoxa tend to remain localised, spores of A. gracilis are probably carried great distances by water, as their floating ability is enhanced by the presence of a tail.

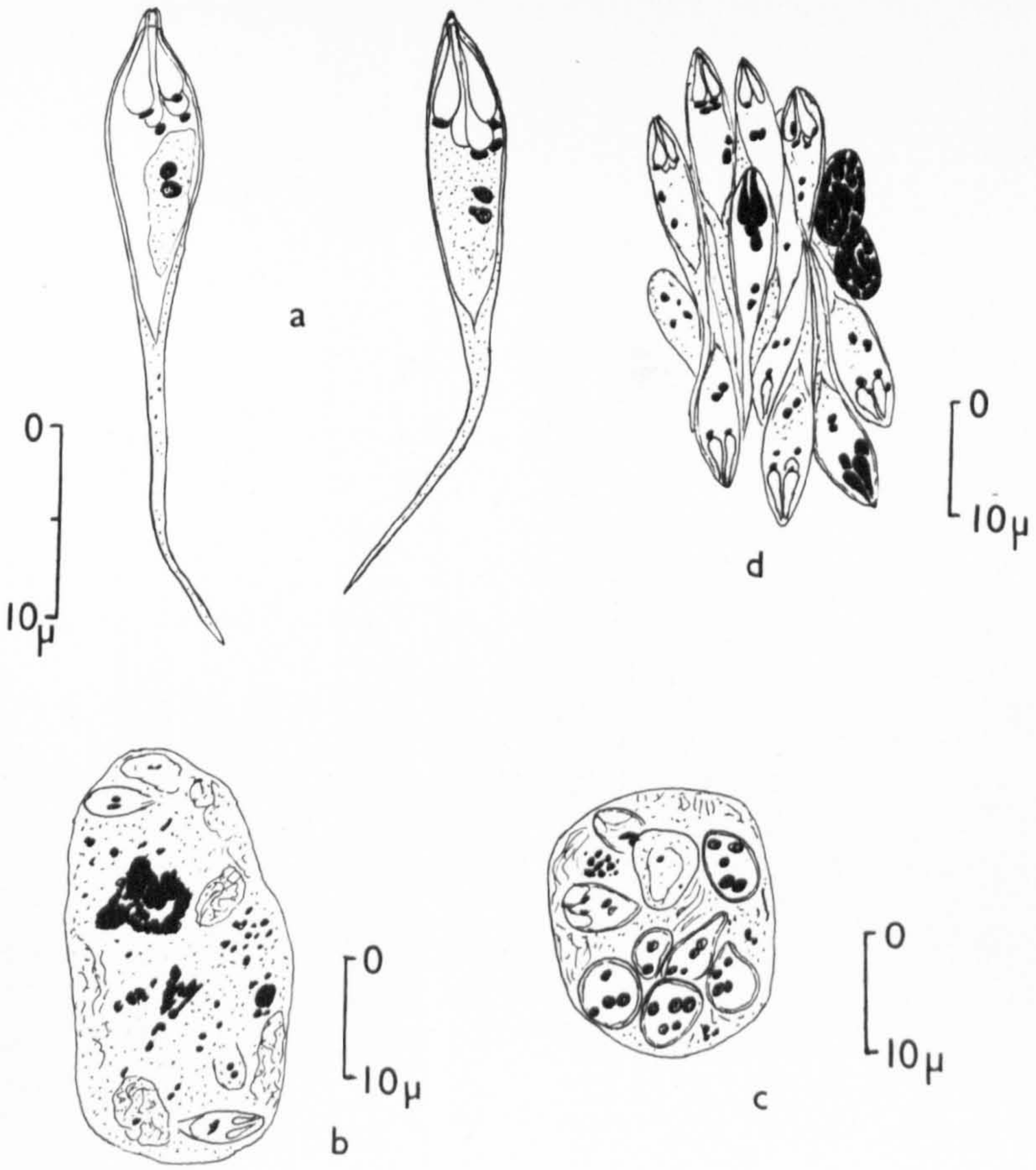


FIGURE 9.

Agarella gracilis from Lepidosiren paradoxa.

Giemsa's stain.

a. Spores.

b. Early trophozoite.

c. Late trophozoite.

d. Mature trophozoite.

(ii) Myxosporida of British fish.

a. Myxobolus sp. 4 from Rutilus rutilus.

Young and adult specimens of Rutilus rutilus collected in three localities in the London area, viz: St. James's Park, the river Colne near Rickmansworth, and the Brocket Estate near Welwyn, were examined for Myxosporida at frequent intervals between September, 1964 and December, 1966. All fish showed spores in the liver, spleen, kidney and musculature, and some contained spores in the gall-bladder, the testis and the alimentary canal. For the reasons given under Myxobolus sp. 1, the spores could be ascribed either to the genus Myxobolus or to the genus Myxosoma. Spores were drawn and measured from fresh preparations, and trophozoites examined and drawn from stained smears and sectioned preparations. Characteristics of the species were as follows:-

Spores: In all three localities irregularly oval spores characterised by two unequal polar capsules were seen (Fig: 10a, b). The polar capsules lay in the sutural plane and opened at the anterior end, to one side of the midline. The anterior polar capsule was invariably smaller than the posterior one. The spore valves were thin. No vacuole was visible in the sporoplasm of freshly mounted spores or in spores mounted in Gram's or Lugol's iodine. In sectioned preparations stained with Best's carmine, Lugol's iodine and with the Bauer-Feulgen technique, however, some spores were seen to possess vacuoles. The proportions of spores with vacuoles are analysed in detail and discussed in part 3 of this section. In Giemsa-stained smears and sections, and in sections

stained with Ehrlich's haematoxylin and eosin, two nuclei were visible in the sporoplasm (Fig: 10b).

In addition to the asymmetrical spores described in the above paragraph, regularly shaped spores (Fig: 10d, e) were found in fish collected in the Brocket Estate. In all fish examined from this locality, both spore types were invariably found together in the same site. The regular spores were oval in shape with a rounded or bluntly pointed anterior end. The two polar capsules were equal in size and opened to the anterior, in the midline. The spore-valves were slightly thicker than those of the asymmetrical spores. The appearance of the spores in smears and sections stained with Giemsa's stain, Ehrlich's haematoxylin and eosin, Best's carmine, Lugol's iodine and the Bauer-Feulgen technique was similar to that described for the asymmetrical spore type.

Measurements of each type of spore (in μ) were as follows: -

Asymmetrical spores (50 measured): -

	Length	Breadth	Thickness	Length of large polar capsule
Average	11.26	7.53	5.82	6.16
Range	10.00-12.00	6.50-8.00	4.75-6.50	5.50-7.50

	Length of small polar capsule	Breadth of large polar capsule	Breadth of small polar capsule
Average	4.83	3.36	2.93
Range	4.00-5.50	3.00-4.00	2.25-3.50

Symmetrical spores (Brocket Estate, 50 measured): -

	Length	Breadth	Thickness	Polar capsule length	Polar capsule breadth
Average	10.94	8.91	6.35	5.84	3.23
Range	10.00-12.00	7.75-9.50	5.75-7.25	4.75-6.75	2.75-4.00

Extrusion of polar filaments (Fig: 10c) was caused by mounting fresh spores in a saturated solution of urea (Lom, 1964) or in a 5% solution of potassium hydroxide (Hoffman et al., 1965). In these conditions, polar filament extrusion took place after approximately 1 minute. 85% of spores examined after 10 minutes showed extrusion of both filaments, and a further 10% extrusion of one filament only. After extrusion, some reduction in the size of the polar capsules was noted. The adhesive quality of the polar filaments, mentioned by Lom (1964), was also noted in the present study. When extruded, filament tips frequently adhered to the slide or coverslip. Spores with extruded polar filaments were also seen frequently in tissue smears which had been dried in air, fixed in methanol and stained with Giemsa's stain. Measurements of polar filaments of the asymmetrical spores were as follows:-

	Large capsule	Small capsule
Average	14.40 μ	11.90 μ
Range	12.00-20.00 μ	10.00-15.00 μ

Trophozoites: Trophozoites were found in the liver, spleen, kidney and musculature of all fish, and occasionally in the gall-bladder walls and testis.

In fresh preparations of liver, spleen and kidney, trophozoites appeared to consist of regions of a diffuse yellow material in which spores were embedded. They were of irregular shape, with no cyst wall, and measured up to 100 μ in diameter. In Giemsa-stained sections (Fig: 11a, b), trophozoites took up a light green colour: besides spores, nuclei and scattered pigment granules could be seen. In sections stained by the P.A.S. method, the trophozoites appeared light pink, while the sporoplasm of developing spores took up a deeper pink colour. There was no clear indication of developing pansporoblasts in any of the sections examined.

In Giemsa-stained smears, the only clearly recognisable forms other than mature spores were disporous pansporoblasts in an advanced stage of development (Fig: 11c, d). In these, two sporoblasts, each with six nuclei and two developing polar capsules, were surrounded by the pansporoblast envelope.

From the smears and sections examined, it was not clear whether each of the large regions of diffuse material, described above, represented one trophozoite with several developing spores (polysporous), or aggregations of smaller trophozoites, each being disporous as pictured in Fig: 11 c. and d. It is possible that this species possesses both poly- and di-sporous trophozoites, this situation being fairly common among the Myxosporida.

Trophozoites found in the general body musculature were usually of elongated shape, lying between muscle fibres (Fig: 12a). They were of variable size, sometimes measuring up to 200 μ in length, and were not surrounded by a cyst wall. All trophozoites examined in this site

appeared to be fully developed, as few inclusions other than mature spores were seen.

In the gall-bladder walls and testis, trophozoites were similar to those in the liver, spleen and kidney.

Spore distribution: Free spores were frequently seen between the cells of the organs in which trophozoites were found. In addition, they were seen free in the intestinal contents, between the epithelial cells of villi (Fig: 12 c, d) and in the tissue in the centre of villi (Fig: 12b). In these sites, there was no indication of trophozoites or of host reaction around the spores. In all spores examined in and around intestinal villi, the polar capsules appeared to be intact.

These observations suggest a way in which spores of histozoic Myxosporida may leave their host before its death. After maturing in a trophozoite, they may be carried by the blood or lymphatic system to become lodged in a villus. They may then pass into the epithelial layer, and be shed into the intestinal contents with epithelial cells, to leave with the faeces.

Identification: The species described here was provisionally placed in the genus Myxosoma by the present author (Walliker, 1966). The results of tests with Lugol's iodine, Best's carmine and the Bauer modification of the Feulgen technique show the presence of a vacuole in certain spores, indicating that it may be a Myxobolus species. Because of the great similarity between these genera, species of both Myxobolus and Myxosoma with characters comparable to the present material are considered here. Table 2 lists the species of these genera described

so far from R. rutilus.

It has been shown above that in all three localities in which R. rutilus was examined, spores characterised by unequal and asymmetrically positioned polar capsules were present. In addition, in one locality (the Brocket Estate), another type of spore, with equal and symmetrical polar capsules, was seen. As it is possible that the two spore types represent two species inhabiting the same site, comparisons will be drawn between each type with previously described species.

Asymmetrical spores: Of the species listed in table 2, Myxobolus dispar Thélohan, 1895, M. musculi Keysselitz, 1908 and M. pseudodispar Gorbunowa, 1936 have unequal polar capsules. Of these, the polar capsules of M. dispar and M. musculi open to the anterior and have an intercapsular process. These two species, therefore, can be eliminated from further comparison. The spores of M. pseudodispar are closely similar to those of the present material, with polar capsules opening to one side of the midline. The polar capsules, however, are smaller than those of the present species, the larger measuring 4.50 - 5.60 u by 3.00 u and the smaller 3.90 - 4.20 u by 2.70 u. Another difference is in the trophozoites which are described (by Bykhovskaya-Pavlovskaya et al., 1962) as round or oval cysts in the kidney and musculature, measuring up to 1 mm. in size.

Among other species of Myxobolus with an asymmetrical spore type, M. uniporus Fujita, 1927 and M. kostiri Herrick, 1936 are similar to the present species. M. uniporus, described from Parasilurus asotus (L.)

(family Siluridae) in Japan, and Liocassis ussuriensis (Dybowskii) and Pseudobagrus fulvidraco (Richardson) (both in family Bagridae) in the U.S.S.R., has spores more comparable to the symmetrical spore type of the present material, but, according to the description given by Bykhovskaya-Pavlovskaya et al. (1962), "freak spores, with unequal polar capsules, are very often encountered". The trophozoites of this parasite are described as round cysts surrounded by a fine, striated, membrane, and are found in the intestine walls, kidneys and subcutaneous connective tissue. M. kostiri, from Micropterus dolomieui Lacepede (family Centrarchidae) in the U.S.A., was described by Herrick (1936) as a cyst in subcutaneous connective tissue near the branchiostegal rays. The spore shape and arrangement of the polar capsules are closely similar to the present material, although the dimensions are less. Spores measure 8.80 - 11.20 μ by 6.40 - 8.00 μ and are 4.90 - 5.80 μ thick. Polar capsule measurements are 4.10 - 4.90 μ x 2.40 - 3.30 μ (larger) and 3.30 - 4.90μx 1.60 - 2.80 μ (smaller).

Among species of Myxosoma with asymmetrical spores, M. hudsonis Bond, 1938 and M. asymmetrica (Parisi, 1912) have comparable features to the present species. M. hudsonis, from Fundulus heteroclitus (L.) (family Cyprinodontidae) in the U.S.A. is described as having spores of two distinct types, always in the same cyst; these types are distinguished by spore size and shape, and in both types the polar capsules open to one side of the midline. The polar capsules, however, are equal in size and slightly smaller than those of the present material (4.00 - 5.00 μ by 2.25 μ). This species forms cysts between the surfaces of scales

covering the fin bases. M. asymmetrica, from Crenilabrus tinca (L.) (family Labridae) in Italy is similar to M. hudsonis in possessing equally-sized polar capsules opening asymmetrically. The trophozoites are described as small rounded forms with a distinct ecto- and endoplasm containing coarse yellowish globules, and are found in the kidney.

Symmetrical spores: Of the species listed in table 2, Myxobolus cycloides Gurley, 1893 and M. cyprini Doflein, 1898 have spores with dimensions comparable to the present material. M. cycloides has three types of spore, of which that described as type A by Kudo (1919) is the most similar. The spores, however, have an intercapsular appendage and folds along the sutural edge, features not seen in the present species. M. cyprini is more similar, having spores with a rather more variable size range (length, 10.00 - 16.00μ, breadth 8.00 - 12.00μ, polar capsule length 5.20 - 7.00μ) than the present species, and with a thick sutural ridge. The trophozoites of M. cyprini are also similar to those of the present species, being amoeboid and forming diffuse infiltrations in the liver, spleen, kidney, musculature, intestinal walls and mesenteries.

Among species of Myxobolus from other cyprinid hosts, there are four which can be considered for comparison with the present species. M. oviformis Thélohan, 1892, from many cyprinid hosts, forms cysts in the subcutaneous tissue of the fins and in the liver, spleen and kidney. While this differs from the type of trophozoite found in the present material, spores of M. oviformis are very similar (measurements: 10.50 -

11.00 μ long by 7.50 - 8.00 μ broad, polar capsules 5.00 - 6.00 μ by 3.00 μ, according to Wegener's description, 1910). M. exiguus Thélohan, 1895 also forms cysts in many cyprinid hosts, occuring in the gills, intestinal walls, spleen and kidney. Again, spores are very similar to those of the present species (measurements: 8.00 - 12.00 μ long by 6.00 - 9.30 μ broad, 4.50 - 5.50 μ thick, polar capsules 4.00 - 7.00 μ by 2.50 - 2.70 μ, according to the description given by Bykhovskaya-Pavlovskaya et al., 1962). M. amurensis Akhmerov, 1960, found in the gills, fins, intestinal walls and gonads of Cyprinus carpio L. and Abbottina rivularis (Basilewskii) in the U.S.S.R. has spores which are rather more rounded than those of the present species, but of two different types, with equal and unequal polar capsules. The trophozoites of this species are described as spherical cysts or diffuse infiltrations in musculature. M. hyborhynchi Fantham, Porter and Richardson, 1939, described from a cyst in bone in the mandible of Pimephales notatus (Raf.) has spores measuring 9.10 - 10.90 μ by 7.30 - 8.60 μ and polar capsules 4.10 - 5.90 μ by 2.30 - 2.50 μ.

Of species of Myxobolus from non-cyprinid hosts, only M. plectroplites Johston and Bancroft, 1919 described from Plectroplites ambiguus (Richardson) (family Serranidae) in Australia has comparable spores. The spores contain a vacuole described as ? not iodinophilous. The species forms cysts in the kidney, and spores are also found in the gall-bladder.

Among previously described species of Myxosoma, none from cyprinid hosts are entirely similar to the present species. From non-cyprinid

hosts, M. endovasa Davis, 1947, M. catostomi Kudo, 1926, and M. microthecum Ucrlitsch, 1942 show certain similarities. M. endovasa, from lamellar capillaries of the gills of Ictiobus bubalus (Raf.) (family Catostomidae) in the U.S.A. has slightly more rounded spores (9.00 μ by 8.00 μ in formalin-fixed preparations). M. catostomi, from the muscles of Catostomus commersoni (Lacepede) (family Catostomidae) differs in having folds or thickenings on the spore walls and in forming cysts. M. microthecum, from the mesenteries and peritoneum of Minytrema melanops (Raf.) (family Catostomidae) in the U.S.A. differs in having an intercapsular process in the spores.

Conclusions: Assuming that the two spore types found in M. rutilus represent two distinct species, the following conclusions can be drawn.

The species with asymmetrical spores can be identified as Myxobolus pseudodispar. It differs from the descriptions given by Gorbunowa (1936) and Rykhovskaya-Pavlovskaya et al. (1962) in inhabiting a wider range of organs and in having a diffuse type of trophozoite rather than an encysted form. A close similarity to Myxobolus kostiri can also be seen.

The species with symmetrical spores can be identified as Myxobolus cyprini, which differs only in its possession of a large sutural ridge. Close similarities are also shared by Myxobolus oviformis, M. oviformis and M. hyborhynchi.

If the two spore types belong to a single species, this is best identified as Myxobolus pseudodispar, as the asymmetrical type of spore was found in all the localities investigated. The form with two spore types, present in the Brocket Estate, may be regarded as a separate strain of M. pseudodispar.

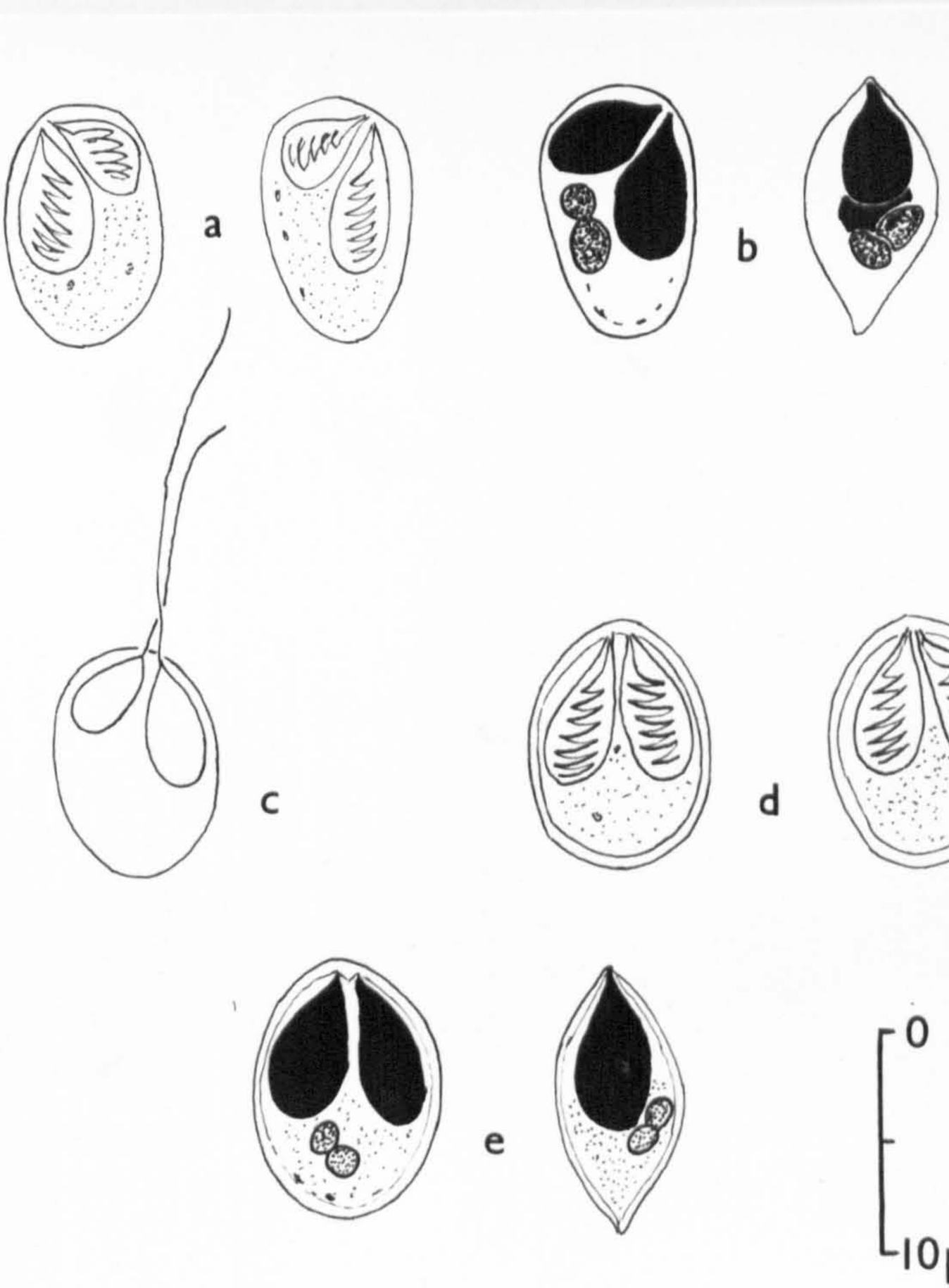


FIGURE 10.

Myxobolus sp. 4. from Rutilus rutilus

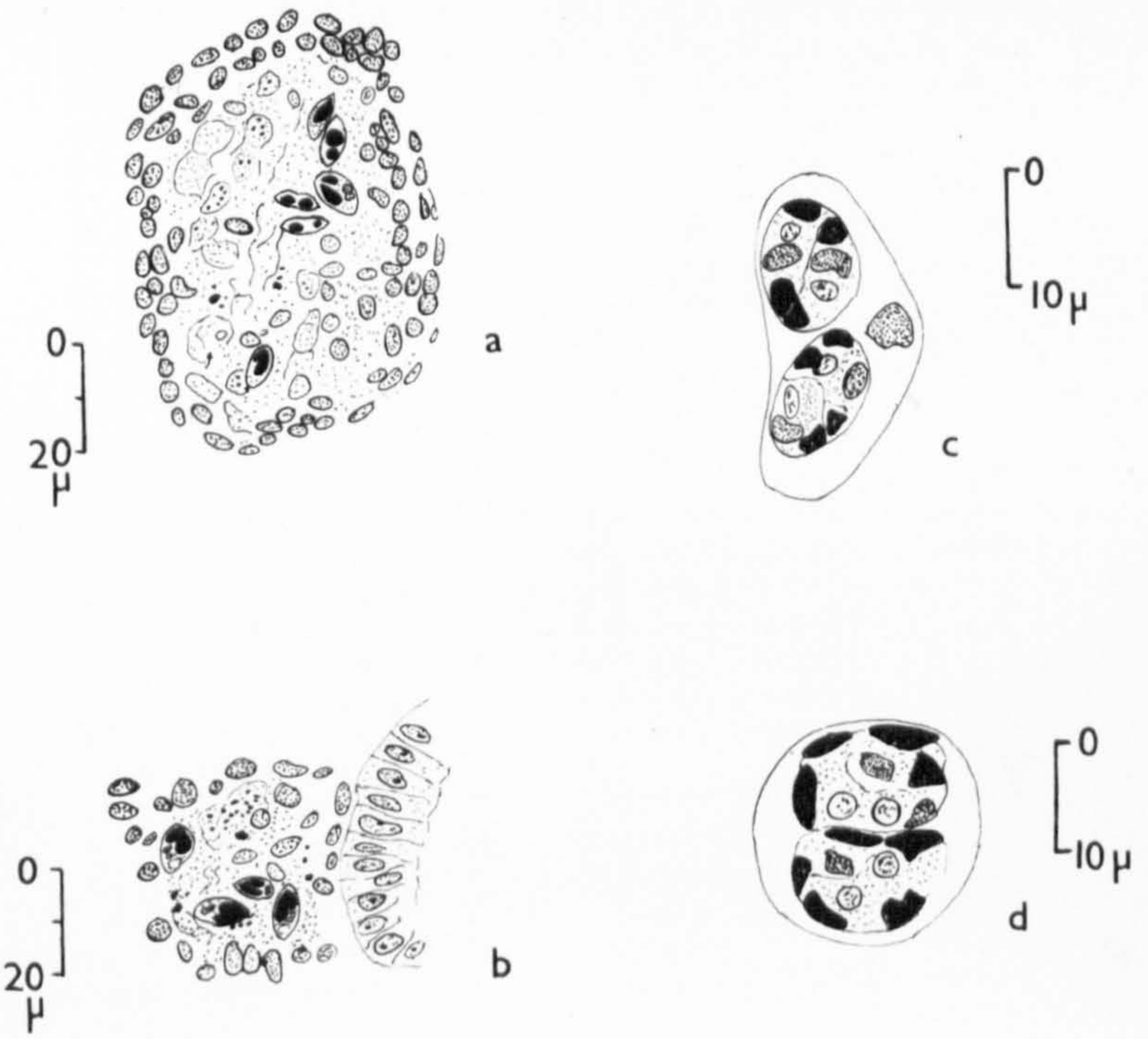
a. Asymmetrical spores. Fresh preparations.

b. Asymmetrical spores, front and side view.
Giemsa's stain.

c. Asymmetrical spore with extruded polar filaments.
Fresh preparation mounted in saturated solution
of urea.

d. Symmetrical spores. Fresh preparations.

e. Symmetrical spores, front and side view. Giemsa's
stain.



F I G U R E 11.

Myxobolus sp. 4 from Rutilus rutilus.

Giemsa's stain.

a. Trophozoite in spleen.

b. Trophozoite in kidney.

c, d. Pansporoblasts.

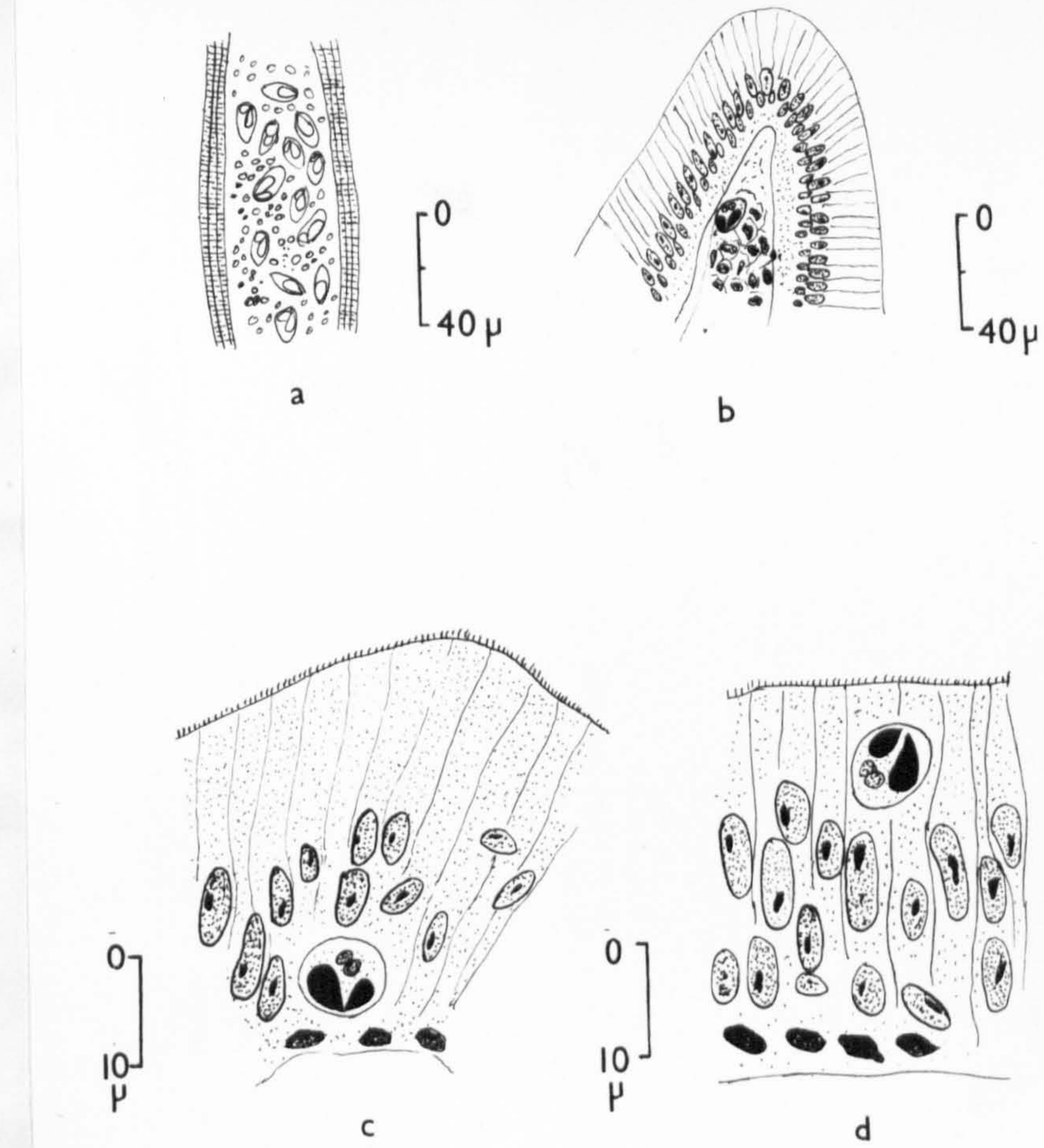


FIGURE 12.

Myxobolus sp. 4 from Rutilus rutilus

a. Mature trophozoite in musculature.

b. Spore in central tissue of intestinal villus.

Giemsa's stain.

c, d. Spores in villus epithelium.

T A B L E 2.

Species of Myxobolus and Myxosoma described from Rutilus rutilus.

Species	Author	Site of infection
Myxobolus cycloides	Gurley, 1893	Branchiae, pseudobranchiae, operculum, kidney.
Myxobolus cyprini	Doflein, 1898	Intestinal wall, mesentery, musculature, liver, spleen, kidney.
Myxobolus dispar	Thélohan, 1895	Skin, gills, musculature, intestinal wall, kidney.
Myxobolus ellipsoides	Thélohan, 1892	Gills, branchial arches, operculum, intestinal wall, gall - bladder wall, mesentery, liver, spleen, kidney, urinary bladder wall, genital glands, musculature, eye.
Myxobolus macrocapsularis	Reuss, 1906	Gills, skin, swim - bladder wall, kidney.
Myxobolus muelleri	Bütschli, 1882	Gills, operculum, skin, intestine, urinary bladder, swim-bladder, gall-bladder wall, liver, kidney, gonads.
Myxobolus musculi	Keysselitz, 1908	Musculature.
Myxobolus pseudodispar	Gorbunowa, 1936	Musculature, kidney.
Myxobolus sp.	Lom, 1961	Kidney.
Myxosoma branchialis.	(Markevich, 1932)	Gills, musculature.
Myxosoma dujardini	Thélohan, 1892	Gills.
Myxosoma sp.	Lom, 1961	Gills

b. Myxobolus cyprini Doflein, 1898 from Gobio gobio (L.)

Specimens of Gobio gobio, collected in the river Colne in July, 1965, were examined for Myxosporida. Fresh preparations of liver, spleen, kidney and musculature revealed spores in all these sites which, for the reasons given under Myxobolus sp.1, could be allocated to either the genus Myxobolus or the genus Myxosoma. For the further reasons given below, the species could be identified as Myxobolus cyprini. Spores were drawn and measured both from fresh preparations and from methanol-fixed and Giemsa - stained smears. Trophozoites were examined and drawn from sectioned preparations.

Spores (Fig: 13a, b, c):- Spores oval in front view, and slightly narrowed at the anterior end. Two polar capsules lying in the sutural plane and opening to the anterior. Two capsulogenous nuclei and two sporoplasm nuclei visible in sections stained with Ehrlich's haematoxylin and eosin (Fig: 13 c). A vacuole visible in some Giemsa - stained spores (Fig: 13a) and in a few spores stained by Lugol's iodine, Best's carmine and the Bauer - Feulgen technique. Spores with extruded polar filaments common in methanol - fixed and Giemsa - stained smears. Spore measurements (in μ) were as follows;-

Fresh spores (14 measured)

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	12.09	9.27	6.52	3.26
Range	10.50 - 13.50	8.00 - 10.25	6.00 - 7.50	3.00 - 3.50

Methanol - fixed spores (25 measured)

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	11.81	8.64	5.83	2.86
Range	10.00 - 13.50	7.75 - 10.00	4.00 - 7.00	2.50 - 3.25

Trophozoites (Fig: 13 d,e);- Trophozoites were similar to those described under Myxobolus sp.4 from Rutilus rutilus. In the liver, spleen and kidney, little structural detail could be elucidated other than mature spores. They consisted of diffuse protoplasmic regions, with no surrounding cyst wall, in which the spores together with lumps of dark - brown pigment were embedded. In fresh preparations, they were a yellow - brown colour, and in Giemsa - stained sections light green. The average diameter of trophozoites in sections was approximately 50 μ, although some measured up to 100 μ. No developing pansporoblasts were observed in smears or sections.

In musculature, clusters of mature spores only were seen. These lay between muscle fibres, in the manner described in Myxobolus sp.4, and probably represented mature trophozoites. No surrounding cyst wall was visible.

Identification;- Table 3 lists the species of Myxobolus described so far from Gobio gobio; no species of Myxosoma has been recorded from this host.

A comparison of the present material with species in this list shows

that on grounds of spore shape and size, M. muelleri Bütschli, 1882, M. subepithelialis Weiser, 1949 and M. ellipsoides Thélohan, 1892 can be considered for identification. Of these, spores of M. muelleri possess a prominent intercapsular process, a feature not seen in any of the present spores. M. subepithelialis, described only from the sub - cutaneous connective tissue of the head of Gobio gobio in Czechoslovakia, has spores of very similar shape and size, although with a slightly greater size range (8.00 - 12.00μlong by 6.00 - 10.00μbroad). The trophozoites of M. subepithialis form small cysts. M. ellipsoides is closely similar to the present species in its trophozoites, which form cysts or diffuse infiltrations in several organs including liver, spleen, kidney and musculature. The spores of M. ellipsoides, however, differ from those of the present species in possessing polar capsules, which are rather less than half of the length of the spore, and in their shape, which is often narrowed at the posterior end.

Among species of Myxobolus described from other cyprinid fishes, only three, M. cyprini, M. hyborhynchi and Myxobolus sp. Akhmerov, 1960 have spores of comparable shape and size. Of these, Myxobolus sp. Akhmerov, described from spores only, from the gills and fins of Cyprinus carpio in the U.S.S.R., has slightly smaller polar capsules (length 4.00 - 5.30 μ). M. hyborhynchi has slightly shorter spores (9.10 - 10.90 μ) and was described from a cyst in bone in the mandible of Pimephales notatus in Canada. M. cyprini, described from numerous cyprinids including Gobio albipinnatus in Europe and the U.S.S.R., is closely similar, as it inhabits liver, spleen, kidney and musculature in the

form of diffuse infiltrations. The only difference in the spores, according to the description given by Bykhovskaya - Pavlovskaya et al. (1962), is the presence of a thick sutural ridge. As the spores of the present species possess fairly thick valves, this difference is not considered of importance here. A close similarity could also be seen between the present spores and the symmetrical type of spore described in Myxobolus sp.1 which was also shown to be similar to M. cyprini.

While the present species can be identified with some certainty as M. cyprini, it is worth recording here other species of Myxobolus and Myxosoma with which it may be confused on grounds of spore shape and size.

Among other species of Myxobolus, from non - cyprinid hosts, the following are similar, but differ in the features listed;-

M. mesentericus Kudo, 1919 (from the mesentery, liver, spleen, gall - bladder, stomach, intestine and pyloric caecum of Lepomis cyanellus Raf. in the U.S.A.) differs in possessing folds along the sutural edge.

M. nemachili Weiser, 1949 (from connective tissue of the head of Nemacheilus barbatulus (L.) in Czechoslovakia) has sutural markings around the edge of the valves.

M. catostomi Fantham, Porter and Richardson, 1939 (from the musculature of Catostomus commersoni (Lacépède) in Canada) possesses one or two lateral barbs on the sutural edge.

M. sparoidis Otto and Jahn, 1943 (from the intestine of Pomoxis sparoides Lacépède in the U.S.A.) has several fat globules between the polar capsules.

Among other species of Myxosoma, the following are similar, but differ in the features mentioned;-

M. microthecum Meglitsch, 1942 (from the mesenteries of Minytrema melanops (Raf.) in the U.S.A.) possesses a long , slender intercapsular process.

M. catostomi Kudo, 1926 (from the musculature around the pectoral fins of Catostomus commersoni in the U.S.A.) differs in having folds on the spore valves.

M. endovasa Davis, 1947 (from the gills of Ictiobus bubalus Raf. in the U.S.A.) differs only in its site, in lamellar capillaries.

Conclusion; - The species described from Gobio gobio may be identified as Myxobolus cyprini, a species common in cyprinid fishes of Europe; this, however, is believed to be the first record of its presence in Gobio gobio. Myxobolus ellipsoides, a species described previously from G. gobio, is also comparable to the present material, but differs slightly in polar capsule size and spore shape.

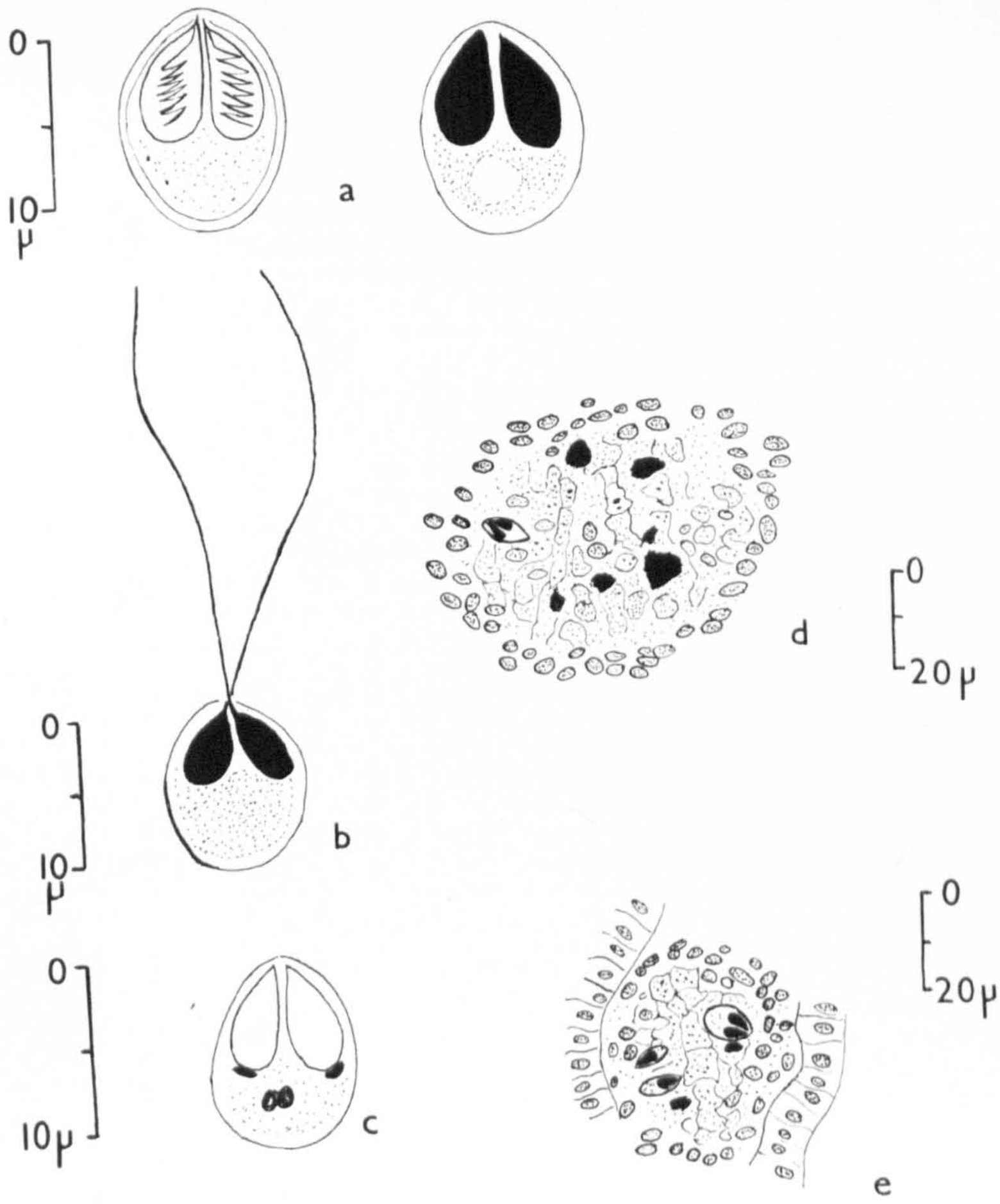


FIGURE 13.

Myxobolus cyprini from Gobio gobio.

a. Spores. Fresh, and in Giemsa's stain.

b. Spore with extruded polar filaments. Giemsa's stain

c. Spore stained with Ehrlich's haematoxylin and eosin

d. Trophozoite in spleen. Giemsa's stain.

e. Trophozoite in kidney. Giemsa's stain.

T A B L E 3.

Species of Myxobolus described from Gobio gobio.

Species	Author	Site of infection
M. cycloides	Gurley, 1893	Branchiae, pseudobranchiae, operculum, kidney.
M. dispar	Thélohan, 1895	Skin, gills, musculature, intestinal walls, kidney.
M. ellipsoides	Thélohan, 1892	Gills, branchial arches, operculum, intestinal walls, mesentery, gall - bladder walls, liver, spleen, kidney, urinary bladder walls, genital glands, musculature, eye.
M. lussi	Akhmerov, 1960	Gills, fins.
M. macrocapsularis	Reuss, 1906	Skin, gills, swimbladder wall, kidney.
M. muelleri	Bütschli, 1882	Gills, operculum, skin, intestine, urinary bladder, swim - bladder, gall - bladder walls, liver, kidney, gonads.
M. multiplex	Akhmerov, 1960	?
M. oviformis	Thélohan, 1892	Gills, fins, musculature, mesentery, liver, spleen, gonads.
M. pseudorasborae	Akhmerov, 1960	Gill - cover, fins, musculature, kidney.
M. rotundus	Nemeczek, 1911	Gills.
M. subepithelialis	Weiser, 1949	Head (sub-cutaneous).
M. vescus	Akhmerov, 1960	?

c. Myxobolus muelleri Bütschli, 1882 from Leuciscus leuciscus (L.)

One specimen of Leuciscus leuciscus, caught in the Lea Navigation Canal near Hertford, was examined for Myxosporida. Spores were found in fresh preparations of spleen and kidney which, for the reasons given under Myxobolus sp. 1, could be allocated either to the genus Myxobolus or to the genus Myxosoma. For the reasons given below, the species was identified as Myxobolus muelleri. Spores were drawn and measured from fresh preparations only, and trophozoites examined and drawn from sectioned preparations.

Spores (Fig: 14a,b,c);- Spores of variable shape and size, but all possessing two polar capsules lying in the sutural plane and opening to the anterior. Two nuclei visible in the sporoplasm of spores stained with Giemsa's stain. No vacuole seen in fresh spores, but a vacuole visible in some spores stained with Lugol's iodine, Best's carmine and the Bauer - Feulgen technique.

Spores of two size ranges could be distinguished. The smaller (Fig: 14b) were more numerous. They were usually of oval shape, but occasionally showed a bluntly pointed anterior end. The spore valves were relatively thick. Polar capsules were generally equal in size, although spores with unequal polar capsules were common. A small intercapsular process was visible in some spores. The polar filament was coiled 6 - 7 times. Measurements of these spores (in μ) were as follows (33 spores measured);-

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	9.80	7.00	4.80	2.60
Range	8.50 - 10.75	6.50 - 7.75	4.00 - 5.50	2.00 - 3.25

The second type of spore (Fig; 14 a) was larger and less numerous. The spore shape was also variable, being either rounded or slightly pointed at the anterior end. The spore valves were fairly thick, and an intercapsular process was sometimes seen. The polar capsules were equal in size, and contained a polar filament coiled 12 - 13 times. Measurements of these spores (in μ) were as follows (8 spores measured);-

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	14.69	10.50	7.55	3.94
Range	13.00 - 16.00	8.50 - 12.50	6.50 - 8.00	3.25 - 4.75

Trophozoites (Fig: 14 d);- Trophozoites were very similar to those of Myxobolus sp.4 and M. cyprini, and were found only in the spleen and kidney. They consisted of diffuse regions of yellow material (appearing light green in Giemsa - stained preparations) in which mature spores, nuclei and lumps of dark - brown pigment could be distinguished. No cyst wall was visible, and no developing pansporoblasts were found in either sections or smears. In sections, trophozoites measured up to 50 μ in diameter.

Identification;- Table 4 lists the species of Myxobolus and Myxosoma described so far from L. leuciscus.

The chief feature of the spores of this species is their variable size and shape. As indicated above, the spores fall into two groups, small and large, which will be treated separately in this section, as they may represent two species inhabiting the same site.

Small spores;- Of the species listed in table 4, Myxobolus musculi Keysselitz, 1908, M. dispar Thelohan, 1895 and M. muelleri show similarities to the present species. M. musculi and M. dispar are characterised by polar capsules of different sizes, a feature seen in some of the present spores. In M. musculi, however, the spores are wider than in the present species (8.00 - 11.00 μ), and in M. dispar the difference in polar capsule size is much greater than in the present spores (larger polar capsule, 5.00 - 8.00 μ, smaller, 2.50 - 4.50 μ). M. muelleri, according to the description given by Bykhovskaya - Pavlovskaya et al. (1962), has spores which are extremely variable in size, measuring 6.00 - 14.50 μ in length and 7.00 - 12.00 in breadth. The spore shape of M. muelleri may be round, oval or tapered at the anterior, and an intercapsular process is present. The trophozoites are described as milk - white cysts of various sizes and form.

The small spores, therefore, can be identified with some certainty as M. muelleri, chiefly on the grounds of their variable shape. The trophozoites differ from those of previous descriptions of this parasite in being diffuse infiltrations rather than cysts. This is also believed to be the first record of M. muelleri in the spleen.

There are several other species of Myxobolus and Myxosoma whose spores or trophozoites may be confused with the present material.

These are considered briefly here.

Of species of Myxobolus from other cyprinid hosts, the following are comparable:-

M. cyprini (See description in 2(ii)b of this section) differs in possessing slightly larger and more regularly shaped spores (length 10.00 - 16.00 μ, breadth 8.00 - 12.00 μ, polar capsule length 5.20 - 7.00 μ). Trophozoites are similar.

M. exiguus, described from several families, infects the gills, intestinal walls, spleen and kidney. Spores are of similar dimensions, although the sutural edge has folds not seen in the present material. The trophozoites also differ in forming cysts.

M. hyborhynchi also has spores of comparable dimensions, although of less variable range (length 9.10 - 10.90 μ, breadth 7.30 - 8.60 u). The species was described from a cyst in bone of the mandible of its host Pimephales notatus in Canada.

M. balleri Reuss, 1906 (from the gills of Abramis ballerus (L.) in the U.S.S.R.) varies in the spore width (9.25 - 10.00 μ) and in the trophozoites which forms a cyst. M. balleri is considered by Bykhovskaya - Pavlovskaya et al. (1962) to be synonymous with M. muelleri.

Among species of Myxobolus from non - cyprinid hosts, the following are comparable:-

M. plectroplites (from the kidney and gall - bladder of Plectroplites ambiguus in Australia) has slightly larger spores (length 10.00 - 12.00 μ, breadth 7.00 - 8.00 μ) and forms cysts in the kidney.

M. uniporus (from the intestinal walls, kidney, gonads and

subcutaneous connective tissue of Parasilurus asotus, Liocassis ussuriensis and Pseudobagrus fulvidraco in the U.S.S.R.) has spores of comparable dimensions. According to Bykhovskaya - Pavlovskaya et al. (1962), "freak" spores, with unequal polar capsules, are often seen, a feature shared by the present species. The trophozoites of M. uniporus differ in forming cysts surrounded by a striated membrane.

No species of Myxosoma described from cyprinid hosts entirely satisfies the features of the present material. Of species from non - cyprinid hosts, only M. commersonii (from the skin of Catostomus commersoni in Canada) has comparable spores. These, however, are slightly larger (9.50 - 16.50 μ long and 7.00 - 11.40 μ broad). The trophozoites also differ in forming cysts.

Large spores;- These spores were invariably found together with the small spores, but were less numerous. On dimensional grounds they could also be identified as Myxobolus muelleri, although the largest spore seen (length 16.00 μ, breadth 12.50 μ) was slightly larger than the extreme of the size range of M. muelleri given by Bykhovskaya - Pavlovskaya et al. (1962), which was 14.50 μ by 12.00 μ. Their great difference in size from the small spores, however, suggests that they could belong to a separate species.

Of the species listed in table 4, only Myxobolus carassii Klokačewa, 1914 has spores of comparable shape and size. This species differs from the present species in its sites of infection and in its trophozoites, which form cysts. The trophozoites, however, are described as yellowish in colour by Kudo (1919).

Among other species of Myxobolus, Myxobolus sp. Gurley, 1894, described from spores only in the body cavity of Carassius carassius (L.) (family Cyprinidae) in Germany has spores measuring 14.00 μ long by 10.00 μ broad. Kudo (1919) points out that this species is very similar to M. carassii.

Myxobolus lintoni Gurley, 1893, from superficial musculature and sub - cutaneous connective tissue of Cyprinodon variegatus Lacépède (family Cyprinodontidae) in the U.S.A. has spores measuring 13.90 μ long by 11.00 μ broad. The trophozoites of this species, according to Kudo (1919), form "fungoid masses of an irregular shape".

Another species with certain similarities to the present material is Myxobolus cuvenoti Naville, 1928 described from the gills of Perca fluviatilis L. (family Percidae) in Switzerland. The spores of this species measure 14.50 - 16.00 μ by 11.00 - 12.00 μ. The trophozoites form small white cysts.

Conclusions;- Both the large and small spores of the present species can be identified as Myxobolus muelleri. The trophozoites differ from those described previously for M. muelleri in being diffuse infiltrations, and they are believed to be recorded from the spleen for the first time.

If the two spore types represent two infections by different species, the small spores are probably identified most satisfactorily as M. muelleri, and the large spores as Myxobolus carassii.

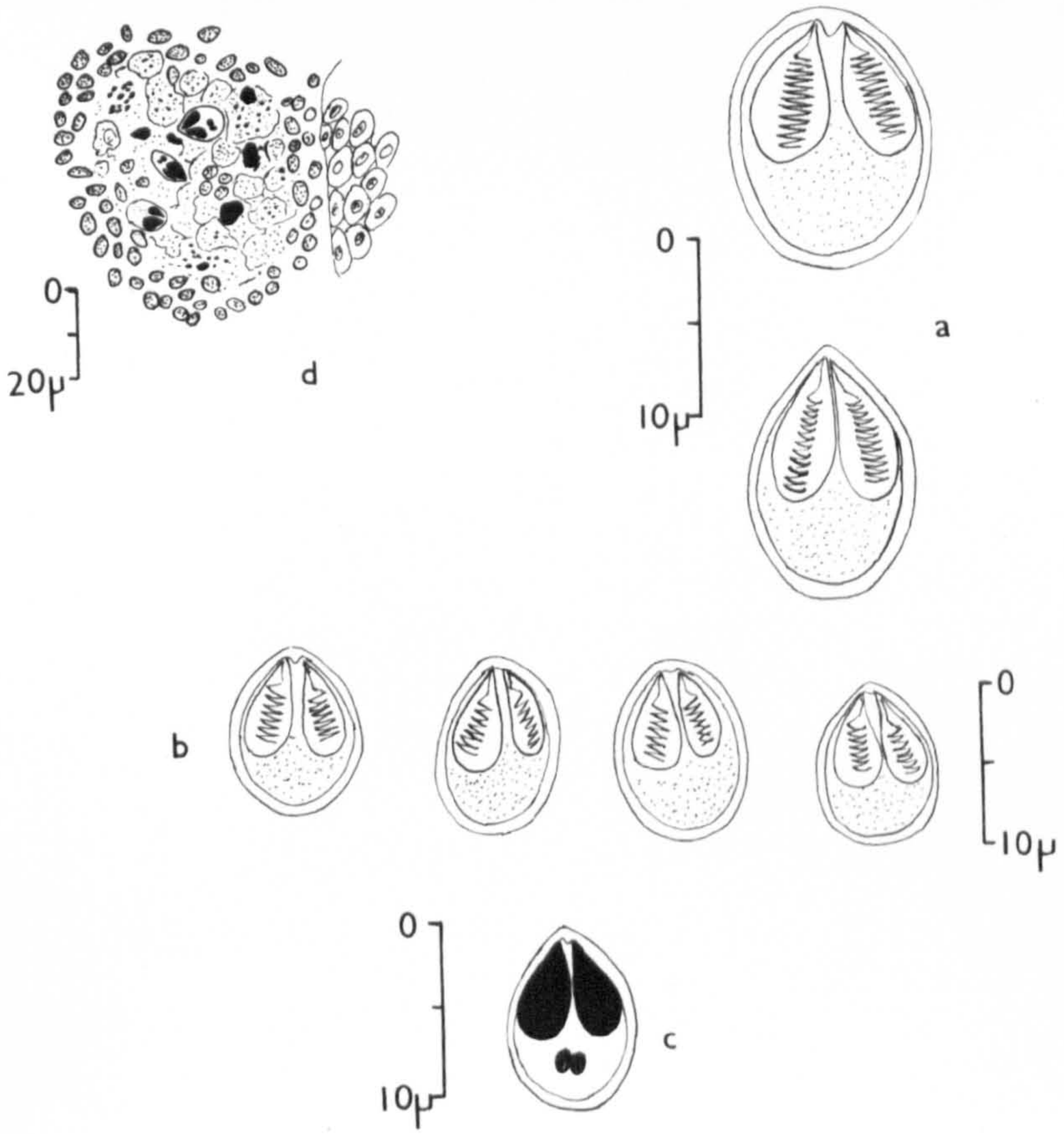


FIGURE 14.

Myxobolus muelleri from Leuciscus leuciscus.

a. Large spores. Fresh preparations.

b. Small spores. Fresh preparations.

c. Spore. Giemsa's stain.

d. Trophozoite in spleen.

T A B L E 4.

Species of Myxobolus and Myxosoma described from Leuciscus leuciscus.

Species	Author	Site
Myxobolus carassii	Klokačewa, 1914	Body cavity, gills, branchial arches, mesentery, intestinal wall, liver.
Myxobolus dispar	Thélohan, 1895	Skin, gills, musculature, intestinal walls, kidney.
Myxobolus minutus	Nemeczek, 1911	Gills.
Myxobolus muelleri	Bütschli, 1882	Gills, operculum, skin, intestine, urinary bladder, swim-bladder, gall-bladder, walls, liver, kidney, gonads.
Myxobolus musculi	Keysselitz, 1908	Musculature.
Myxobolus nemeczeki	Schulman, 1962	Gills.
Myxobolus sp.	Donets, 1962	Musculature, heart.
Myxosoma dujardini	Thélohan, 1892	Gills.

d. Myxidium rhodei Léger, 1905 from Rutilus rutilus.

In all specimens of Rutilus rutilus collected in St. James's Park between September, 1964 and December, 1966, spherical white cysts measuring up to 0.15 mm were seen in fresh preparations of the kidney. The majority of cysts were packed with myxosporidan spores which, for the resons given below, could be identified as Myxidium rhodei (sub - order Bipolarina, family Myxididae according to Tripathi's classification, 1948). Spores were drawn and measured both from fresh preparations and from sections fixed in 10% formol saline and stained with Giemsa's stain. Cysts were examined and drawn from fresh and sectioned material.

Spores (Fig: 15 a,b,c);- Spores fusiform in shape with pointed or slightly rounded ends. Many spores slightly constricted in the centre. Two polar capsules, one opening at each end. Two thin spore valves, with six longitudinal striations on each valve. Sutural line indistinct. Two capsulogenous nuclei and two sporoplasm nuclei visible in sections stained with Ehrlich's haematoxylin and eosin (Fig: 15 c). No vacuole in the sporoplasm in sections stained with Lugol's iodine, Best's carmine, or the Bauer - Feulgen technique. Spore measurements (in μ) were as follows;-

Fresh spores (30 measured)

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	12.69	4,47	4.22	2.52
Range	11.00 - 13.50	3.50 - 5.50	3.50 - 5.25	2.00 - 3.50

Formol saline fixed spores (20 measured)

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	11.48	3.61	3.84	2.10
Range	10.50 - 12.50	3.00 - 4.00	3.50 - 4.50	2.00 - 2.50

Attempts were made to extrude polar filaments by using 5% potassium hydroxide solution, a saturated solution of urea, and bile, gastric fluid and intestinal fluid from the host. These fluids were added to spores mounted in distilled water on slides. Spores were dissected out from cysts, placed in distilled water at room temperature, and tested daily for fourteen days after extraction. In none of these tests were extruded polar filaments seen, although the polar capsules appeared empty in approximately 1% of the spores in each test.

Trophozoites:- The majority of trophozoites were in the form of spherical cysts, which appeared white in fresh preparations. In early cysts (Fig: 16a), the contents consisted mainly of scattered nuclei and pigment granules in a diffuse cytoplasm. Thin layers of fibrous tissue surrounded these cysts. In later cysts (Fig:16b), groups of nuclei were visible, which were probably pansporoblasts. A few mature spores were also present. The surrounding fibrous tissue was fairly thick. Mature cysts (Fig:16c), which measured up to 0.15 mm. in diameter, contained few inclusions other than mature spores, which numbered up to 500. The fibrous tissue surrounding these cysts was thick. In many fresh preparation cysts were yellow in colour and appeared to be degenerating, being

overgrown by dense fibrous tissue.

Identification;- Two species of Myxidium have been described from R. rutilus. M. rhodei Léger, 1905 (first described from Rhodeus amarus (Bloch) in France) forms cysts in the kidney. M. pfeifferi Auerbach, 1908 (first described from Tinca tinca L. in Germany) forms small discoid trophozoites in the gall - bladder. The two species have almost identical spores, and have often been considered as one. For example, Lom (1961) and Markevich (1951) described the occurrence of M. pfeifferi in both organs of R. rutilus. Bykhovskaya - Pavlovskaya et al. (1962), however, consider the two species to be distinct.

The present species was identified by the present author (Walliker, 1966) as M. pfeifferi, after reference to the accounts of this parasite by Lom and Markevich. It is clear, however, that if M. pfeifferi and M. rhodei are two distinct species, it should be identified as M. rhodei. The species would appear to be distinct from the present investigations, as the parasite was found only in the kidney of the fish examined, and not in the gall - bladder. Some experimental evidence, described in part 4 of this section, suggests that the two species may, in fact, be one. If this should be the case, the correct identification of the present species woud remain M. rhodei, as this was named before M. pfeifferi.

The present material differs only slightly from the description of M. rhodei given by Bykhovskaya - Pavlovskaya et al. (1962) in the size of the spores; M. rhodei spores, according to these authors, measure 11.00 - 18.00 μ long by 4.50 - 6.50 μ broad. This difference is not

considered significant here.

There are several other species of Myxidium which may be confused with M. rhodei on grounds of spore shape and size. These are considered briefly here.

M. barbatulae Cépède, 1906 (from the kidney of Nemacheilus barbatulus L. in France) differs only in possessing slightly wider spores (6.00 µ). Bykhovskaya - Pavlovskaya et al. (1962) consider that M. barbatulae can probably be synonymised with M. rhodei.

M. histophilum Thélohan, 1895 (from the kidney and ovary of Phoxinus phoxinus (L.) in France) has slightly longer spores (15.00 µ) than the present material, but is otherwise similar. The trophozoite of this species is described, by Kudo (1919), as a small mass.

M. kudoi Meglitsch, 1937 (from the gall - bladder of Ictalurus furcatus (LeSueur)in the U.S.A.) differs in having shorter polar capsules (2.50 - 3.50 µ) and in possessing flattened trophozoites.

M. oncorhynchi Fujita, 1923 (from the gall - bladder of Oncorhynchus masou Brevoort in Japan) differs in having wider spores (5.00 - 8.00 µ) and a ridge along the suture line of the spores.

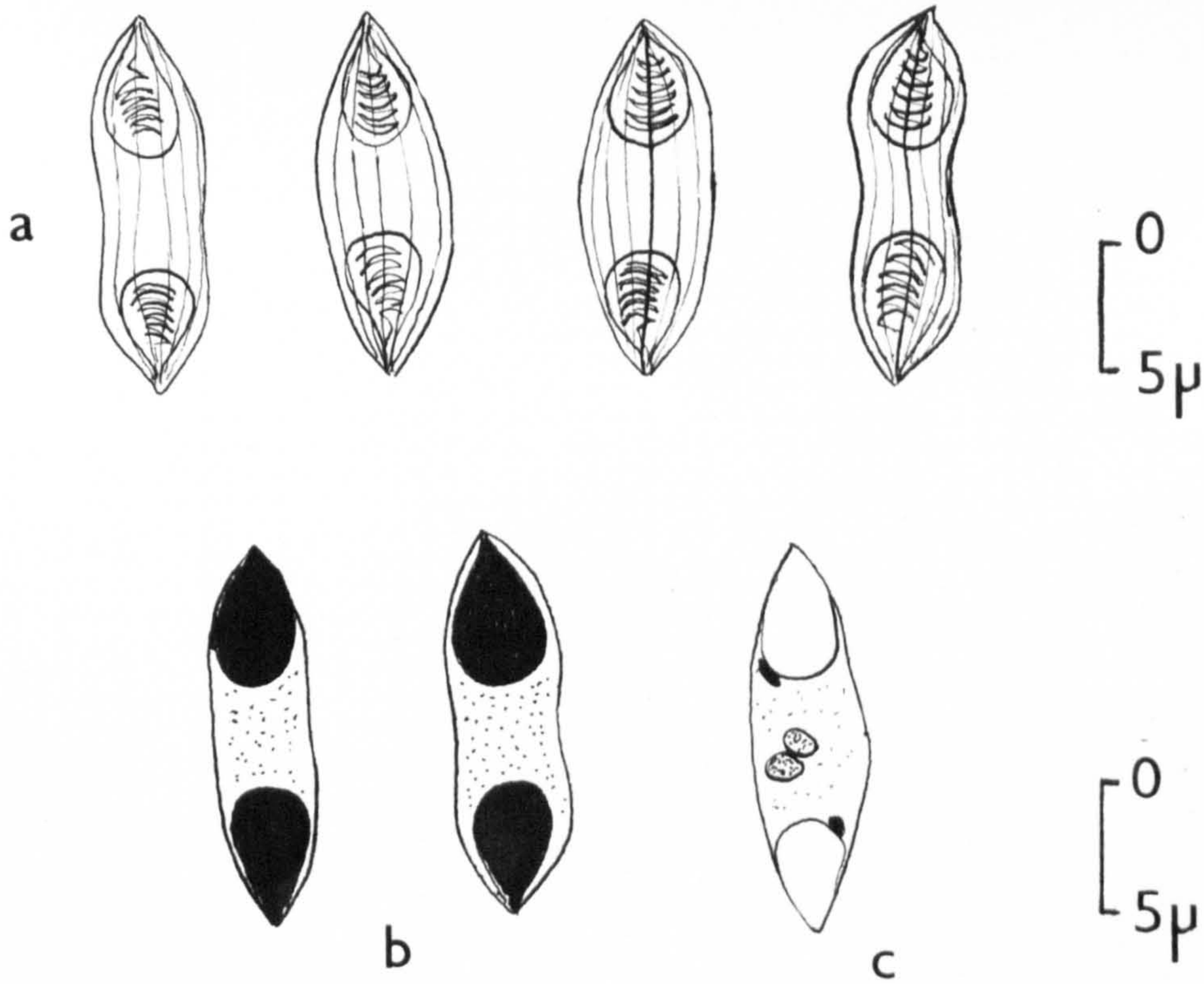
M. ophiocephali Akhmerov, 1960 (from the gall - bladder of Ophicephalus argus warpachowskii Berg in the U.S.S.R.), described from spores only, has spores which have similar dimensions to the present material, but which never constricted in the middle.

M. ventricosum Schulman, 1962 (from the kidney tubules of Thymallus arcticus (Pallas) in the U.S.S.R.) has wider spores (6.50 - 7.00 µ) and longer polar capsules (5.20 - 6.00 µ) than the present material.

M. rimskykorsakowi Schulman, 1962 (from the urinary bladder of Percottus flehni Dybowski in the U.S.S.R.) differs in having slightly wider spores (6.50 - 7.00 μ) and in having a curved suture line.

Myxidium sp. Schulman, 1962 (from the gall - bladder of Pseudobagrus fulvidraco (Richardson) in China) differs in possessing spores which are expanded in the central region.

Conclusion;- The species of Myxidium found in R.rutilus in St. James's Park is M. rhodei. M. pfeifferi may be synonymous with M. rhodei. Close similarities are noted between M. rhodei and several other species, particularly M. barbatulae and M. histophilum.



F I G U R E 15.

Myxidium rhodei from Rutilus rutilus.

a. Spores. Fresh preparations.

b. Spores. Giemsa's stain.

c. Spores. Ehrlich's haematoxylin and eosin stain.

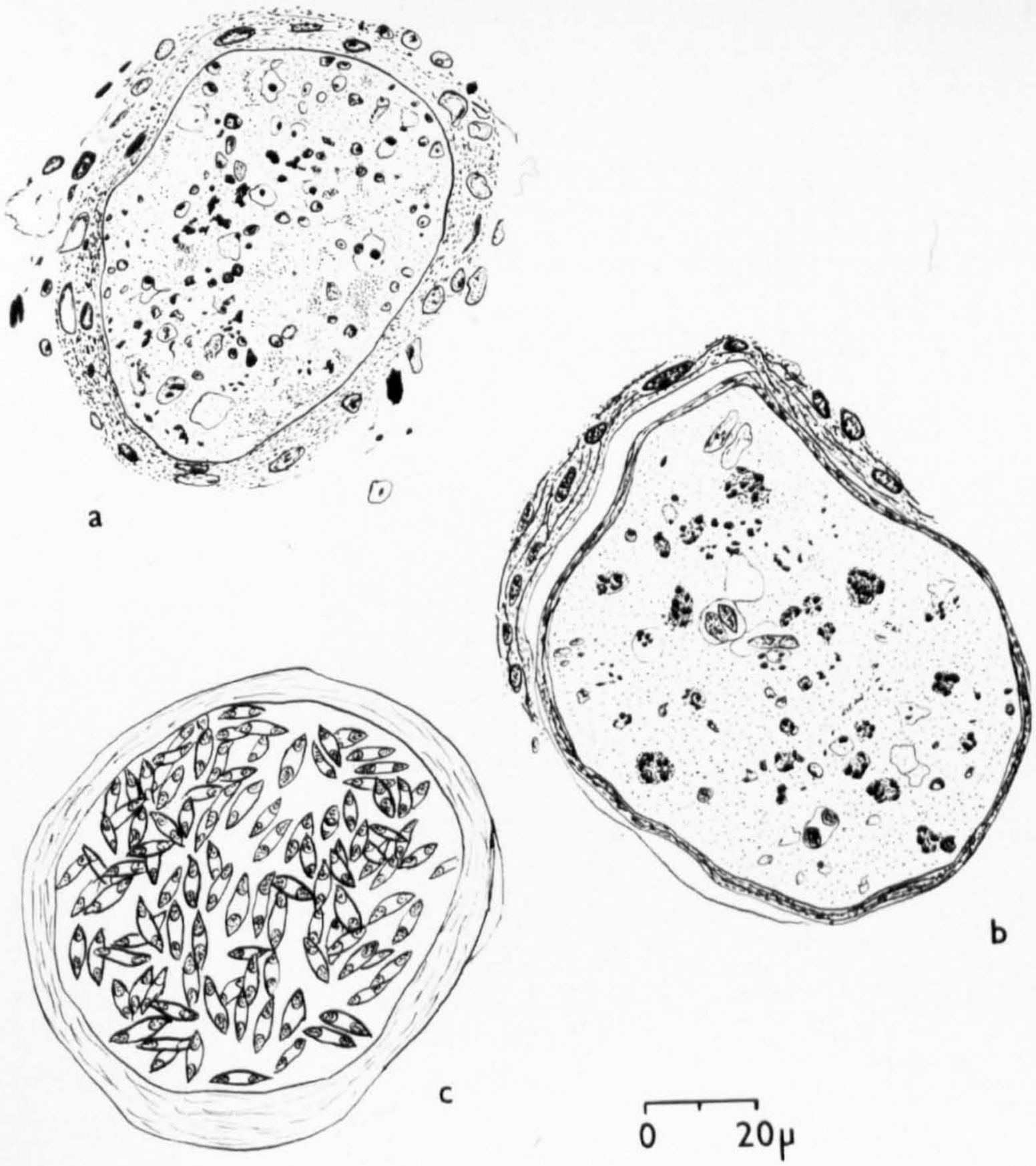


FIGURE 16.

Myxidium rhodei from Rutilus rutilus.

a. Early cyst. Ehrlich's haematoxylin and eosin stain.

b. Later cyst. Ehrlich's haematoxylin and eosin stain.

c. Mature cyst. Giemsa's stain.

e. Myxidium sp.2 from Gobio gobio (L.)

In one specimen of Gobio gobio collected in the river Colne in July, 1965, myxosporidan spores were seen in Giemsa - stained smears of the kidney which could be identified as belonging to the genus Myxidium. Examination of smears and sectioned preparations revcaled developing pansporoblasts and mature cysts. Spores and developmental stages were drawn and measured from methanol - fixed, Giemsa - stained smears, and cysts from sectioned preparations.

Spores (Fig: 17a);- Spores fusiform in shape with pointed ends. Two polar capsules, one opening at each end. Two spore valves, with eight longitudinal striations parallel to the sutural line on each valve. Sporoplasm and capsulogenous nuclei visible only in immature spores stained with Giemsa's stain (Fig: 17.1). Spore measurements (in μ) from methanol - fixed and Giemsa - stained smears, were as follows (30 spores measured);-

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	16.31	5.10	4.98	3.30
Range	15.00 - 17.75	4.50 - 6.00	4.00 - 5.50	3.00 - 4.00

Trophozoites;- Trophozoites formed cysts (Fig: 17 b) in the kidney which were surrounded by a thin cellular wall and measured up to 100 μ in diameter. The cysts differed from those of M. rhodei in Rutilus rutilus in having no apparent development of fibrous tissue around them. In all cysts, developing pansporoblasts and mature spores

were visible. Giemsa - stained smears revealed these developmental stages more clearly (Fig: 17c - m). The earliest stage seen was the binucleate pansporoblast (Fig: 17c), and several stages between this and multinucleate pansporoblasts could be traced (Fig: 17d - j). In the multinucleate bodies, nuclei appeared to be differentiated into two types, large and small. Maturing spores could be followed from the six - nucleate condition (Fig: 17k), in which the two sporoplasm nuclei, two capsulogenous nuclei and two valvular nuclei could be differentiated in each developing spore. The pansporoblasts were disporous. Mature spores (Fig:17 m) showed no trace of any nuclei.

Identification;- The only species of Myxidium described so far from G. gobio is M. pseudomacrocapsulare Gvozdev, 1950. This species occurs in the ureter of its host, and differs markedly from the present material in its spores which are S - shaped and only 11.00 - 12.00 μ long. Another species of gudgeon, G. albipinnatus Iuk., is parasitized by M. orientalis Schulman, 1962 in the gall - bladder, but this species differs from the present material in possessing much shorter spores (10.00 - 11.00 μ).

The present species corresponds well, on dimensional grounds, with M. rhodei. The spores are larger than those found in R. rutilus, but are embraced by the size range of M. rhodei spores given by Bykhovskaya - Pavlovskaya et al. (1962) (11.00 - 18.00 μ long by 4.50 - 6.50 μ broad). They differ slightly in their proportions; the polar capsules of the present species are relatively shorter than those found in R. rutilus, and no spores are constricted in the middle. It is possible

that this species is a different strain of M. rhodei from that in R. rutilus

The present material is also comparable with several other species, which are discussed briefly here.

M. barbatulae, which, as mentioned under the description of M. rhodei in part 2(ii)d, may be synonymous with M. rhodei, differs in having shorter spores (12.00 - 15.00 μ) and narrower polar capsules (2.50 - 3.00 μ). M. barbatulae forms cysts in the kidney of its host, Nemacheilus barbatulus.

M. histophilum (from the kidney and ovary of Phoxinus phoxinus) differs in its spore shape, which is constricted at the centre.

M. cruzi Penido, 1927 (from the gall - bladder of Chalcinus nematurus Kner. in Brazil) has slightly larger spores (17.00 - 18.00 μ long by 5.00 - 7.00 μ broad) than the present species.

M. ctenopharyngodonis Akhmerov, 1960 (from the kidney tubules of Ctenopharyngodon idella (Valenciennes) in the U.S.S.R.) has spores of very similar appearance to the present species, but with a greater size range (13.00 - 21.00 μ long by 5.00 - 6.50 μ broad). The trophozoites are described as "? round cysts" by Bykhovskaya - Pavlovskaya et al. (1962).

M. heteropneustesi Chakravarty, 1943 (from the gall - bladder of Heteropneustes fossilis (Bloch) in India) has spores of slightly different dimensions (14.42 μ long by 6.18 μ broad) from the present species.

Myxidium sp. Yasutake and Wood, 1957 (from the kidney of Oncorhynchus kisutch (Walbaum) in the U.S.A.) has broader spores (6.50 - 8.00 μ), which are also more pointed than those of the present species.

Myxidium sp. Schulman, 1962 (from the gall - bladder of Pseudobagrus fulvidraco in China)differs in having shorter spores (12.00 - 13.00 μ). The trophozoites of this species were not described.

Conclusion; The species of Myxidium found in G. gobio cannot be identified with certainty as a previously described species. It may be a strain of M. rhodei different from that found in Rutilus rutilus in St. James's Park. Among other comparable species, it is most similar to M. ctenopharyngodonis.

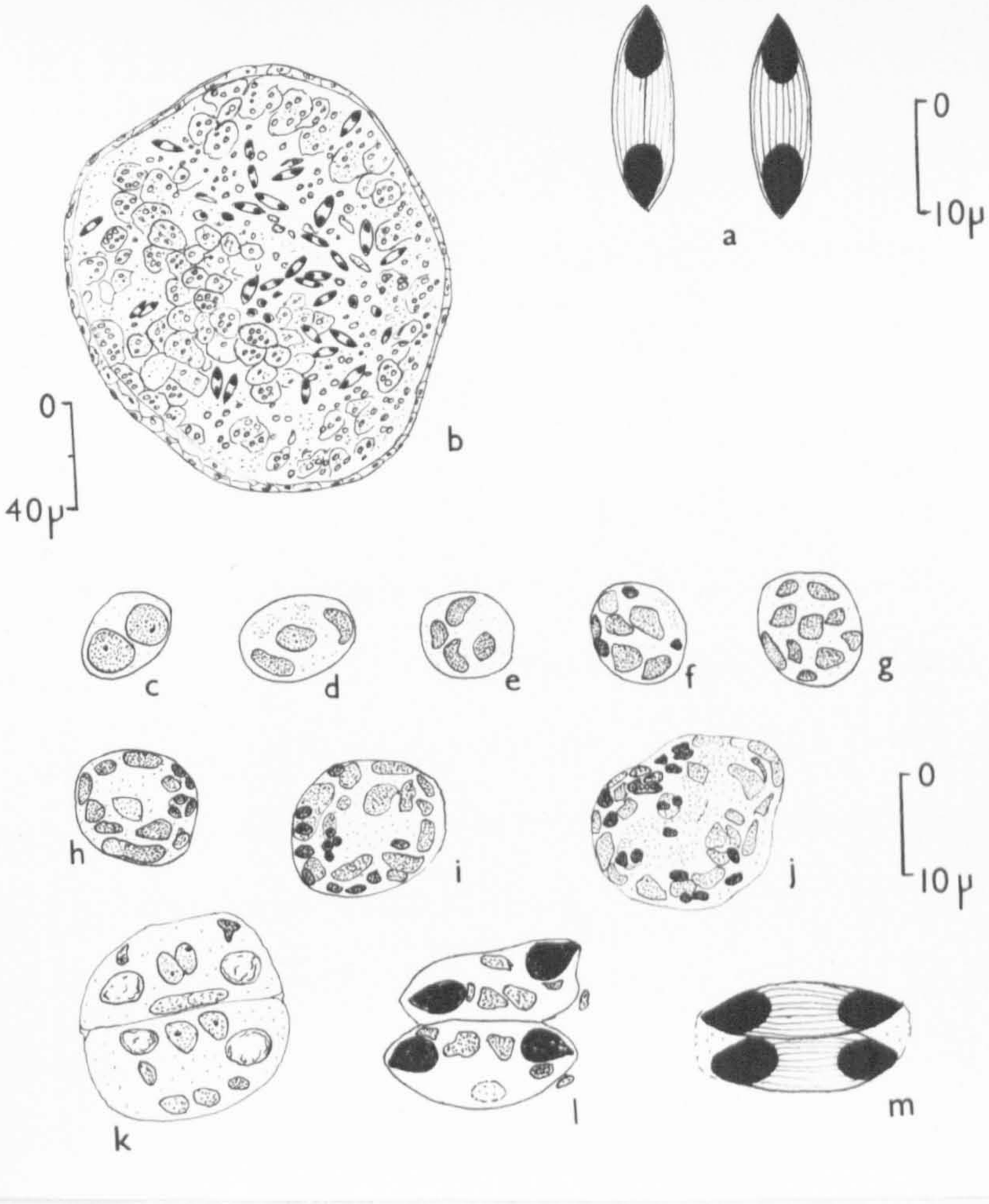


FIGURE 17.

Myxidium sp. 2 from Gobio gobio.
Giemsa's stain.

a. Spores.

b. Cyst.

c - j. Stages in development of pansporoblasts.

k, l. Developing spores.

m. Mature spores.

f. Myxidium sp. 3 from Salmo salar L.

In June, 1965, a specimen of Salmo salar, collected in a river in Northern Ireland and sent to the Veterinary Research Division of the Ministry of Agriculture for Northern Ireland in Belfast, was found to be infected with a myxosporidan parasite in the liver. A section of the infected liver was sent to the London School of Hygiene and Tropical Medicine, where the parasite was identified as a species of Myxidium. During the following winter and spring, livers from British salmon were collected from a firm of salmon importers in London and examined for the parasite. In April, 1966, large numbers of infected livers were found. In general, the infection was confined to one lobe of the liver, and was recognisable macroscopically by the yellow pustular appearance of the lobe. Slight pressure on infected regions liberated spores as a dense yellow fluid. Spores were drawn and measured both from fresh smears and from smears fixed in methanol and stained with Giemsa's stain. Trophozoites were examined and drawn from sectioned preparations.

Spores (Fig: 18a,b,c,d.); Spores oval in front view, with rounded ends. Two polar capsules, one opening at each end. In side view, suture line slightly S - shaped, and polar capsules opening diagonally to the longitudinal axis of the spore. Spore valves thin and slightly pointed at the polar capsule openings. 8 - 10 fine longitudinal striations on each valve. Two sporoplasm nuclei and two capsulogenous nuclei visible in sections stained with Ehrlich's haematoxylin and eosin (Fig: 18c).

Spores in methanol - fixed and Giemsa - stained smears (Fig: 18d) were seen to have more pointed ends than fresh spores, and also to be slightly longer. This is unusual, as spores normally shrink under the action of fixatives (Kudo, 1921 b).

Spore measurements (in μ) were as follows;-

Fresh spores (50 measured)

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	10.50	6.11	3.99	3.02
Range	9.50 - 11.00	5.00 - 7.00	3.25 - 4.50	2.50 - 3.25

Methanol - fixed spores (15 measured)

	Length	Breadth	Polar capsule length	Polar capsule breadth
Average	11.33	6.43	3.78	2.58
Range	10.50 - 12,00	6.00 - 7.00	3.50 - 4.00	2.25 - 3.00

Extrusion of polar filaments (Fig: 18e) was caused by mounting spores in a saturated solution of urea or in a 5% solution of potassium hydroxide. In these fluids, extrusion took place after approximately 1 minute. After 20 minutes, 60% of the spores mounted in urea had extruded both polar filaments, and a further 10% 1 filament only. Of the spores mounted in 5% potassium hydroxide, 95% had extruded both filaments after 20 minutes, and a further 4% 1 filament only. The average length of an extruded filament was 33.05 μ, the range being 28.00 - 37.00 μ (20 filaments measured). After discharge of the filaments, the

polar capsule volume was reduced.

Trophozoites (Fig: 19 a,b,c);- The livers available for examination were not fresh. In sectioned preparations, it was found that the parenchyma cells had degenerated too far to be recognisable. Some details of the trophozoites could be elucidated, however.

Trophozoites appeared to be flattened, leaf - like, structures in the centre of which spores were differentiated. They measured up to 60 μ in thickness. They were surrounded by a thin cyst wall, 1 cell - layer in thickness, outside which was some development of fibrous tissue (Fig: 19c). Within the cyst wall several trophozoites appeared to be enclosed. From the sectioned preparations available, however, it was not possible to determine whether these represented only one large trophozoite, folded around itself.

In sections, trophozoites appeared to be polysporous. In smears, spores were commonly seen in pairs (Fig: 18h), suggesting disporous development. Immature spores, with six nuclei (Fig: 18g), and multinucleate bodies (Fig: 18f), which were probably early pansporoblasts, were also visible in smears.

Identification; The only species of Myxidium described so far from S. salar is M. oviforme Parisi, 1912 which occurs in the gall - bladder. According to the description given by Bykhovskaya - Pavlovskaya et al. (1962), M. oviforme can also extend into the hepatic ducts and cause intense inflammation of the liver. The parasite differs from the present species in three respects; the spores are wider (7.00 - 9.00 μ),

the polar capsules relatively narrower (2.00 - 3.00 μ), and the trophozoites are small spherical plasmodia of diameter 10 - 12 μ.

Another species of Myxidium which occurs in salmonid fishes is M. minteri Yasutake and Wood, 1957. Although spore dimensions of this species are similar to those of the present species, it differs in even more respects than M. oviforme; M. minteri spores have smaller polar capsules (2.30 - 3.80 μ long) and a straight suture,and trophozoites occur in the kidney tubules. M. minteri was described from the north - west States of the U.S.A. in the "coho" salmon, Oncorhynchus kisutch (Walbaum).

There are several species of Myxidium from families other than the Salmonidae which compare with the present material. These are considered briefly here.

The most similar species in M. folium Bond, 1938, which occurs in the hepatic ducts of Fundulus heteroclitus (L.) (family Cyprinodontidae) in the U.S.A. The spore dimensions and features correspond almost exactly with those of the present species, with the exception of a small protuberance at the opening of the polar capsules; a similar but less obvious, protuberance occurs in the present spores. The trophozoites of M. folium are described as green and leaf - like, with polysporous and disporous spore development.

M. moxostomatis Kudo, 1921, from the gall - bladder of Moxostoma sp. (family Cyprinodontidae) in the U.S.A., also compares well with the present species in its trophozoites, which are leaf - like. Spore features are very similar, although they are slightly smaller (8.50 - 10. 50 μ long by 5.00 - 6.00 μ broad).

M. kudoi Meglitsch, 1937, from the gall - bladder of Ictalurus furcatus (family Ictaluridae) in the U.S.A., is another species with similar trophozites, described as flattened, yellowish and often rolled up. Spores are found in the inner region, and polysporous development occurs. The polar capsules are slightly shorter than in the present species (2.50 - 3.50 μ), and also differ in opening in the line of the longitudinal axis.

M. aplodinoti Kudo, 1934, from the gall - bladder of Aplodinotus grunniens Raf. (family Sciaenidae) in the U.S.A., was described from spores only. These are identical with the present spores in their features and measurements.

M. parvoviforme Fantham, 1930, found in the bile of Johnsonius hololepidotus (Lacépède) (family Sciaenidae) in South Africa, has slightly smaller spores (8.00 - 11.00 μ long by 5.50 - 7.00 μ broad) and also differs in having polar capsules opening in the line of the longitudinal axis of the spore.

M. macrocapsulare Auerbach, 1910, from the gall - bladder of Scardinius erythrophthalmus L. (family Cyprinidae) and Aplodinotus grunniens (family Sciaenidae) in Europe, differs in some spore festures; the spore is more S - shaped in side view than the present spores, the valves are thicker, and the polar capsules are broader (3.80 μ). The trophozoites of this parasite were not described.

M. pseudomacrocapsulare Gvozdev, 1950, from the ureter of Gobio gobio (family Cyprinidae) in the U.S.S.R., has slightly larger spores (11.00 - 12.00 μ long by 6.00 - 8.00 μ broad), which are also more S - shaped in side view.

M. striatum Cunha and Fonseca, 1917, from the gall - bladder of Menticirrhus americanus (L.) and Bairdiella ronchus (C. and V.) (both in family Sciaenidae) in Brazil, differs in having spores which are thickened at each end, and which are slightly larger (10.00 - 14.00 μ long by 6.00 - 8.00 μ broad).

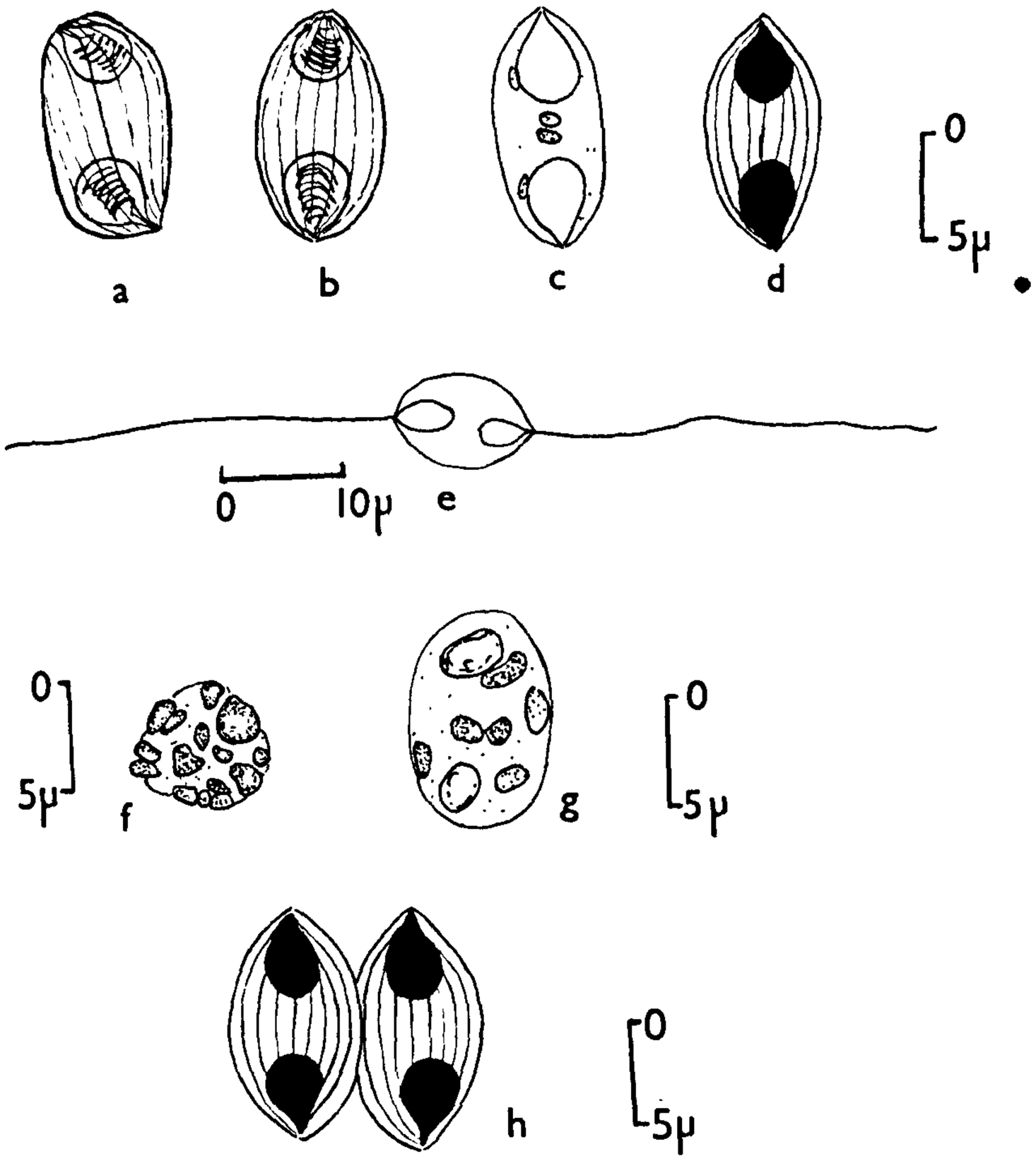
M. glutinosum Davis, 1917, from the gall - bladder of Cynoscion regalis (Bloch and Schneider) (family Sciaenidae) in the U.S.A. has a transparent envelope around the spores, and slightly shorter polar capsules (3.00 μ). The trophozoite also differs in being small (20 μ in diameter).

M. melum Otto and Jahn, 1943, from the bile of Ictalurus melas (Raf.) (family Ictaluridae) and Pomoxis sparoides (family Centrarchidae) in the U.S.A., has slightly smaller polar capsules (3.00 μ long), but the spores are similar otherwise. The trophozoites are described as white, measuring 550 μ by 1,070 μ.

Conclusions; It can be seen that the species described here as Myxidium sp. 3 cannot be identified with certainty, as it resembles several species fairly closely. Of Myxidium species from salmonid fishes, the nearest is M. oviforme, but this differs markedly in the spore width, and in its trophozoites. Among other species of Myxidium the closest is M. folium, but close similarities are also seen in M. moxostomatis and M. aplodinoti.

Economic importance; During 1965, many salmon in the British Isles died from an unidentified disease, which was first noticed in Southern

Ireland. The species of Myxidium described above was found in many Irish salmon during the early summer of 1966. The Veterinary Research Division of the Ministry of Agriculture for Northern Ireland found particularly heavy infections in the River Foyle. It seems possible that the parasite could have been at least a contributary factor to the heavy mortalities, because of the damage caused to the liver; in heavy infections, the surface of an infected lobe was covered with small abcesses. However, infections were usually confined to one lobe, and the parasite was, therefore, unlikely to have been the primary cause of death.



F I G U R E 18.

Eyzidium sp. 3 from Salmo salar.

a. Spore in side view. Fresh preparation.

b. Spore in front view. Fresh preparation.

c. Spore. Ehrlich's haematoxylin and eosin stain.

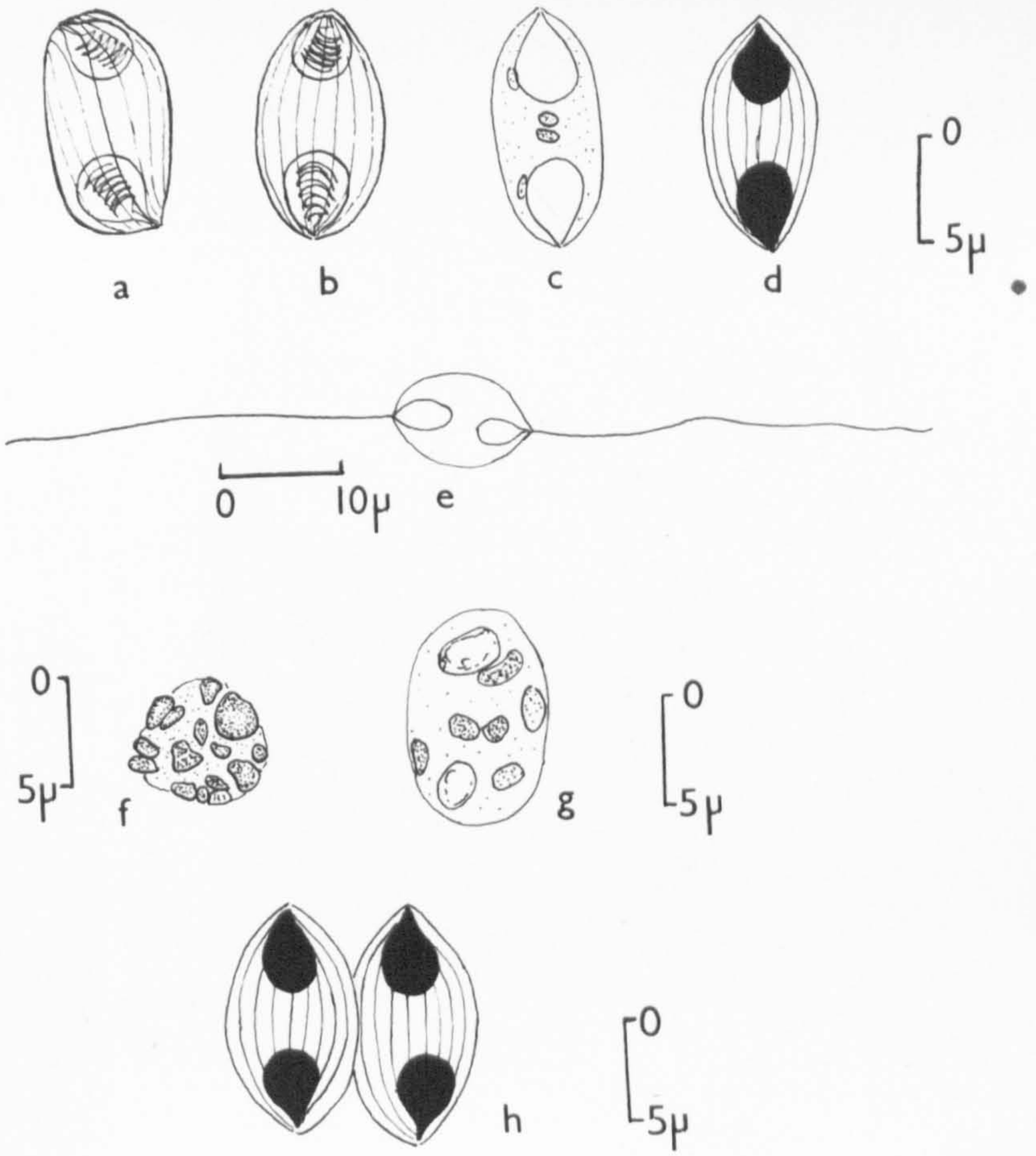
d. Spore. Giemsa's stain.

e. Spore with extruded polar filaments. Fresh preparation mounted in saturated solution of urea.

f. Early pansporoblast. Giemsa's stain.

g. Developing spore. Giemsa's stain.

h. Mature spores. Giemsa's stain.



F I G U R E 18.

Myxidium sp. 3 from Salmo salar.

a. Spore in side view. Fresh preparation.

b. Spore in front view. Fresh preparation.

c. Spore. Ehrlich's haematoxylin and eosin stain.

d. Spore. Giemsa's stain.

e. Spore with extruded polar filaments. Fresh preparation mounted in saturated solution of urea.

f. Early pansporoblast. Giemsa's stain.

g. Developing spore. Giemsa's stain.

h. Mature spores. Giemsa's stain.

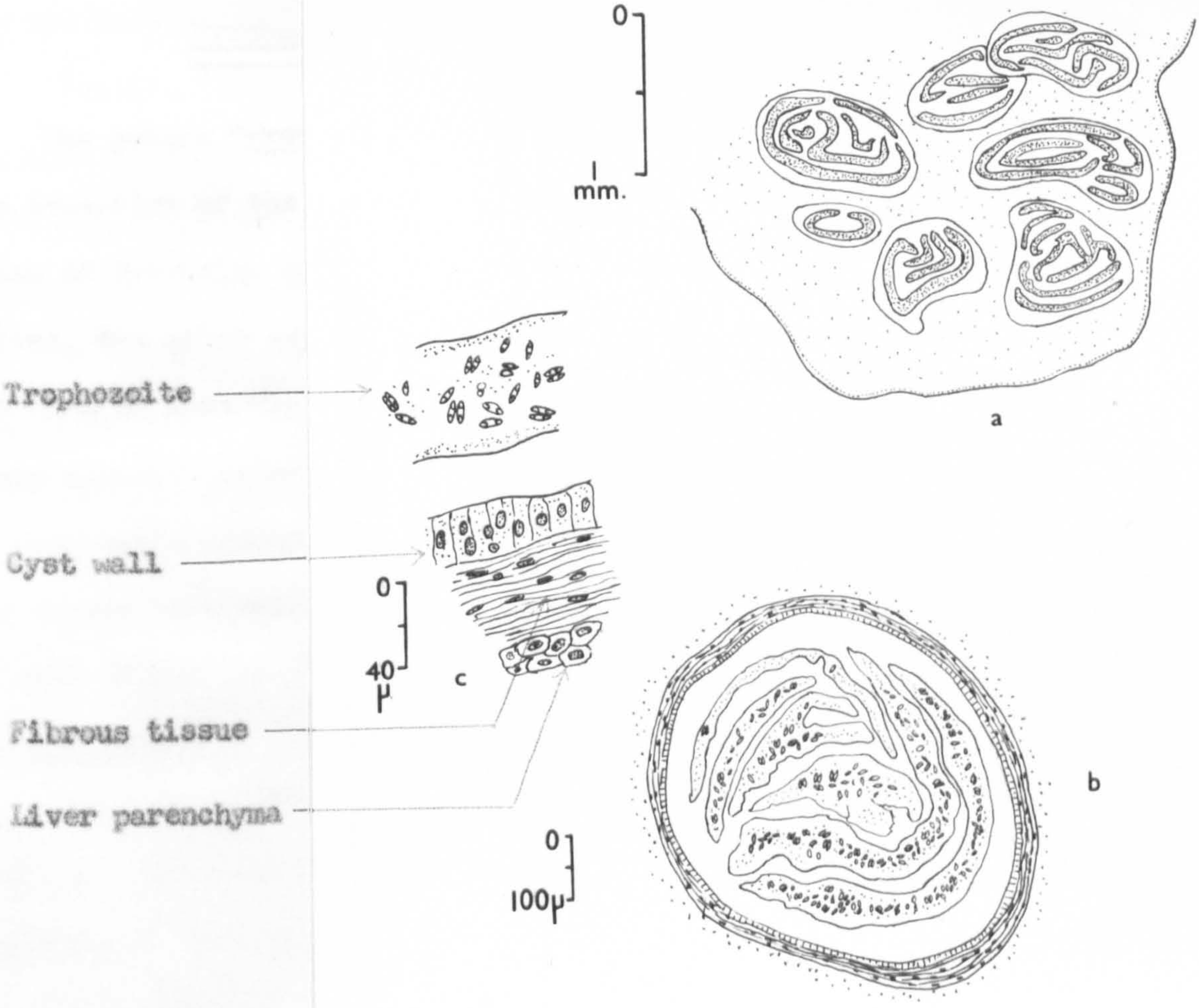


FIGURE 19.

Myxidium sp. 3 from Salmo salar.

Sections of trophozoites in liver. Ehrlich's haematoxylin and eosin stain.

a. Low - power view of infected lobe.

b. Cyst enclosing leaf - like trophozoites.

c. Section through trophozoite and cyst wall.

3. An investigation of the genera

Myxobolus Bütschli, 1882 and Myxosoma Thelohan, 1892

The genera Myxobolus and Myxosoma are similar in all respects, with the exception of the presence of an iodinophilous vacuole in the sporoplasm of Myxobolus spores. Thus, the spores of each genus have two valves, two polar capsules opening at the anterior end, and are ovoidal and more or less flattened in shape. Despite this great similarity, in every classification of Myxosporida proposed so far, with the exception of Auerbach's system (1911) and the proposals of Akhmerov (1960), the two genera have been placed in separate families, and sometimes separated at even higher levels.

(i) The nature of the iodinophilous vacuole.

The iodinophilous vacuole was first seen and illustrated by Muller (1841). Butschli (1881) noted the vacuole in spores of Myxobolus muelleri and called it a nucleus. He found that it became more distinct on the addition of acetic acid or iodine solution, but failed to stain it with nuclear stains, attributing this to the failure of the stain to penetrate the spore valves.

Thelohan (1889) also noted that the vacuole was barely visible in the fresh state, but became more apparent under the action of alcohol, acetic, nitric and osmic acids and a 2% solution of silver nitrate. He found that it was unaffected by nuclear stains; in iodine, however, the vacuole took up a red-brown colour while the remainder of the spore became yellow. Thelohan pointed out the similarity of this reaction to the glycogen reaction with iodine and further showed that the vacuole contents

were insoluble in alcohol, soluble in alkalies and altered by acids, iodine having no effect after acid treatment.

Perugia (1891) confirmed Thélohan's results, showing that the vacuole stained red-brown in iodine. Curley (1894) found that the staining reaction was best obtained with an aqueous solution of iodine in potassium iodide. He also considered that the contents probably served as a food reservoir for the sporoplasm, pointing out that the vacuole was a feature of the sporoplasm only, and not of the trophozoite.

Kudo (1921a) considered that the vacuole was unlikely to be a food store, and carried out several tests on it, with the following results. In Lugol's iodine, the vacuole stained an orange-brown colour which disappeared on warming. After spores had been kept in distilled water for six hours, Lugol's stain was ineffective. Using Lubarsch's method, the vacuole, polar capsules and spore valves stained deep blue-violet. With Best's carmine, the vacuole took up a faint pink colour. With Delafield's haematoxylin and Lugol's stain, a red-brown colour was seen. Kudo concluded that the contents of the vacuole were similar to glycogen, and further showed that in Henneguya salminicola Ward, 1919 the glycogenous substance was inconspicuous in the pansporoblast, but sharply outlined in mature spores.

Bond (1940) carried out a series of studies to show the presence of glycogenous material in spores of species from families other than the Myxobolidae. He stained smears and sections of spores of Myxosoma subtecalis Bond, 1938, M. funduli Kudo, 1919, and Myxidium folium Bond, 1938 with Lugol's iodine, Best's carmine and the Bauer modification of

the Feulgen technique. In all cases, there was an uptake of stain, showing a diffuse or particulate distribution in each species.

In the present work, the presence or absence of glycogen was investigated in the spores of selected myxosporidan species, using methods similar to those of Bond (1940). Sectioned preparations of infected tissues, fixed in Carnoy's fluid or in formol saline, were stained with Lugol's iodine, Best's carmine or, in selected species, with the Bauer modification of the Feulgen technique. Control slides were immersed in saliva for 30 minutes prior to staining.

Results

The most satisfactory results were seen when Best's carmine was used. The appearances of the species examined by this technique were as follows.

a. Species of Myxobolus from Rutilus rutilus, Gobio gobio and Leuciscus leuciscus (Carnoy fixation).

In all species, the spores showed either a clearly outlined vacuole, stained bright red (Fig: 20), a particulate (Fig: 21a) or diffuse (Fig: 21b) stain uptake, or no staining reaction at all. The percentage of spores examined showing each type of reaction is given in tables 5a and 6.

b. Myxobolus sp. 1. from Perrasalmus rhombeus (Formol saline fixation).

The majority of spores were unstained; in a few, however, a clearly outlined red vacuole or a diffuse stain uptake was seen. The percentage of spores showing each type of reaction is given in table 6. As glycogen may be dissolved out by aqueous fixatives, the absence of stain may not

necessarily indicate a lack of glycogen.

c. Agarella gracilis from Lepidosiren paradoxa (Carnoy fixation).

The majority of spores were unstained, although a particulate stain distribution was noted in some spores. Small red-stained droplets were also seen around and between clumps of spores. The proportion of spores showing stain uptake is given in table 7.

d. Myxidium rhodei from Rutilus rutilus (Carnoy fixation).

All spores were unstained, but small red-stained globules were seen in cysts, around the spores.

e. Myxosoma heterospora Baker, 1963 from the spleen of Tilapia esculenta Graham, 1928 (Carnoy fixation).

The spores showed either a clearly outlined red-stained vacuole, a diffuse or particulate staining reaction, or no stain uptake. The percentages of spores showing each type of reaction are given in table 7.

The Bauer modification of the Feulgen technique gave similar results to Best's carmine, the colour, in this case, being a less intense red or mauve. Table 5c gives the percentages of spores showing each type of staining reaction.

Lugol's iodine was the least satisfactory stain employed as, besides the sporoplasm, the remainder of the spore, the trophozoite and the surrounding tissue took up a yellow or brown stain. This, together with some loss in clarity, rendered the distinction of spore inclusions difficult. A further disadvantage of the technique was that iodine-

stained preparations tended to fade rapidly. The percentages of spores showing vacuoles, diffuse stain uptake and no staining reaction are given in tables 5b and 6.

In none of the control slides, on which salivary digestion had been carried out, was uptake of stain noted.

Discussion

The chief conclusion that can be drawn from these results is that glycogen, or a glycogenous material, is present in spores of all species of Myxobolus examined, and in Agarella gracilis and Myxosoma heterospora, but absent from spores of Myxidium rhodei. Of particular interest is the variable amount of glycogenous material in the species containing it, there being apparently none in some cases. There appeared to be little variation in the proportions of spores containing glycogen from one organ to another or from one locality to another.

Bond (1940) considered that when spores remained unstained by Best's carmine, the stain had failed to penetrate the spore valves; he suggested that staining was obtained only when the spores were cut in section. He also found that the Bauer modification of the Feulgen technique gave the most accurate results. In the present study, reference to table 5 shows little difference between the Best's carmine and the Bauer-Feulgen results. Further, when stain uptake occurred, the quantity of material stained was very variable, ranging from a comparatively large vacuole to one or two small globules. It seems more probable, therefore, that some spores contain glycogen and some do not.

From the material examined, it was difficult to determine whether

the quantity of glycogen varied according to the maturity of the spore, as all types of stain uptake occurred in apparently mature spores. As glycogen is employed as a food storage substance by many higher animals, it may possibly be used either in the maturation of the spore, or in the future development of the sporoplasm. If the former, spores without glycogen would be mature, but if the latter those with the greatest glycogen content would be mature.

(ii) The use of the iodinophilous vacuole in classification.

The iodinophilous vacuole was first used by Thelohan in his classification (1892) to separate the "Myxobolees" (including the genus Myxobolus) with one or two polar capsules on the one hand, from the "Myxidiees" (including the genus Myxosoma) with two polar capsules and the "Chloromyxees" with four polar capsules on the other. The terms "Myxobolees","Myxidiees" and "Chloromyxees" were subsequently made over into the families Myxobolidae, Myxididae and Chloromyxidae by Gurley (1893). These distinctions were retained in the systems of classification suggested by Labbé (1899), Doflein (1899) and Auerbach (1910).

Auerbach (1911) altered his system of 1910 by discarding all families. This brought the genera Myxobolus, Myxosoma and Lentospora Plehn, 1905 into the same "legion", the Polysporea . (The genus Lentospora was subsequently synonymised with Myxosoma by Kudo, 1933). Poche (1913) considered that the family was an "obligatory" category in any system of classification, and erected the family Myxosomatidae for Myxosoma and Lentospora, defining the family as Polysporea in which the sporoplasm does not contain an iodinophilous vacuole. Davis (1918) did

not notice the creation of this family by Poche, but included exactly the same genera in his family Myxosomidae.

Kudo (1919), basing his classification entirely on characters of the spore, placed the families Myxobolidae and Myxosomatidae into a new sub-order, the Platysporea. In his paper of 1933, he amended these families slightly by synonymising Lentospora with Myxosoma and creating the genus Thelohanellus for Myxobolidae with only one polar capsule. The composition of the two families was then as follows:-

Family	Myxosomatidae	Poche, 1913.
Genus	Myxosoma	Thelohan, 1892.
"	Agarella	Dunkerly, 1915.
Family	Myxobolidae	Thelohan, 1892.
Genus	Myxobolus	Bütschli, 1882.
"	Thelohanellus	Kudo, 1933.
"	Henneguya	Thelohan, 1892.
"	Hoferellus	Berg, 1898.

Tripathi (1948) considered that the presence or absence of an iodinophilous vacuole in the sporoplasm was indicative of a fundamental difference in metabolism, and used this character alone to distinguish two super-families in his sub-order Unipolaria, the Ceratomyxoidea without a vacuole, and the Myxoboloidea with one. His classification was as follows:-

Super-family	Ceratomyxoidea	n. superfam.
Family	Ceratomyxidae	Doflein, 1899 sensu emend.
Sub-family	Myxosomatinae	n. subfam.
Genus	Myxosoma	
Super-family	Myxoboloidea	n. superfam.
Family	Myxobolidae	
Genus	Myxobolus	
"	Hennegnya	
"	Hoferellus	

Tripathi transferred the genus Agarella from the Myxosomatidae to the Chloromyxidae, and Thelohanellus to a new family, the Thelohanellidae.

In his textbook (1954), Kudo used his classification of 1933, with the addition of certain new genera. Schulman (1959) included several changes in his system, but retained the families Myxosomatidae and Myxobolidae in the sub-order Platysporea. Meglitsch (1960) also retained the two families, but followed Tripathi in transferring Agarella to the Chloromyxidae. Kudo (1966), in accordance with the recommendations of the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists (1964), used the sub-orders Unipolarina Tripathi and Bipolarina Tripathi, and also followed Tripathi in placing Agarella in the Chloromyxidae. The composition of the families Myxosomatidae and Myxobolidae, according to Kudo (1966), is as follows:-

Family Myxosomatidae

Genus Myxosoma

Family Myxobolidae

Genus Myxobolus

" Thelohanellus

" Henneguya

" Hoferellus

" Unicauda Davis, 1944.

" Trigonosporous Hoshina, 1952.

" Neohenneguya Tripathi, 1953.

The difficulties resulting from the use of the iodinophilous vacuole to distinguish Myxobolus from Myxosoma have caused much confusion in the past. Examples are given here to illustrate this:-

a. Gurley (1894), in his description of Myxobolus oblongus Gurley, 1893, stated:- "Vacuole: I could not detect this structure, but do not wish, on the strength of the material available, to positively assert its absence."

b. Kudo (1919) described as Myxobolus funduli and Myxosoma funduli forms with and without a vacuole respectively from the same host in the same locality. Bond (1938) found that spores of Myxosoma funduli stained with Dobell's or Lugol's iodine solution failed to show any evidence of a vacuole, although a slight indication of a vacuole could sometimes be recognised in fresh material.

c. Guyenot and Naville (1922), in their description of Myxobolus ranae, stated that the spores contained a binucleate sporoplasm, consisting of a homogeneous granular protoplasm without a vacuole. They

placed the species, however, in the genus Myxobolus.

d. In the original descriptions of the following species of Myxobolus, an iodinophilous vacuole was neither mentioned in the text, nor illustrated:- M. permacnus Wegener, 1910, Myxobolus sp. Wegener, 1910, M. associatus Nemeczek, 1926, M. chondrophilus Nemeczek, 1926, M. cunhai Penido, 1927, M. pyrocentris Penido, 1927 and M. inaequalis Gurley, 1893.

e. Gurley (1894) mentioned that the vacuole of Myxobolus globosus Gurley, 1893 was not clearly contoured, and of M. transovalis Gurley, 1893, difficult to detect.

f. Bykhovskaya-Pavlovskaya et al. (1962) mention that in Myxobolus sprostoni Schulman, 1962 the vacuole is small and not always readily seen, and in Myxobolus sp. Akhmerov, 1960 it is not encountered in all spores.

g. In the following species of Myxobolus, the vacuole is described as taking up no stain, or difficult to stain:- M. diaphana Fantham, Porter and Richardson, 1940, M. dispar Thélohan, 1895, M. exiguus Thélohan, 1895, M. plectroplites Johnston and Bancroft, 1919, M. nemeczeki Schulman, 1962, and M. ribbosus Herrick, 1941.

h. Myxobolus conspicuus Kudo, 1929 is described as having a single vacuole, or one, two or three smaller vacuoles.

i. The following species of Myxosoma are allocated to the genus Myxobolus by Bykhovskaya-Pavlovskaya et al. (1962):- M. gigi (Fujita, 1927), M. kawabatae (Fujita, 1927) and M. lobatum Nemeczek, 1911.

j. Myxobolus anurus Cohn, 1895 is placed in the genus Myxosoma by Bykhovskaya-Pavlovskaya et al. (1962).

Doubts concerning the usefulness of the iodinophilous vacuole as a taxonomic criterion were hinted at by Gurley (1894) who, observing that the "vacuolate genera" tended to be absent from the excretory tract, queried "are the present generic references of some species correct, and are their structural characters accurately determined?" Akhmerov (1960) pointed out that in smears of Myxobolus spores, some spores showed a vacuole while others did not. He considered that the quantity of glycogen in the spores varied according to their maturity. Akhmerov further pointed out that in many species of helminths the glycogen content of the parasite varies with that of the organ it inhabits. From these observations, Akhmerov proposed that species of the sub-family Myxosomatinae Tripathi (containing the single genus Myxosoma) should be transferred to the genus Myxobolus.

As mentioned previously, the system of classification proposed by Kudo (1966) retains the families Myxosomatidae and Myxobolidae. Bykhovskaya-Pavlovskaya et al. (1962) also retain these families, adopting Schulman's classification (1959); in their key to parasites of freshwater fish of the U.S.S.R., the following species of Myxobolus, described as species novae by Akhmerov (1960), are transferred to the genus Myxosoma:- M. circulus, M. bilis, M. versandis, M. confessus, M. ornatus, M. soldatovi.

Results of the present work

The results of this study are in accordance with the observations of Akhmerov (1960). Table 8 shows the proportions of Myxobolus spores from Rutilus rutilus, Gobio gobio and Leuciscus leuciscus with clearly outlined vacuoles, as observed in sectioned preparations of spleen and

kidney stained with Best's carmine, Lugol's iodine and the Bauer modification of the Feulgen reaction. These proportions are analysed in more detail in tables 5 and 6.

Two features of particular importance to the classification of these species can be seen in these tables:-

a. In no preparation examined did the majority of spores possess a clearly outlined vacuole. The maximum detected in any one fish was 26.5% in Leuciscus leuciscus, and the maximum in a specific organ 33.6%, in the kidney of Leuciscus leuciscus.

b. In Rutilus rutilus, there was a considerable variation in the proportions of spores with vacuoles both from one fish to another and from one locality to another. Of the three fish examined from St. James's Park, only 0.6% of all spores examined from fish no: R. 1. contained vacuoles, while in R. 3. 18.5% contained vacuoles. In fish from the river Colne, very few vacuolate spores were seen, the maximum proportion being 0.8% of all spores examined in R. 9. In fish from the Brocket Estate, however, the minimum total of vacuolate spores recorded was 15.0% in R. 4.

These variations cause some difficulty in classification. Before the above studies, the species of Myxobolus from Rutilus rutilus was provisionally allocated to the genus Myxosoma (Walliker, 1966), chiefly from material from the river Colne, stained with Lugol's iodine. As shown above, very few vacuolate spores were found in this locality, and, as pointed out previously, Lugol's iodine was the least satisfactory stain employed for the detection of the vacuole.

Further, table 7 shows that clearly outlined vacuoles are present in 4.0% of spores of Myxosoma heterospora, stained with Best's carmine, Baker (1963) was unable to detect vacuoles in this species using Lugol's iodine.

Conclusion

From these observations, it is clear that no importance should be attached to the presence or absence of the so-called iodinophilous vacuole in myxosporidan taxonomy. It is proposed, therefore, following the suggestion of Akhmerov (1960), that the family Myxosomatidae Poche, 1913 and genus Myxosoma Thélohan, 1892 should be sunk, and that species of Myxosoma should be transferred to the genus Myxobolus Bütschli, 1882.

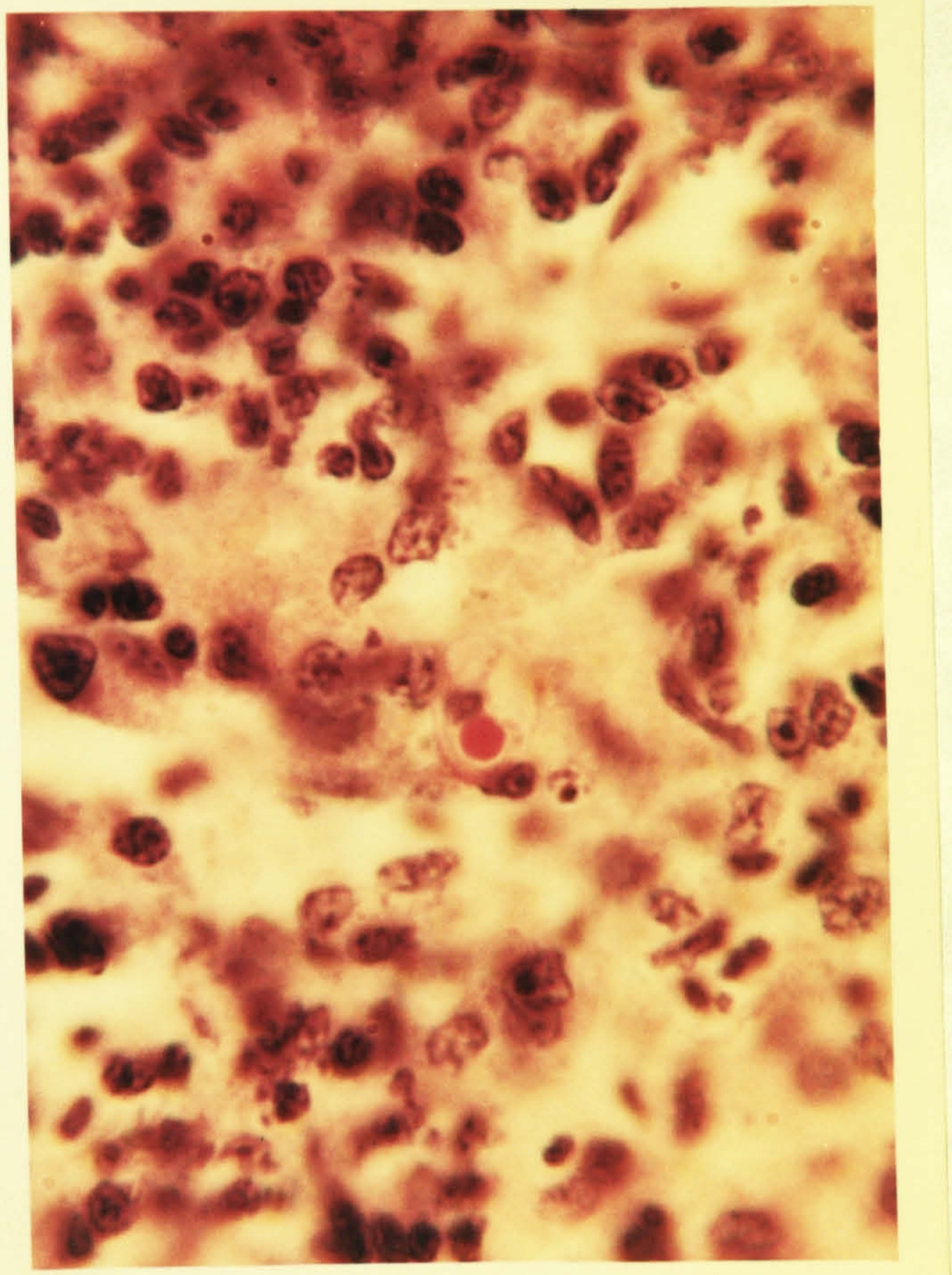


FIGURE 20.

Myxobolus sp. 4 from Hutilus rutilus.

Spore in section of spleen showing clearly outlined vacuole.

Best's carmine stain.

FIGURE 20.

Myxobolus sp. 4 from *Rutilus rutilus*.

Spore in section of spleen showing clearly outlined vacuole.

Best's carmine stain.

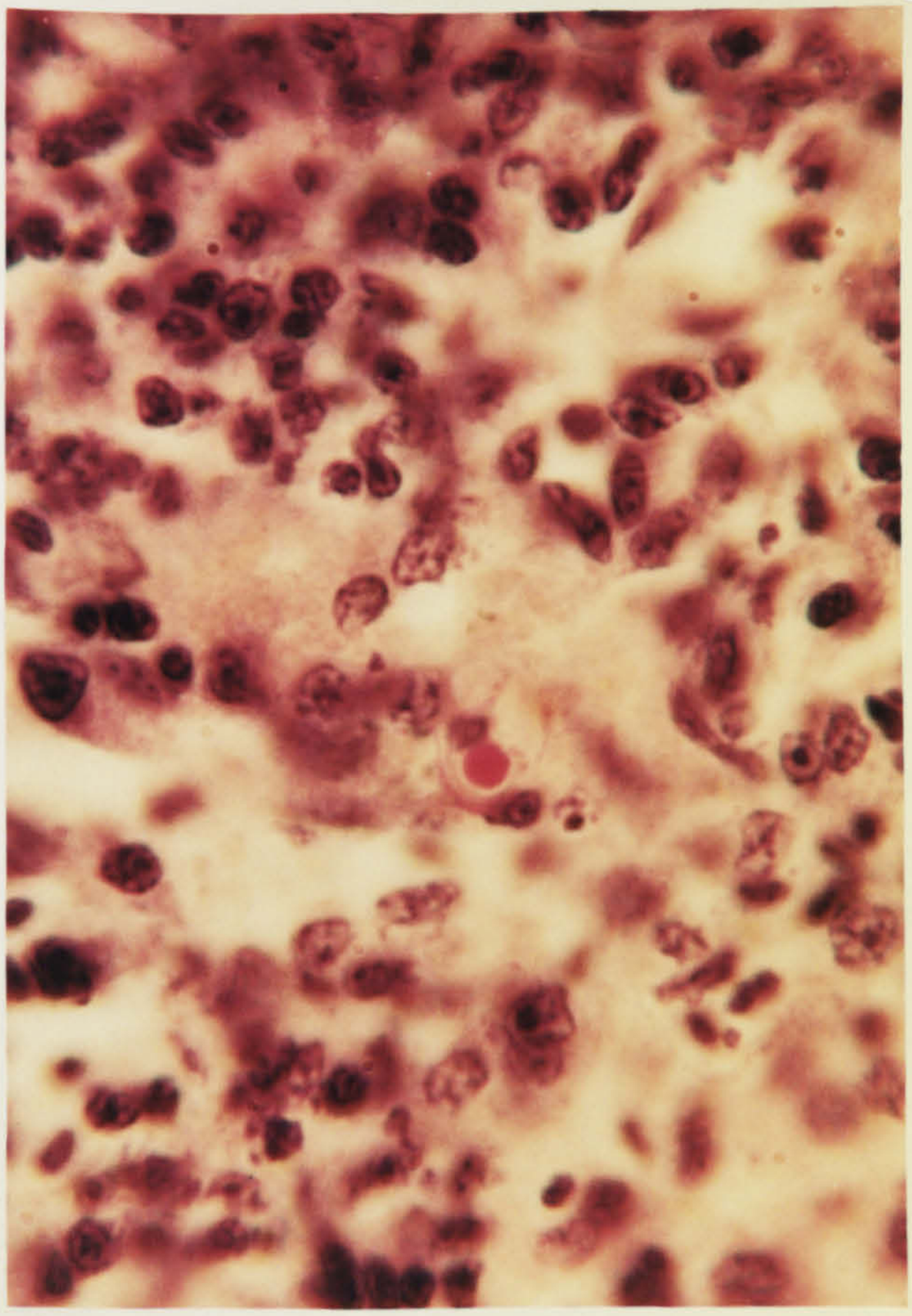


FIGURE 20.

Myxobolus sp. 4 from Rutilus rutilus.

Spore in section of spleen showing clearly outlined vacuole.

Best's carmine stain.

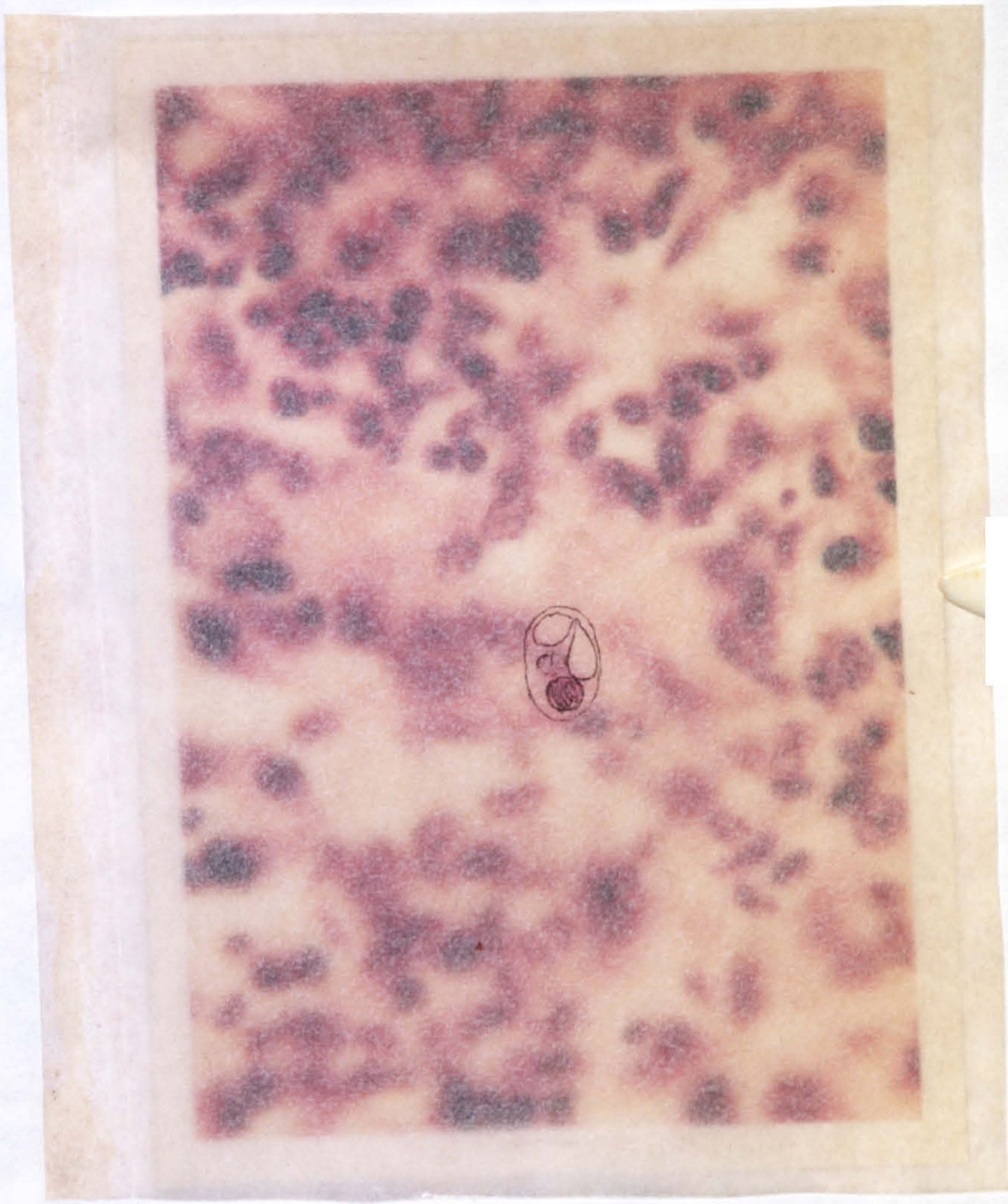


FIGURE 20.

Myxobolus sp. 4 from *Rutilus rutilus*.

Spore in section of spleen showing clearly outlined vacuole.

Best's carmine stain.

D

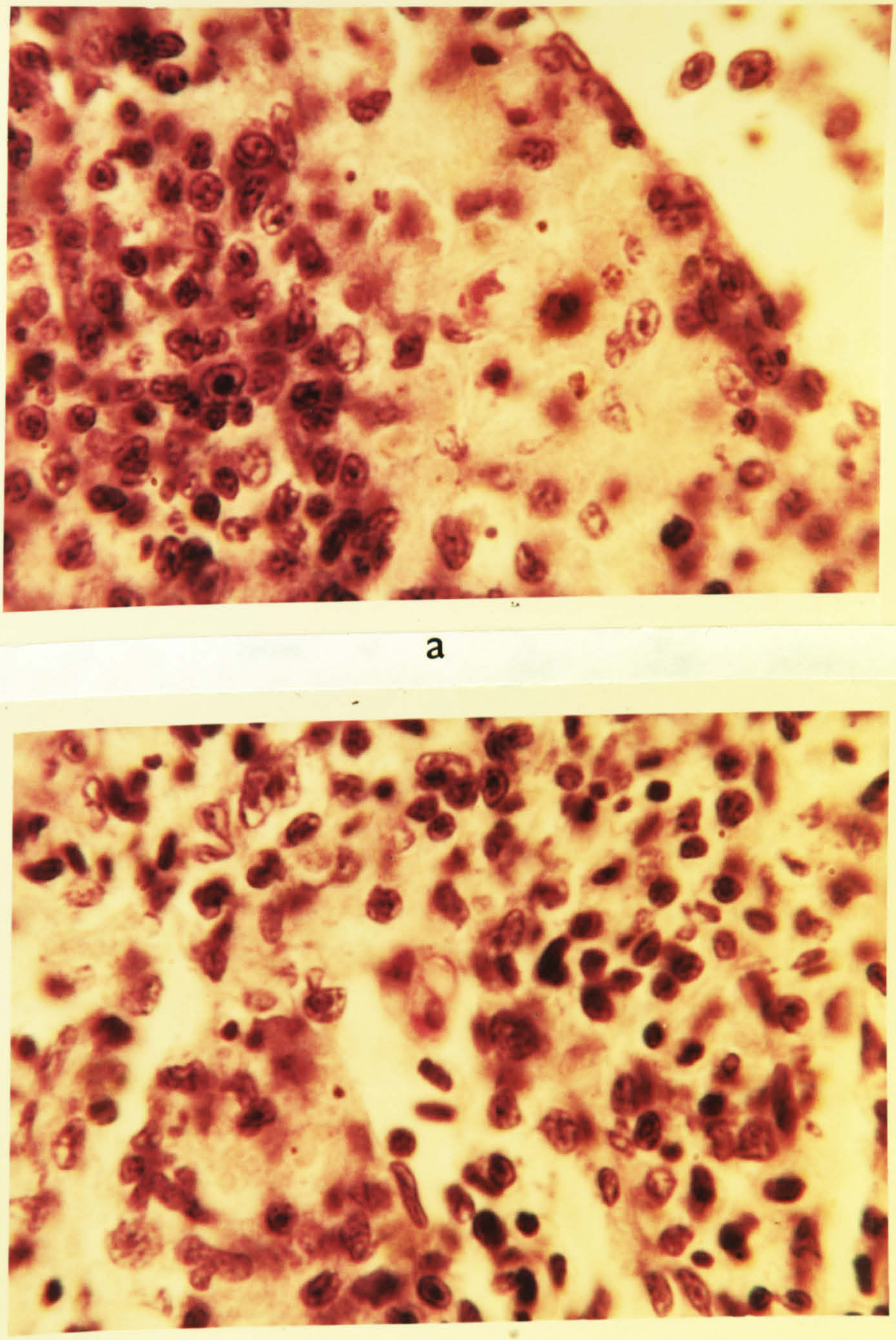
FIGURE 21.

Myxobolus sp. 4 from Rutilus rutilus

Spores in sections of spleen. Best's carmine stain

a. Particulate stain uptake.

b. Diffuse stain uptake.



a

b

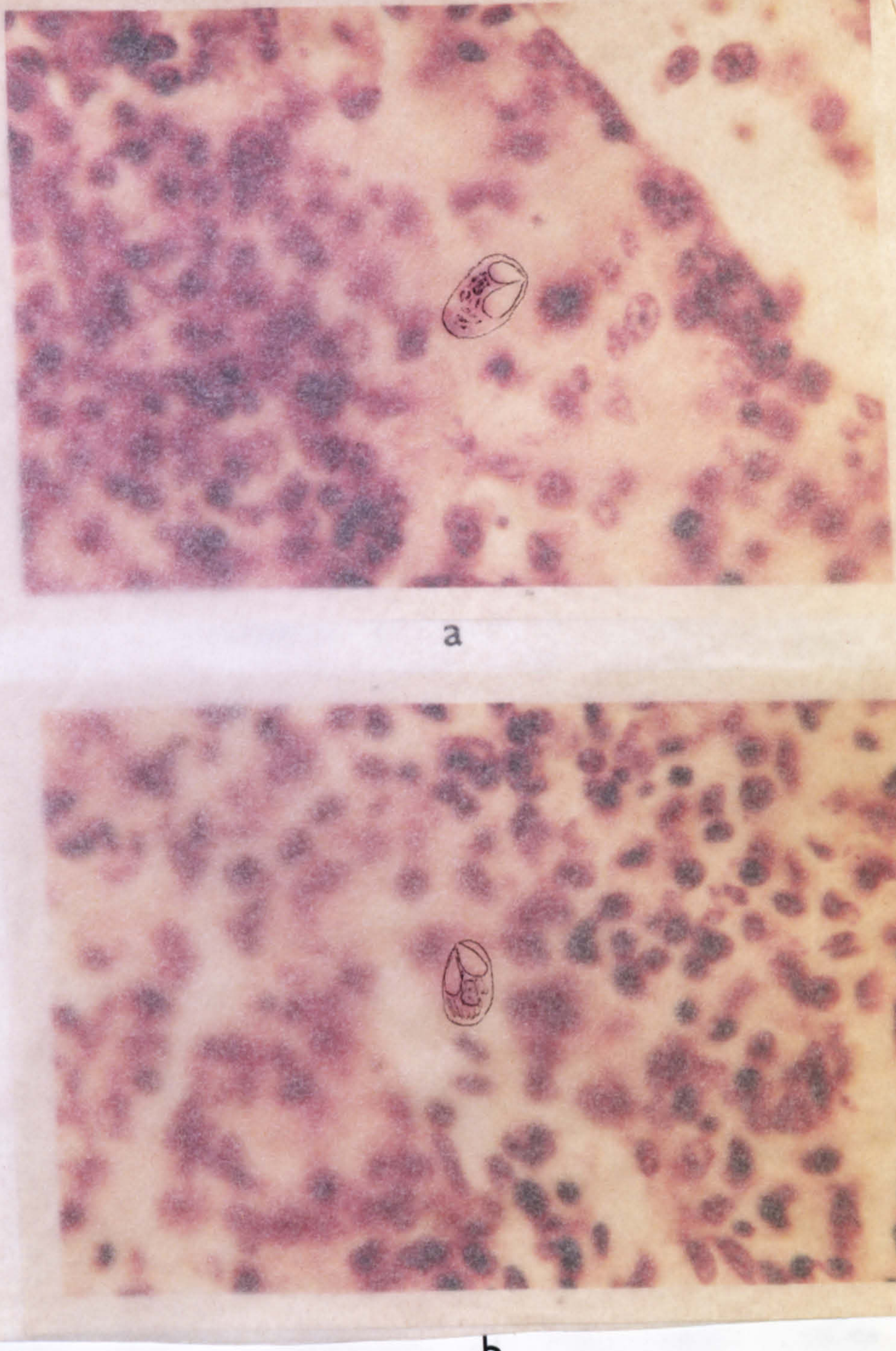
FIGURE 21.

Myxobolus sp. 4 from Rutilus rutilus

Spores in sections of spleen. Best's carmine stain.

a. Particulate stain uptake.

b. Diffuse stain uptake.



a

b

FIGURE 21.

Myxobolus sp. 4 from Rutilus rutilus

Spores in sections of spleen. Best's carmine stain

a. Particulate stain uptake.

b. Diffuse stain uptake.

TABLE 5a.

TABLE 5

Results of tests for glycogen in spores of Myxobolus sp. 4 from Rutilus rutilus

a. Best's carmine

Locality	Host	Organ	Total examined	Total showing vacuoles	Total showing diffuse or particulate staining	Total unstained
St. James's Park	R.1	Kidney	42	0 (0%)	6 (14.3%)	36 (85.7%)
		Spleen	98	0 (0%)	52 (53.1%)	46 (46.9%)
	R.2	Kidney	95	4 (4.2%)	30 (31.6%)	61 (64.2%)
		Spleen	92	0 (0%)	32 (34.8%)	60 (65.2%)
	R.3	Kidney	67	12 (17.9%)	40 (59.7%)	15 (22.4%)
		Spleen	98	21 (21.4%)	50 (51.0%)	27 (27.6%)
Brocket Estate	R.4	Kidney	80	18 (22.5%)	10 (12.5%)	52 (65.0%)
		Spleen	75	2 (2.7%)	28 (37.3%)	45 (60.0%)
	R.5	Kidney	97	12 (12.4%)	34 (35.0%)	51 (52.6%)
		Spleen	77	2 (2.6%)	42 (54.5%)	33 (42.9%)
	R.6	Kidney	105	21 (20.0%)	40 (38.1%)	44 (41.9%)
		Spleen	111	16 (14.4%)	37 (33.3%)	58 (52.3%)
River Colne	R.7	Kidney	44	0 (0%)	1 (2.3%)	43 (97.7%)
		Spleen	91	0 (0%)	33 (36.3%)	58 (63.7%)
	R.8	Kidney	49	0 (0%)	4 (8.2%)	45 (91.8%)
		Spleen	82	0 (0%)	36 (43.9%)	46 (56.1%)
	R.9	Kidney	64	0 (0%)	30 (46.9%)	34 (53.1%)
		Spleen	90	1 (1.1%)	46 (51.1%)	43 (47.8%)

TABLE 5b.

TABLE 5 (Continued)

b. Lugol's iodine

Locality	Host	Organ	Total examined	Total showing vacuoles	Total showing diffuse or particulate staining	Total unstained
St. James's Park	R.1	Kidney	41	0 (0%)	37 (90.2%)	4 (9.8%)
		Spleen	62	1 (1.6%)	57 (91.9%)	4 (6.5%)
	R.2	Kidney	62	9 (14.5%)	51 (82.3%)	2 (3.2%)
		Spleen	30	0 (0%)	27 (90.0%)	3 (10.0%)
	R.3	Kidney	41	6 (14.6%)	28 (68.3%)	7 (17.1%)
		Spleen	39	6 (15.4%)	29 (74.3%)	4 (10.3%)
Brocket Estate	R.4	Kidney	59	8 (13.5%)	48 (81.4%)	3 (5.1%)
		Spleen	91	11 (12.1%)	52 (57.1%)	28 (30.8%)
	R.5	Kidney	57	19 (33.3%)	31 (54.4%)	7 (12.3%)
		Spleen	74	18 (24.3%)	34 (46.0%)	22 (29.7%)
	R.6	Kidney	80	23 (28.7%)	28 (35.0%)	29 (36.3%)
		Spleen	93	12 (12.9%)	47 (50.5%)	34 (36.6%)
River Colne	R.7	Kidney	66	0 (0%)	48 (72.7%)	18 (27.3%)
		Spleen	59	0 (0%)	56 (94.9%)	3 (5.1%)
	R.8	Kidney	40	0 (0%)	12 (30.0%)	28 (70.0%)
		Spleen	43	0 (0%)	24 (55.8%)	19 (44.2%)
	R.9	Kidney	35	1 (2.9%)	15 (42.8%)	19 (54.3%)
		Spleen	77	0 (0%)	41 (53.3%)	36 (46.7%)

TABLE 5c.

c. Bauer - Feulgen technique

Locality	Host	Organ	Total examined	Total showing vacuoles	Total showing diffuse or particulate staining	Total unstained
St. James's Park	R.1	Spleen	87	1 (1.2%)	45 (51.7%)	41 (47.1%)
	R.2	Kidney	82	3 (3.7%)	48 (58.5%)	31 (37.8%)
	R.3	Spleen	74	14 (18.9%)	37 (50.0%)	23 (31.1%)
Brocket Estate	R.4	Kidney	109	23 (21.1%)	45 (41.3%)	41 (37.6%)
	R.5	Kidney	85	14 (16.5%)	51 (60.0%)	20 (23.5%)
	R.6	Spleen	116	34 (29.3%)	36 (31.0%)	46 (39.7%)

TABLE 6.

TABLE 6

Results of tests for glycogen in spores of species of Myxobolus from Leuciscus leuciscus, Gobio gobio and Serrasalmus rhombeus

Species	Locality	Host	Organ	Best's carmine				Lugol's iodine			
				Total examined	Total showing vacuoles	Total showing diffuse or particulate staining	Total unstained	Total examined	Total showing vacuoles	Total showing diffuse or particulate staining	Total unstained
M. muelleri	Hertford	Leuciscus leuciscus	Kidney	54	14 (25.9%)	7 (13.0%)	33 (61.1%)	53	22 (41.5%)	19 (35.9%)	12 (22.6%)
			Spleen	11	0 (0%)	1 (9.1%)	10 (90.9%)	37	5 (13.5%)	21 (56.8%)	11 (29.7%)
M. cyprini	River Colne	Gobio gobio G.1.	Kidney	42	1 (2.4%)	22 (52.4%)	19 (45.2%)				
		Gobio gobio G.2.	Kidney	50	2 (4.0%)	19 (38.0%)	29 (58.0%)				
Myxobolus sp. 1.	Rio Negro, Brazil	Serrasalmus rhombeus	Kidney	154	5 (3.2%)	30 (19.5%)	119 (77.3%)				
			Spleen	153	0 (0%)	6 (3.9%)	147 (96.1%)				

TABLE 7

Results of tests for glycogen in spores of Myxosoma heterospora, Agarella gracilis and Myxidium rhodei

Best's carmine stain

Species	Locality	Host	Organ	Total examined	Total showing vacuoles	Total showing diffuse or particulate staining	Total unstained
Myxosoma heterospora	Lake Victoria, Uganda	Tilapia esculenta	Spleen	109	5 (4.6%)	78 (71.8%)	26 (23.8%)
Agarella gracilis	Belem, Brazil	Lepidosiren paradoxa	Testis	159	0 (0%)	38 (23.9%)	121 (76.1%)
Myxidium rhodei	St. James's Park	Rutilus rutilus	Kidney	200	0 (0%)	0 (0%)	200 (100%)

TABLE 8

Percentages of spores of species of Myxobolus with clearly outlined vacuoles

Species	Locality	Host		Percentage of total spores examined		
				Kidney	Spleen	Combined percentage
Myxobolus sp. 4	St. James's Park	Rutilus rutilus	R.1.	0%	0.8%	0.6%
			R.2.	6.7%	0%	4.4%
			R.3.	16.7%	19.4%	18.5%
	Brocket Estate	" "	R.4.	19.8%	7.8%	15.0%
			R.5.	18.8%	13.3%	16.7%
			R.6.	23.8%	19.4%	21.0%
	River Colne	" "	R.7.	0%	0%	0%
			R.8.	0%	0%	0%
			R.9.	1.0%	0.6%	0.8%
M. cyprini	River Colne	Gobio gobio	G.1.	2.4%	-	2.4%
			G.2.	4.0%	-	4.0%
M. muelleri	Hertford	Leuciscus leuciscus		33.6%	10.4%	26.5%

4. Attempts to establish myxosporidan infections

(i) Review of previous work.

While a considerable quantity of information is available on the morphology and sites of infection of Myxosporida, little experimental work has been carried out on their life - cycle. The aim of the present work was to establish infections of selected species, and thence to determine as much as possible of their life - history.

The most likely route of infection has been generally considered to be via the alimentary canal. The evidence for this, however, is largely circumstantial, as very few infections have been established experimentally. Evidence for the theory is based mainly on the observations of several workers that spores split along their suture line to liberate the sporoplasm under certain conditions, notably under the action of digestive juices.

The exit of the sporoplasm from spores was first seen by Lieberkühn (1854) who noted the occurrence in spores of a species of Myxobolus forming cysts on the gills of Tinca tinca (L.) He found, on breaking a cyst, corpuscles moving like amoebae. He further observed, " I have seen, several times, a psorosperm split, and the amoeboid corpuscle leaving". Balbiani (1863) considered that the sporoplasm, " disengaged itself " from the spore with the aid of slow contraction movements. Pfeiffer

(1890) found that spores of Myxidium lieberkuehni Bütschli, 1882, placed in the urine of pike, underwent changes; after 4 to 12 hours, many split shells could be found, and in some individuals the sporoplasm was seen to flow out between the valves.

Thélohan (1894) was the first to investigate changes in spores introduced into the alimentary canal. In his first experiments, he fed fish with infected tissues, and examined the faeces 24 hours later. He fed perch on gill filaments infected with cysts of Henneguya psorospermica Thélohan, 1895, and barbel, bleak and bream apparently free of other infection with muscle tissue infected with a Myxobolus species. He found unchanged spores and empty spore shells in the faeces of all the fish. He continued feeding the barbel, bleak and bream on infected musculature for 20 days and then dissected them. In all the fish, the intestine contained both unchanged spores and empty spore shells.

In a second experiment, Thélohan injected spores of Myxobolus pfeifferi Thélohan, 1895 and M. ellipsoides Thélohan, 1892 into ligatured regions of the intestine of a tench. After 3½ hours, he found spores in the ligatured regions in which the sporoplasm appeared to have contracted away from the spore walls.

In a final group of experiments, Thélohan introduced balls of cotton - wool impregnated with spores of Myxobolus ellipsoides into the stomach of a tench. The balls were attached to a thread and pushed down a glass tube, inserted through the mouth of the host, with the aid of a glass rod. The glass tube was then removed, leaving the thread hanging from the mouth. After 24 hours, he pulled the cotton - wool balls out for examination. He again found spores of both types, normal

and split, and also small amoeboid bodies corresponding in size to the sporoplasm.

From these results, Thélohan concluded that myxosporidan spores probably entered their host via the alimentary canal; the sporoplasm liberated there then then travelled either directly to its final site, in the case of the gall - bladder and swim - bladder, or across the intestinal wall and through the blood or other tissues, in the case of other sites.

Auerbach (1910) carried out similar experiments to Thélohan using pith instead of cotton - wool. Pieces of pith, impregnated with spores of Myxidium bergense Auerbach 1910, were introduced into the stomach and duodenum of Pollachius virens (L.). After 24 and 48 hours, the pieces of pith were pulled out. On examining them, he found that those from the stomach contained mainly complete spores, while those from the duodenum contained empty shell - valves and free amoeboid " embryos ". Auerbach then stated that he could detect these amoebae crawling up the bile - duct into the gall - bladder, the site of trophozoite maturation.

Erdmann (1917) observed liberated amoebulae when she added gastric fluid followed by bile to spores of Chloromyxum leydigi Mingazzini, 1890. Davis (1916) mixed spores of Sinuolinea dimorpha Davis, 1916 with a drop of fluid from the pyloric caeca of their host and observed " sporozoites " emerging with an active amoeboid movement. Georgévitch (1917) noted a similar separation of spore valves in Ceratomyxa coris Georgévitch, 1916.

Kudo (1922) carried out two series of experiments with Leptotheca ohlmacheri (Gurley, 1893), from the frog Rana pipiens Schreber. In the first, he found that the sporoplasms emerged from the spores when

gastric fluid was added to spores in hanging - drop preparations; he also noted that movements of the sporoplasms appeared to cause polar capsule extrusion and separation of the valves. He succeeded in inducing spore germination with a mixture of gastric fluid and bile, with fluid from the duodenum and with weak pepsin and hydrochloric acid, but failed to cause it with fluid from the large intestine or with urine. In the second experiment he introduced pieces of cork impregnated with spores into the alimentary canal and dissected the animals after various intervals of time. Results revealed that spore germination occured in the pylorus or duodenum.

The work described so far indicates that myxosporidan spores split and release the sporoplasm under the action of digestive juices. In none of the experiments mentioned, however, were infections established in hosts known to be parasite - free; it is therefore, only surmise that sporoplasms liberated in the gut proceed to infect organs elsewhere in the body.

Experimentel infections have been attempted by a few workers, using various methods. Auerbach (1910), working on Pollachius virens, found that young fish, under 20 cm. long were rarely infected, while fish over 25 cm. long were heavily infected. He then attempted to infect young fish, assuming them to be parasite - free, with Myxidium bergense, a parasite of the gall - bladder of which the spores are also found in the intestinal contents. In one series of experiments, he fed seven of these fish with the gall - bladder and intestinal contents of an infected adult. After 3½ weeks, three were dissected and found to be infected.

The remainder were kept 1½ months, starved for a few days, and given more infected gall - bladder. After a few more days, two were found infected and two uninfected. In a second experiment, Auerbach kept six small fish in a tank with heavily infected fish. At the end of the experiment (after an unspecified time) all fish were infected, spores being found in the gall - bladder and the intestine.

From this work, Auerbach concluded that he had established infections in clean hosts, and that infections arose in the natural state by uptake of spores via the mouth.

Erdmann (1912) carried out infection experiments using Chloromyxum leydigi, parasitic in the gall - bladder of Torpedo torpedo (L.); she used young specimens and assumed them to be free of the myxosporidan. In a series of experiments, she fed fish with spores, and young and mature trophozites in gelatine capsules. In some fish, an intestinal fistula was made so that gut contents could be examined daily in living specimens; other fish were dissected at various intervals after feeding. On the second day after feeding, she found empty spore shells and amoeboid embryos. In 6 to 10 days she found young forms in the bile - duct and gall - bladder, and after 39 days mature spore - producing trophozoites in the gall - bladder.

Shiba (1934) attempted to demonstrate cross - infection from one fish to another by placing, apparently uninfected fish into the same tank as Macronodus chinensis (Bloch) infected with Henneguya macronodi Shiba, 1934. The healthy fish become infected in 50 days, when cysts were visible.

The work of Auerbach, Erdmann and Shiba is open to the criticism that the experimental animals were not known to be definitely parasite-free. As mentioned above, Auerbach used fish from a population of which a small poportion were known to be infected. Erdmann's fishes were assumed to be free of Myxosporida, although no control fishes were mentioned. The source of the experimental fishes used by Shiba was not given.

Bond (1939) carried out a series of experiments using several myxosporidan species parasitic in Fundulus heteroclitus (L.) and F. diaphanus (Le Sueur) by making use of F. heteroclitus raised from eggs under artifical conditions and fed on infusoria; specimens of these fish, dissected at intervals before experiments were commenced, were found to be free of infection with Myxosporida.

In his first experiment, Bond fed the young F. heteroclitus on tissues infected with five Myxosporida (Myxosoma funduli Kudo 1918, M. subtecalis Bond, 1938, Myxidium folium Bond, 1938, Myxobolus bilineatum Bond, 1938, and Sphaerospora renalis Bond, 1938) for five days. Subsquent examinations revealed early stages of Myxosoma funduli on the gills after 36 days, and mature, spore - producing trophozoites of M. funduli on the gills and M. subtecalis in the brain and kidney after 50 days. The M. subtecalis infections were described as "surprisingly light".

In a second experiment, Bond fed F. diaphanus with infected tissue of F. heteroclitus; in this case, however, the experimental F. diaphanus came from a group of fish of which a sample of 23 were negative for all

Myxosporida and 2 were positive for M. funduli only. After 125 days, Bond found heavy infections of M. funduli, M. subtecalis and S. renalis in the experimental fish.

In a third experiment, Bond put 8 F. heteroclitus, known to be infected with M. funduli, M. subtecalis, M. bilineatum and M. folium, in a aquarium with 12 F. diaphanus from a collection of which a sample had shown 14 to be negative for all Myxosporida and 2 positive for M. funduli only. Of the 8 F. diaphanus alive 2 months later, Bond found 4 to be infected with M. subtecalis in the liver, 5 with M. subtecalis on the fins, 4 with M. funduli on the gills, and 5 with M. funduli in the brain.

Another successful experimental infection is reported by Uspenskaya (1966). After unsuccessful attempts at infecting trout with spores of Myxosoma cerebralis (Hofer, 1903) newly extracted from cartilage, she allowed spores to "age" in water for varying lengths of time. "Aged" spores were then introduced to artifically reared trout fingerlings either as a suspension in the water, or through a tube through the mouth into the stomach. Using spores "aged" for 4 months, she found that 5 out of 16 fish contained trophozoites of M. cerebralis after 17 days.

A successful infection of a different nature was achieved by Wagh (1961). After unsuccessful attempts to infect Notemigonus crysoleucas (Mitchill) orally and by spraying the gills with spores of Myxosoma ovalis (Davis, 1923) extracted from cysts in Ictiobus bubalus Raf., he injected a suspension of spores in distilled water hypodermally near

the pelvic fins. After 57 days, he observed trophozoites in the musculature, together with live spores. More trophozoites were seen on the 68th and 78th day after injection. Thélohan (1894) inoculated spores of Myxobolus inaequalis and M. muelleri into the skin and musculature of roach, and recovered fresh spores; he considered, however, that the spores recovered were those injected previously, having found no trace of trophozoites.

(ii) Description of the present work.

In this study, procedures similar to those of Thélohan (1894), Bond(1939), Wagh (1961) and Uspenskaya (1966) were used in attempts to establish infections in healthy fish.

a. Attempted infections of Myxidium - free Rutilus rutilus with Myxidium rhodei.

Four procedures were adopted in this investigation. In the first, the fate of spores was followed shortly after their introduction into the alimentary canal of two fish. In the second, spores were introduced into the stomach of 36 fish, which were examined for signs of infection after various intervals of time. In the third, spores were added to the water in a tank inhabited by eight fish. In the fourth, spores were introduced into the body - cavity of four fish by hypodermic injection.

In all these experiments, the experimental fish were specimens of R. rutilus, measuring approximately 15 cm. in length, collected in the

Brocket Estate during January and February, 1966. Dissection of 21 fish of sizes up to 30 cm. from this source at the time of collection revealed no Myxidium infection in any organs. From this, it was assumed that Myxidium species infective to R. rutilus were probably absent from the lake. The situation in the Brocket Estate could be contrasted with that in St. James's Park, where all fish examined were found to be infected with M. rhodei in the kidney.

Spores of M. rhodei were obtained and treated similarly in all four experiments. The kidneys of infected fish from St. James's Park were dissected out, and portions were mounted on a slide in distilled water, and flattened with a coverslip. After removal of the coverslip, cysts were dissected out with the aid of mounted entomological pins and placed in distilled water in a watch - glass. They were then punctured to liberate the spores. Approximately 20 cysts were used for each millilitre of distilled water. The spore suspension was thoroughly mixed, with the aid of a fine pipette, to obtain an even spread of spores. It was found that the majority of spores sank to the bottom within 30 minutes; the suspension, therefore, was remixed before each inoculation.

Experiment 1. The fate of spores in the alimentary canal.

In this experiment, two R. rutilus were starved for 24 hours. A small, hard, polythene tube was inserted through the mouth of each fish into the stomach. The spore suspension was taken up into a syringe, and the needle inserted into the tube, 0.25 ml. was then inoculated into each fish, and the tube removed. The fish were then fed on "Tetramin".

Each fish was dissected after one and two hours respectively. Portions of the stomach, duodenum and intestine were taken, some to be examined fresh and others to be sectioned and stained. In the examination of fresh material, the contents of the gut portions were placed on a slide and mounted in distilled water. Scrapings of the gut wall were also examined both fresh and in stained smears.

Result; - The examination of both fresh and stained material was rendered difficult by the presence of partly digested food. In fresh preparations, however, many complete and apparently unchanged spores were found in all regions of the gut other than the stomach. Similar spores were also detected in the sectioned material. In no preparations were split spores or spores with extruded polar filaments seen.

Experiment 2. Attempted infection by the introduction of spores into the stomach.

On March 3rd, 1966, 36 specimens of R. rutilus were each inoculated with 0.20 ml. of the spore suspension, in the manner described in experiment 1. 20 other fish were kept as controls in a separate room. The experimental and control fish were subsequently fed regularly on "Tetramin". The water in the tank was changed approximately once every two weeks.

The experimental fish were examined by dissection over the following 11 months. The kidney and gall - bladder were examined for developing trophozoites by both fresh and stained smears and by stained sectioned preparations. Two fish were dissected after one day. Three were then examined at weekly intervals. until April 8th (six weeks), and then two

fish weekly until April 29th (nine weeks). After this date, examinations were irregular, two being dissected on June 6th (14½ weeks), two on November 11th (37½ weeks) and the remaining five on January 25th, 1967 (49 weeks). One fish died 10 days after inoculation.

Of the 20 control fish, 16 died on June 19th, due to a failure in the aeration system. These were examined by fresh preparations of kidney and gall - bladder, on the same day. The remaining four were examined at the end of the experiment, on January 25th, 1967.

Result; - No spores or trophozoites of M. rhodei were detected in either the kidney or gall - bladder of any fish, experimental or control.

Experiment 3. Attempted infection by the addition of spores to the water.

On May 2nd, 1966, portions of infected kidney from fish in St. James's Park, together with 5 ml. of the spore suspension, were added to the water in a tank inhabited by six R. rutilus from the Brocket Estate. The fish were then fed on "Tetramin" for 5½ weeks. The water remained unchanged during this time.

Examinations of kidney and gall - bladder were carried out during this period, by fresh preparations, smears and sections. Two fish were dissected after 23 days, two after 30 days and two after 37 days.

Result; - In none of the fish examined was evidence seen of infection by M. rhodei, either in the kidney or in the gall - bladder.

Experiment 4. Attempted infection by hypodermic injection of spores.

On November 25th, 1966, 0.25 ml. of the spore suspension was inoculated into the body cavity of each of four R. rutilus. The needle

was inserted near the posterior margin of the pelvic fins. Four fish were kept in a separate tank as controls. All fish were then fed regularly on "Tetramin" until the end of the experiment. The water was changed once every two weeks.

Two experimental fish were dissected on December 29th (after five weeks), one on January 5th, 1967 (six weeks) and one on January 20th (eight weeks). The four control fish were all dissected on January 25th. The kidney of each experimental fish was examined both by fresh and by sectioned preparations, and the gall - bladder, liver and spleen by fresh preparations only.

Result; - In none of the four experimental fish were spores of M. rhodei found in the kidney. In one of the two dissected after five weeks, and in those dissected after six and eight weeks, tissue similar to that of the cyst - wall of M. rhodei was seen, encircling regions of diffuse material which appeared yellow in fresh preparations and light green in Giemsa - stained sections. Apart from the "cyst - wall" tissue, however, there was nothing to suggest that these regions represented sites of trophozoite development.

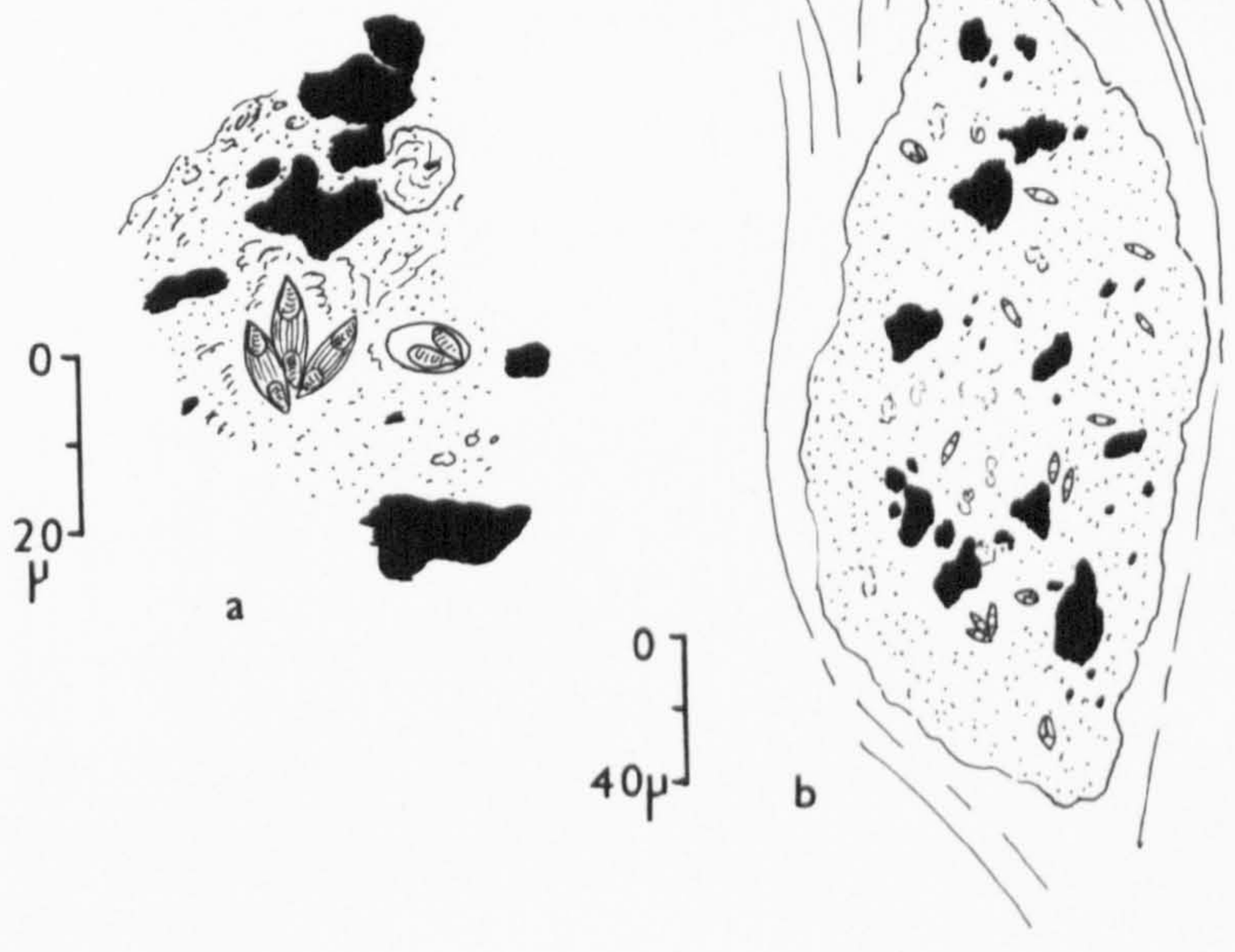
In one of the experimental fish examined after five weeks, and the one examined after eight weeks, spores corresponding in shape and size to those of M. rhodei were seen in fresh preparations of the gall - bladder. They were surrounded by yellow - coloured material in which lumps of dark - brown material and spores of Myxobolus sp. 4 were also present (Fig: 22 a,b).

In none of the other organs examined were spores of M. rhodei or

developing trophozoites seen. None of the four control fish showed *M. rhodei* in either the kidney or the gall - bladder.

Discussion; The negative result of experiments 1,2 and 3 are similar to those obtained by Wagh (1961), Hoffman *et al.* (1965)and Uspenskaya (1966). With the exception of Bond's work (1939) and Uspenskaya's results(1966) when "aged" spores were used, no myxosporidan infections have been established under laboratory conditions by feeding healthy fish on infected material, or by introducing spores into the alimentary canal. There are many reasons why such sttempts should fail, as the natural environment in which fish live is difficult to re - create in the laboratory. The present results, however, cast some doubt on the generally accepted method of infection, in which spores are presumed to germinate in the alimentary canal.

While the result of experiment 4 suggests that newly mature spores may be able to initiate infections from within their host, the results of experiments 1, 2 and 3 suggest that they are unable to do so when ingested. It may be necessary for spores to undergo some form of aging before becoming infective to a new host by ingestion, as suggested by Uspenskaya. There is also a possibility that a second, intermediate, host is invblved, as suggested by Hoffman *et al.* (1965). This theory provided the basis for the following experiment, in which the copepod *Cyclops* was fed on spores of *M. rhodei*.



F I G U R E 22.

Fresh preparation of gall - bladder wall of Rutilus rutilus, eight weeks after hypodermic injection of spores of Myxidium rhodei.

a. High - power view showing M. rhodei spores, Myxobolus sp. 4 spore and lumps of pigment surrounded by diffuse yellow material.

b. Low - power view.

b. Attempted infections of species of Cyclops with spores of Myxidium rhodei.

Because of the possibility that invertebrates may act as transport or intermediate hosts of Myxosporida, a short experiment was devised to test whether spores of M. rhodei showed any changes when ingested by species of the copepod Cyclops. Cyclops was chosen for this test as it is a bottom - feeder; as mentioned previously, spores of M. rhodei sink in water.

Specimens of Cyclops were collected with the aid of a plankton net in Regent's Park lake, London. They were placed in lake water in a large petri - dish, and 5 ml. of the spore suspension, made up as described in part a., was added. After 24 hours, 25 specimens of Cyclops were removed, fixed in Duboscq - Brazil fluid, sectioned and stained with Giemsa's stain.

Result; - In five specimens of Cyclops, spores of M. rhodei were recognised in the gut contents. Spores appeared to be complete; no extruded polar filaments were seen. No evidence of spores or spore germination was seen in other regions of the body.

Conclusion; As Cyclops and other invertebrates form part of the diet of R. rutilus, they may well act as transport hosts of myxosporidan spores. Further investigations will be necessary to determine whether they are necessary to complete the parasite's life - cycle.

c. Attempted infection of Salmo salar with Myxidium sp. 3.

In this experiment, portions of liver infected with Myxidium sp. 3 were fed to one - year old Salmo salar in an attempt to establish an infection.

One - year old salmon were obtained from the Ministry of Agriculture and Fisheries in London and taken to Winches Farm on June 9th, 1966. Two were dissected and found to free of myxosporidan infection in the liver, gall - bladder, spleen and kidney. 14 experimental fish were then placed in one pond, and 10 controls in the other. All were fed daily on a salmon - feed marketed by Astra - Hewlett Ltd. A continuous flow of tapwater through each pond was maintained for the duration of the experiment.

Liver infected with Myxidium sp. 3 was obtained from a firm of salmon importers in London. Spores were assumed to be viable as they extruded their polar filaments in a saturated solution of urea and in 5% potassium hydroxide solution. The infected liver was cut into small portions and fed to the experimental salmon on June 24th. The salmon were then examined by dissection over the following four months.

Two salmon were examined on each of the following dates; July 1st (after one week), July 8th (two weeks), July 21st (four weeks), August 19th (seven weeks) and September 9th (nine weeks). The remaining four were examined on November 11th (17½ weeks). The liver of each fish was examined by fresh preparations and by stained smears and sections. Examinations were also made of fresh preparations of the gall - bladder and spleen.

The ten control fish all died on August 20th, probably because of pollution by a toxic chemical. They were all examined on the same day, by fresh preparations of the liver and gall - bladder.

Result; In none of the fish examined, experimental or control, was any evidence seen of infection by Myxidium sp. 3.

Discussion; Several reasons can be put forward for the negative results of this experiment.

It has already been suggested that spores may have to "age" in water before becoming infective, and that invertebrates may play some part in the myxosporidan life - cycle. In this experiment, the conditions under which fish were kept allowed for these possibilities to some extent, as pieces of liver not consumed by the fish lay on the bottom for several weeks, and a community of invertebrates built up in the ponds during the period of the experiment. After a few weeks, in fact, it was noticed that the salmon were feeding on debris on the bottom and on invertebrates in preference to the salmon feed. Had the experiment continued, it is possible that infections might have become established.

An alternative reason for the negative result is that under natural conditions salmon may become infected while at sea. In this case, spores may have to undergo some form of maturation in salt - water.

5. Check - lists

Since the publication of Kudo's revised classification of Myxosporida (1933), no comprehensive check - list of species has been compiled. Tripathi (1953) listed specific names only, giving no authors or references. Bykhovskaya - Pavlovskaya et al. (1962) give a key to Myxosporida of Russian fishes only. Baker (1963) gives a check - list of species of Myxosoma with authors and references.

In the present study, some emphasis has been given to species of the genera Myxobolus, Myxosoma and Myxidium. In the following pages, check - lists of species of Myxobolus and Myxidium are given, with authors, hosts and countries. A list of species of Myxosoma described since 1963 and not included in Baker's paper is also given.

The host names in these lists are those in current use (1967) according to information provided by the British Museum (Natural History). The British Museum was unable to trace or record the validity of the following names in the lists:-

Acheilognathus chankaensis

Leporinus mormyrops

Myoxocephalus axillaris

Necrophis annulatus

Xystrias principalis

(i) Check - list of species of the genus Myxobolus Bütschli, 1882.

This list does not include species which, prior to 1919, were synonymised with species listed by Kudo (1919); neither does it include species originally ascribed to the genus Myxobolus which Kudo (1933) transferred to the genus Thelohanellus Kudo, 1933.

Certain species, characterised by spores with unequal polar capsules, were placed in the genus Disparospora Akhmerov, 1954 by Akhmerov (1954, 1960). These are retained here in the genus Myxobolus.

Check-list of species of the genus Myxobolus Bütschli

Species	Author	Host	Country
M. muelleri 1 24	Bütschli, 1882	Abramis ballerus A. brama A. sapa Alburnus alburnus Aspro asper Barbus vulgaris Blicca bjoerkna Carassius auratus gibelio Chalcalburnus chalcoides Cottus gobio Crenilabrus melops Gobio gobio Leuciscus cephalus L. idus L. leuciscus L. souffia agassizii Lota lota Mugil auratus M. cephalus Phoxinus phoxinus Pseudaspius leptocephalus Rhodeus sericeus Rutilus frisii R. rutilus Scardinius erythrophthalmus Silurus glanis Stizostedion lucioperca Tinca tinca Vimba vimba	France, Germany, Italy, Switzerland, U.S.S.R.
* M. ellipsoides 2 3	Thélohan, 1892	Abbottina rivularis Abramis brama A. sapa Acanthorhodeus asmussi Acerina cernua Alburnus alburnus Blicca bjoerkna Carassius auratus gibelio	France, Germany, Czechoslovakia, Italy, U.S.S.R.

Species	Author	Host	Country
		Chalcalburnus chalcoides	
		Chondrostoma nasus	
		Cobitis taenia	
		Coregonus ussuriensis	
		Cyprinus carpio	
		Gobio gobio	
		Hypophthalmichthys molitrix	
		Idus melanotus	
		Leuciscus cephalus	
		L. idus	
		Pelecus cultratus	
		Perca fluviatilis	
		Rhodeus sericeus	
		Rutilus rutilus	
		Scardinius erythrophthalmus	
		Tinca tinca	
		Vimba vimba	
* M. oviformis 5	Thélohan, 1892	Abramis brama Alburnus alburnus Aspius aspius Blicca bjoerkna Cyprinus carpio Gobio gobio Scardinius erythrophthalmus Tinca tinca Vimba vimba	France, Germany, Switzerland, U.S.S.R.
M. cycloides 1 4 19	Gurley, 1893	Abramis brama Alburnus alburnus Blicca bjoerkna Gobio gobio Lota lota Rhodeus amarus Rutilus rutilus Scardinius erythrophthalmus Vimba vimba Vimba vimba	France, Germany.
M. globosus	Gurley, 1893	Erimyzon oblongus E. sucetta	U.S.A.
M. inaequalis	Gurley, 1893	Pimelodus clarias Synodontis schall	Guiana, Surinam.
1 M. lintoni	Gurley, 1893	Cyprinodon variegatus	U.S.A.

Species	Author	Host	Country
M. obesus	Gurley, 1893	Alburnus alburnus	France U.S.S.R.
M. oblongus	Gurley, 1893	Frimyzon oblongus F. sucetta	U.S.A.
M. sphaeralis	Gurley, 1893	Coregonus lavaretus C. nasus	Switzerland
M. transovalis	Gurley, 1893	Clinostomus funduloides	U.S.A.
Myxobolus sp.	Gurley, 1894	Nais lacustris	?
Myxobolus sp.	Gurley, 1894	Carassius carassius	Germany
Myxobolus sp.	Gurley, 1894	Stizostedion lucioperca	Germany
* M. anurus	Cohn, 1895	Esox lucius	Germany
18 * M. dispar 25	Thélohan, 1895	Abbottina rivularis Abramis brama Alburnus alburnus Aspius aspius Blicca bjoerkna Carassius carassius C. auatus gibelio Cyprinus carpio Gobio gobio Hypophthalmichthys molitrix Leuciscus cephalus L. leuciscus L. idus Lota lota Pelecus cultratus Perca fluviatilis Rutilus rutilus Scardinius erythrophthalmus Stizostedion lucioperca	France, Austria, Germany, U.S.S.R.
* M. exiguus	Thélohan, 1895	Abramis brama Blicca bjoerkna Chondrostoma nasus Leuciscus idus Mugil auratus M. cephalus M. labrosus	France, Germany, Switzerland, Italy, U.S.S.R.

Species	Author	Host	Country
		M. ramada M. saliens Scardinius erythrophthalmus	
* M. pfeifferi 9	Thélohan, 1895	Barbus barbus B. canito B. plebejus	Germany, Italy, U.S.S.R.
* M. cyprini	Doflein, 1898	Abramis brama Cyprinus carpio Gobio albipinnatus Pseudorasbora parva Rutilus rutilus Tinca tinca	Germany, Austria, U.S.S.R.
* M. neurobius	Schuberg & Schröder, 1905	Oncorhynchus nerka Salmo trutta	Germany, U.S.S.R.
* M. aeglefini	Auerbach, 1906	Gadus esmarkii G. merlangus G. morhua callarias Melanogrammus aeglefinis Merlangius merlangus Molva molva Trisopterus esmarkii	Norway, U.K. U.S.S.R.
* M. gigas	Auerbach, 1906	Abramis brama Blicca bjoerkna Leuciscus cephalus Tinca tinca	Germany, Italy, U.S.S.R.
* M. balleri 1	Reuss, 1906	Abramis ballerus	U.S.S.R.
* M. bramae 1	Reuss, 1906	Abramis brama	U.S.S.R.
* M. cyprinicola	Reuss, 1906	Cyprinus carpio Pseudorasbora parva	U.S.S.R.
* M. macro-capsularis 5	Reuss, 1906	Abbottina rivularis Blicca bjoerkna Cyprinus carpio Gobio gobio Hypophthalmichthys molitrix Opsariichthys uncirostris Rutilus rutilus Scardinius erythrophthalmus	U.S.S.R.

	Species	Author	Host	Country
* 5	M. physophilus	Reuss, 1906	Scardinius erythrophthalmus	U.S.S.R.
* 6	M. sandrae	Reuss, 1906	Stizostedion lucioperca S. volgense	U.S.S.R.
* 1	M. scardinii	Reuss, 1906	Scardinius erythrophthalmus	U.S.S.R.
* 6	M. volgensis	Reuss, 1906	Stizostedion volgense	U.S.S.R.
*	M. cordis	Keysselitz, 1908	Barbus plebejus	Germany, U.S.S.R.
* 7	M. musculi	Keysselitz, 1908	Acanthorhodeus asmussi Barbus plebejus Carassius auratus gibelio Leuciscus waleckii L. leuciscus Phoxinus percnurus P. phoxinus Rutilus rutilus	Germany U.S.S.R.
*	M. squamae	Keysselitz, 1908	Barbus barbus B. plebejus Cyprinus carpio Schizothorax intermedius	Germany, U.S.S.R.
*	Myxobolus sp.	Miyairi, 1909	Misgurnus anguillicaudatus	Japan
8	Myxobolus sp.	Wegener, 1910	Perca fluviatilis	Germany
	M. permagnus.	Wegener, 1910	Perca fluviatilis Phoxinus percnurus Leuciscus waleckii Scardinius erythrophthalmus	Germany
*	M. minutus	Nemeczek, 1911	Leuciscus leuciscus L. cephalus Perca fluviatilis	Austria, U.S.S.R.
*	M. rotundus	Nemeczek, 1911	Abramis brama Gobio gobio	Austria, U.S.S.R.
*	Myxobolus sp.	Lobzelter, 1912	Thymallus thymallus	?
*	M. magnus	Awerinzew, 1913	Acerina cernua	U.S.S.R.

	Species	Author	Host	Country
* 9	M. carassii	Klokačewa, 1914	Alburnus alburnus Carassius carassius C. auratus gibelio Leuciscus idus L. leuciscus	U.S.S.R.
* 17	M. toyamai	Kudo, 1915	Cyprinus carpio	Japan, U.S.S.R.
10	Myxobolus sp.	Southwell, 1915	Rasbora daniconius	India.
*	M. pleuronectidae	Hahn, 1917	Pseudopleuronectes americanus	U.S.A.
	M. capsulatus	Davis, 1918	Cyprinodon variegatus	U.S.A.
	M. nodularis	Southwell & Prasad, 1918	Rasbora daniconius	India.
*	M. hylae	Johnston & Bancroft, 1918	Hyla aurea	Australia.
	M. lutzi	Aragao, 1919 (see Pinto, 1928a)	Poecilia vivipara	Brazil.
*	M. aureatus	Ward, 1919	Notropis anogenus	U.S.A.
27	M. discrepans	Kudo, 1919	Carpiodes velifer Ictiobus bubalus	U.S.A.
11	M. funduli	Kudo, 1919	Fundulus heteroclitus F. majalis	U.S.A.
12	M. koi	Kudo, 1919	Acanthorhodeus asmussi Cyprinus carpio Squaliobarbus curriculus	Japan, U.S.S.R.
	M. mesentericus	Kudo, 1919	Lepomis cyanellus	U.S.A.
	M. miyairii	Kudo, 1919	Parasilurus asotus Silurus soldatovi	Japan. U.S.S.R.
	M. orbiculatus	Kudo, 1919	Notropis dorsalis	U.S.A.
*	M. plectroplites	Johnston & Bancroft, 1919	Plectroplites ambiguus	Australia
	M. destruens	Schuurmans-Stekhoven, 1920	Scardinius erythrophthalmus	Holland

Species	Author	Host	Country
M. ranae	Guyénot & Naville, 1922	Rana temporaria	Switzerland.
M. elongatus	Fujita, 1924	Carassius carassius	Japan.
M. associatus	Nemeczek, 1926	Leporinus mormyrops	Brazil.
M. chondrophilus	Nemeczek, 1926	Sardinella anchovia	Brazil.
M. cunhai	Penido, 1927	Pimelodus clarias Serrasalmus piraya	Brazil.
M. pygocentris	Penido, 1927	Serrasalmus piraya	Brazil.
M. uniporus	Fujita, 1927	Liocassis ussuriensis Parasilurus asotus Pseudobagrus fulvidraco	Japan. U.S.S.R.
M. kawabatae	(Fujita, 1927)	Liocassis brashnikowi L. ussuriensis Pseudobagrus fulvidraco	Japan. U.S.S.R.
13			
M. gigi	(Fujita, 1927)	Liocassis brashnikowi L. ussuriensis Parasilurus asotus Pseudobagrus fulvidraco	Japan. U.S.S.R.
14			
M. noguchii	Pinto, 1928	Serrasalmus spilopleura	Brazil.
M. stokesi	Pinto, 1928	Pimelodus sp.	Brazil.
M. guyenoti	Naville, 1928	Perca fluvintilis	Switzerland.
M. conspicuus	Kudo, 1929	Moxostoma breviceps M. aureolum	U.S.A. Canada.
M. intestinalis	Kudo, 1929	Pomoxis sparoides	U.S.A.
M. ovoidalis	Fantham, 1930	Barbus sp. Cyprinus carpio	S. Africa, Canada.
M. luciopercae	Schäferna & Jirovec, 1931	Stizostedion lucioperca	Czechoslovakia.
6			
M. luciopercae	Dogiel & Petrushevskii, 1933	Stizostedion lucioperca	U.S.S.R.
6			
M. lobatus	Dogiel & Bykhovskii, 1934	Barbus barbus B. brachycephalus Hemibarbus maculatus	U.S.S.R.

Species	Author	Host	Country
M. angustus	Kudo, 1934	Pimephales vigilax	U.S.A.
M. bellus	Kudo, 1934	Carpiodes carpio	U.S.A.
M. compressus	Kudo, 1934	Notropis blennius	U.S.A.
M. congesticus	Kudo, 1934	Moxostoma anisurum	U.S.A.
M. gravidus	Kudo, 1934	Moxostoma anisurum	U.S.A.
M. mutabilis	Kudo, 1934	Pimephales notatus	U.S.A.
M. nodosus	Kudo, 1934	Pimephales notatus	U.S.A.
M. obliquus	Kudo, 1934	Carpiodes velifer	U.S.A.
M. ovatus	Kudo, 1934	Ictiobus bubalus	U.S.A.
M. squamosus	Kudo, 1934	Hybopsis biguttata	U.S.A.
M. teres	Kudo, 1934	Notropis whipplei	U.S.A.
M. vastus	Kudo, 1934	Moxostoma aureolum	U.S.A.
M. pseudodispar	Gorbunowa, 1936	Rutilus rutilus	U.S.S.R.
M. kostiri	Herrick, 1936	Micropterus dolomieui	U.S.A.
M. osburni	Herrick, 1936	Micropterus dolomieui Lepomotis gibbosus	U.S.A.
Myxobolus sp.	Bhatia, 1938	Rasbora daniconius	India.
M. bilineatum	Bond, 1938	Fundulus heteroclitus	U.S.A.
M. kudoi	Guimaraes & Bergamin, 1938	Nematognatha (catfish)	Brazil.
M. calbasui	Chakravarty, 1939	Labeo calbasu L. rohita Cirrhinus chinensis	India.
M. mrigalae	Chakravarty, 1939	Cirrhinus chinensis	India.
M. catostomi	Fantham, Porter & Richardson, 1939	Catostomus commersoni	Canada.

Species	Author	Host	Country
M. couesii	Fantham, Porter & Richardson, 1939	Couesius plumbeus	Canada
M. dentium	Fantham, Porter & Richardson, 1939	Esox masquinongy	Canada
M. grandis	Fantham, Porter & Richardson, 1939	Notropis cornutus	Canada
M. hyborhynchi	Fantham, Porter & Richardson, 1939	Pimephales notatus	Canada
M. notropis	Fantham, Porter & Richardson, 1939	Notropis heterolepis	Canada
M. percae	Fantham, Porter & Richardson, 1939	Perca flavescens	Canada
M. poecilichthidis	Fantham Porter & Richardson, 1939	Etherostoma caeruleum	Canada
M. rhinichthidis	Fantham Porter & Richardson, 1939	Rhinichthys atratulus	Canada
M. subcircularis	Fantham Porter & Richardson, 1939	Catostomus commersoni	Canada
M. transversalis	Fantham Porter & Richardson, 1939	Notropis cornutus	Canada
M. heterocapsulatus	Jaczo, 1940	Aspius aspius	Hungary
M. hungaricus	Jaczo, 1940	Abramis brama	Hungary
M. variabilis	Jaczo, 1940	Abramis brama	Hungary

Species	Author	Host	Country
+ M. dogieli	Bykhovskii & Bykhovskii, 1940	Blicca bjoerkna Carassius auratus gibelio Cyprinus carpio Pelecus cultratus Phoxinus percnurus Tinca tinca	U.S.S.R.
+ M. kubanicum 15	Bykhovskii & Bykhovskii, 1940	Cyprinus carpio Carassius auratus gibelio	U.S.S.R.
+ M. karelicus 8	Petrushevskii, 1940	Aspro zingel Perca fluviatilis Stizostedion lucioperca	U.S.S.R.
M. gibbosus	Herrick, 1941	Eupomotis gibbosus	U.S.S.R.
M. catlae	Chakravarty, 1943.	Catla catla Cirrhinus chinensis Labeo rohita	India.
M. clarii	Chakravarty, 1943.	Clarias batrachus	India.
M. bubalis	Otto & Jahn, 1943	Ictiobus bubalus	U.S.A.
M. iowensis	Otto & Jahn, 1943	Pomoxis sparoides	U.S.A.
M. okobojiensis	Otto & Jahn, 1943	Pomoxis sparoides	U.S.A.
M. sparoidis	Otto & Jahn, 1943	Pomoxis sparoides	U.S.A.
M. symmetricus	Rice & Jahn, 1943	Pomoxis sparoides	U.S.A.
M. moxostomi	Nigrelli, 1948	Moxostoma aureolum	U.S.A.
M. bengalensis	Chakravarty & Basu, 1948	Catla catla	India.
+ M. marinus 5 29	Dogiel, 1948	Leuciscus brandti	U.S.S.R.
M. nemachili	Weiser, 1949	Nemacheilus barbatulus	U.S.S.R. Czechoslovakia.

Species	Author	Host	Country
M. subepithelialis	Weiscr, 1949	Gobio gobio	Czechoslovakia.
M. ochridensis	Georgévitch, 1949	Barbel, carp, chub, trout.	Yugoslavia.
M. turbinoidus	Georgévitch, 1949	Barbel, carp, chub, trout.	Yugoslavia.
+ M. thelohanellus	Schulman & Vikhrova, 1952.	Carassius carassius	U.S.S.R.
M. acanthogobii	Hoshina, 1952	Acanthogobius flavimanus	Japan.
M. dermatobius	Hoshina, 1952a	Cyprinus carpio	Japan.
Myxobolus sp. 7	Rostovshchikov, 1952	Alburnoides bipunctatus	U.S.S.R.
Myxobolus sp.	Rostovshchikov, 1952.	Schizothorax sp.	U.S.S.R.
M. galaxii	Szidat, 1953	Galaxias maculatus	Argentina.
M. magellanicus	Szidat, 1953	Galaxias maculatus	Argentina.
M. barbi	Tripathi, 1953	Barbus ticto	India.
M. indicum	Tripathi, 1953	Cirrhinus chinensis	India
M. branchialis	Tripathi, 1953	Puntius sarana	India.
M. sphaericum	Tripathi, 1953	Cirrhinus chinensis	India.
+ M. drjagini 16	(Akhmerov, 1954)	Hypophthalmichthys molitrix	U.S.S.R.
+ M. pavlovskii 16	(Akhmerov, 1954)	Hypophthalmichthys molitrix	U.S.S.R.
M. kisutchi	Yasutake & Wood, 1957	Oncorhynchus kisutch	U.S.A.
+ M. spatulatus	Dogiel & Bogolepova, 1957	Paracottus kneri	U.S.S.R.
+ M. talievi	Dogiel & Bogolepova, 1957	Batrachocottus baicalensis B. nikolskii Cottinella boulengeri Procottus jeitteleisi	U.S.S.R.

Species	Author	Host	Country
+ M. hemibarbi	Dogiel & Akhmerov, 1960	Hemibarbus labeo H. maculatus	U.S.S.R.
+ M. rotundatus	Dogiel & Akhmerov, 1960	Cyprinus carpio Parabramis pekinensis	U.S.S.R.
+ M. chuatsi 18	Dogiel & Akhmerov, 1960	Siniperca chuatsi	U.S.S.R.
+ M. brannaeformis 3	Dogiel & Akhmerov, 1960	Cyprinid fishes	U.S.S.R.
+ M. pseudobagri 14	Dogiel & Akhmerov, 1960	Pseudobagrus fulvidraco	U.S.S.R.
M. amurensis	Akhmerov, 1960	Cyprinus carpio Pseudorasbora parva	U.S.S.R.
M. artus	Akhmerov, 1960	Carassius auratus Cyprinus carpio	U.S.S.R.
M. auctus 2	Akhmerov, 1960	Carassius auratus Hypophthalmichthys molitrix	U.S.S.R.
M. bilis 20	Akhmerov, 1960	Carassius auratus	U.S.S.R.
M. circulus 20	Akhmerov, 1960	Cyprinus carpio	U.S.S.R.
M. confessus 20	Akhmerov, 1960	Acanthorhodeus asmussi	U.S.S.R.
M. confirmatus	Akhmerov, 1960	Acanthorhodeus asmussi	U.S.S.R.
M. ctenopharyngodonis 2	Akhmerov, 1960	Ctenopharyngodon idella	U.S.S.R.
M. dombrovskayae	Akhmerov, 1960	Abbottina rivularis	U.S.S.R.
M. gradicapsularis 13	Akhmerov, 1960	Pseudobagrus fulvidraco	U.S.S.R.
M. liocassii 13	Akhmerov, 1960	Liocassis ussuriensis	U.S.S.R.

Species	Author	Host	Country
M. lussi	Akhmerov, 1960	Erythroculter mongolicus Gobio albipinnatus Gobio gobio	U.S.S.R.
M. microcapsularis 2	Akhmerov, 1960	Carassius auratus gibelio	U.S.S.R.
M. multihospitis 2	Akhmerov, 1960	Acanthorhodeus asmussi Carassius auratus gibelio Cyprinus carpio Hypophthalmichthys molitrix Abbottina rivularis	U.S.S.R.
M. multilococapsularis 14	Akhmerov, 1960	Pseudobagrus fulvidraco	U.S.S.R.
M. multiplex 5	Akhmerov, 1960	Gobio gobio Opsarichthys uncirostris	U.S.S.R.
M. ornatus 20	Akhmerov, 1960	Acanthorhodeus asmussi	U.S.S.R.
M. platyrostris 15	Akhmerov, 1960	Carassius auratus gibelio	U.S.S.R.
M. pseudogobii	Akhmerov, 1960	Abbottina rivularis	U.S.S.R.
M. pseudorasborae 19	Akhmerov, 1960	Abbottina rivularis Gobio gobio Pseudorasbora parva Rhodeus sericeus	U.S.S.R.
M. simplex	Akhmerov, 1960	Acanthorhodeus asmussi	U.S.S.R.
M. soldatovi 21	Akhmerov, 1960	Oncorhynchus keta	U.S.S.R.
M. tunicatus 13	Akhmerov, 1960	Pseudobagrus fulvidraco	U.S.S.R.
M. varius	Akhmerov, 1960	Hyporhthalmichthys molitrix	U.S.S.R.
Myxobolus sp	Akhmerov, 1960	Cyprinus carpio	U.S.S.R.
M. vegrandis 28	Akhmerov, 1960	Acanthorhodeus asmussi	U.S.S.R.
M. vescus 5	Akhmerov, 1960	Gobio gobio Hypophthalmichthys molitrix	U.S.S.R.
Myxobolus sp	Lom, 1961	Rutilus rutilus	Czechoslovakia.

Species	Author	Host	Country
Myxobolus sp. 22	Lom, 1961	Phoxinus phoxinus	Czechoslovakia
+ M. lomi 22	Donets & Kulakowskaya, 1962	Phoxinus phoxinus	U.S.S.R.
+ M. infundibulatus	Donets & Kulakowskaya, 1962	Leuciscus cephalus	U.S.S.R.
+ M. schulmani	Donets, 1962	Abramis sapa	U.S.S.R.
+ M. chondrostomi	Donets, 1962	Chondrostoma nasus	U.S.S.R.
+ Myxobolus sp.	Donets, 1962	Alburnus alburnus Aspius aspius Leuciscus idus L. leuciscus Pelecus cultratus	U.S.S.R.
+ M. petruschewskii	Zhukov, 1962	Myxocephalus axillaris M. platycephalus	U.S.S.R.
+ M. cheisini	Schulman, 1962	Ophicephalus argus	China.
+ M. cheni	Schulman, 1962	Mugil cephalus M. soiuy	China .
+ M. grandiintercapsularis	Schulman, 1962	Hypseleotris swinchonis	China.
+ M. niei	Schulman, 1962	Percottus glehni	China.
+ M. orientalis	Schulman, 1962	Carassius auratus gibelio	Chim.
+ M. parvus	Schulman, 1962	Mugil cephalus M. soiuy	China.
+ M. poljanskii 23	Schulman, 1962	Abbottim rivularis	China.
+ M. problematicus	Schulman, 1962	Acheilognathus (?)chankaensis Paracheilognathus imberbis	China.
+ M. sphaerocapsularis	Schulman, 1962	Acheilognathus (?)chankaensis	China.

	Species	Author	Host	Country
•	M. vini	Schulman, 1962	Misgurnus anguillicaudatus	China.
‡	M. eniscocapsularis	Schulman, 1962	Hemibarbus labeo H. maculatus	U.S.S.R.
‡	M. folius	Schulman, 1962	Pseudaspius leptocerhalus	U.S.S.R.
‡	M. latus	Schulman, 1962	Hypophthalmichthys molitrix	U.S.S.R.
‡	M. phylloides	Schulman, 1962	Hypophthalmichthys molitrix	U.S.S.R.
‡	M. solidus	Schulman, 1962	Carassius carassius	U.S.S.R.
‡	M. arrostoni	Schulman, 1962	Parasilurus asotus Silurus soldatovi	U.S.S.R.
24	M. cristatus	Schulman, 1962	Alburnoides bipunctatus Schizothorax intermedius Varicorhinus capoeta	U.S.S.R.
25	M. disparoides	Schulman, 1962	Schizothorax intermedius	U.S.S.R.
26	M. nemeczeki	Schulman, 1962	Aspius aspius Leuciscus idus L. leuciscus	U.S.S.R.
27	M. obpyriformis	Schulman, 1962	Schizothorax intermedius	U.S.S.R.
28	M. suturalis	Schulman, 1962	Schizothorax intermedius	U.S.S.R.
	M. insidiosus	Wyatt & Pratt, 1963	Oncorhynchus tschawytscha	U.S.A.
	M. musajevi	Kandilov, 1963	Varicorhinus capoeta	U.S.S.R.
	M. notemigroni	Lewis & Summerfelt, 1964	Notemigonus crysoleucas	U.S.A.
	M. aligarhensis	Bhatt & Siddiqui, 1964	Ophicephalus punctatus	India.
	M. ophicephali	Bhatt & Siddiqui, 1964	Ophicephalus punctatus	India

Notes

* Original references not seen. Descriptions of these species, with references, are given by Kudo (1919).

• Original references not seen. Descriptions of these species, with references, are given by Bykhovskaya - Pavlovskaya et al. (1962)

‡ These species are described as spp. nov. Schulman by Bykhovskaya-Pavlovskaya et al. (1962).

1. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. brannae, M. scardinii, M. balleri, some descriptions of M. cycloides and M. lintoni (Rostovshchikov's description, 1952) should all be synonymised with M. muelleri.

2. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. auctus, M. ctenopharyngodonis, M. multihospitis and M. microcapsularis can all be synonymised with M. ellipsoides.

3. Dogiel and Akhmerov (1960) described M. brannaeformis as a new specie Bykhovskaya - Pavlovskaya et al. (1962) consider it to be a subspecies of M. ellipsoides.

4. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. cycloides should be synonymised partly with M. muelleri and partly with M. pseudorasborae.

5. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. physophilu

M. multiplex, M. vescus. M. oviformis (Rostovshchikov's description, 1952), and M. marinus can all be synonymised with M. macrocapsularis.

6. Dogiel and Petrushevskii (1933) described M. luciopercae as a new species without noticing the use of the same name by Schäferna and Jirovec (1931). Bykhovskaya - Pavlovskaya et al. (1962) consider that M. luciopercae Dogiel, 1933, M. luciopercae Schäferna and Jirovec, 1931 and M. volgensis can all be synonymised with M. sandrae. (M. luciopercae Dogiel, 1933 probably refers here to M. luciopercae Dogiel and Petrushevskii, 1933.)

7. Bykhovskaya - Pavlovskaya et al. (1962) consider that an unnamed species described by Rostovshchikov (1952) is M. musculi.

8. Bykhovskaya - Pavlovskaya et al. (1962) consider that Myxobolus sp. Wegener, 1910 can be identified as M. karelicus Petrushevskii, 1940.

9. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. pfeifferi (Akhmerov's description, 1960) can be synonymised with M. carassii.

10. Schuurmans - Stekhoven (1920b) mentions M. rasborae Southwell, 1918 in his check - list (p.338.). This probably refers to Myxobolus sp. Southwell, 1915.

11. M. funduli is a nomen novum for M. musculi Hahn, 1915, the latter species being pre-occupied by Keysselitz (1908).

12. Kakai (1926) described M. koi as a new species, its spores differing in size from those of M. koi Kudo, 1919. Kudo (1933), however

considers Nakai's species to be synonymous with his own.

13. Fujita (1927) described Lentospora kawabatae which is placed in the genus Myxobolus by Bykhovskaya - Pavlovskaya et al. (1962). The latter authors also consider that M. liocassii, M. gradicapsularis and M. tunicatus can all be synonymised with M. kawabatae.

14. Fujita (1927) described Lentospora piri, which is placed in the genus Myxobolus by Bykhovskaya - Pavlovskaya et al (1962). The latter authors also consider that M. pseudobagri, M. multilococapsularis and M. miyairii (Akhmerov's description, 1960) can all be synonymised with M. piri.

15. Bykhovskaya - Pavlovskaya et al (1962) consider that M. platyrostris is synonymous with M. kubanicum.

16. M. drjagini and M. pavlovskii were originally ascribed to the genus Disparospora by Akhmerov (1954).

17. M. toyamai Kudo, 1915 was placed in the genus Thelohanellus by Kudo (1933). Bykhovskaya - Pavlovskaya et al (1962) replaces it in the genus Myxobolus.

18. M. anurus and M. chuatsi are both transferred to the genus Myxosoma by Bykhovskaya - Pavlovskaya et al. (1962).

19. Bykhovskaya - Pavlovskaya et al. (1962) considers that the species described by Müller (1841) and identified as M. cycloides by Gurley (1893) should be identifed as M. pseudorasborae.

20. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. bilis, M. circulus, M. confessus, M. ornatus and M. vegrandis can all be synonymised with Myxosoma branchialis (Markevich, 1932).

21. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. soldatovi can be synonymised with Myxosoma dermatobia (Ishii, 1916).

22. Myxobolus sp. Lom, 1961 is named M. lomi by Donets and Kulakovskaya, 1962 (see Bykhovskaya - Pavlovskaya et al., 1962). M. lomi is spelt M. iomi in the text of the English translation of Bykhovskaya - Pavlovskaya et al. (1962).

23. M. poljanskii is spelt M. poljanski in the text of the English translation of Bykhovskaya - Pavlovskaya et al. (1962).

24. M. cristatus is a nomen novum for M. muelleri (Rostovshchikov's description, 1952) (see Bykhovskaya - Pavlovskaya et al., 1962).

25. M. disparoides is a nomen novum for M. dispar (Rostovshchikov's description, 1952) (see Bykhovskaya - Pavlovskaya et al., 1962)

26. M. nemeczeki is a nomen novum for Myxosoma lobatum Nemeczek, 1911 (see Bykhovskaya - Pavlovskaya et al., 1962)

27. M. obovriformis is a nomen novum for M. disperans Kudo (Rostovshchikov's description, 1952) (see Bykhovskaya - Pavlovskaya et al. 1962). M. disperans appears to be a misspelling of M. discrepans.

28.

28. M. suturalis is a nomen novum for M. dogieli (part after Rostovshchikov, 1952) (see Bykhovskaya - Pavlovskaya et al., 1962)

29. Akhmerov (1960) describes a subspecies of M. marinus from Leuciscus brandti which he names M. marinus subsp. fluviatilis.

(ii) Check - list of species of the genus Myxidium Bütschli, 1882.

Check - list of species of the genus *Myxidium* Bütschli

Species	Author	Host	Country
* 1 *M. lieberkuehni*	Bütschli, 1882	*Anabas testudineus* *Esox niger* *E. lucius* *L. reicherti* *Lota lota*	France, Germany, Italy, U.S.S.R., U.S.A., Canada, India.
* 2 *M. immersum*	(Lutz, 1889)	*Bufo arenarum* *B. granulosus corbigny* *B. marinus* *Dendrophryniscus stelzneri* *Hyla raddiana* *Leptodactylus ocellatus* *L. prognathus* *Paludicola bibroni* *Pseudis mantidactyla*	S. America, N. America.
* *M. incurvatum*	Thélohan, 1892	*Ammodytes tobianus* *Blennius pholis* *Callionymus lyra* *Cyclopterus lumpus* *Fundulus majalis* *Garbusia affinis* *Hippocampus hippocampus* *Limanda limanda* *Mugil caphalus* *Myoxocephalus quadricornis* *M. scornius* *Nerophis annulatus* *N. aequoreus* *N. lumbriciformis* *Pleuronectes flesus* *Scorpaena scrofa* *Syngnathus acus* *S. schlegeli* *S. typhle*	France, Germany, Italy, Norway, U.S.S.R., U.S.A., Japan.
* *M. sphaericum*	Thélohan, 1895	*Belone belone*	France.
* *M. histophilum*	Thélohan, 1895	*Phoxinus phoxinus*	France.
Myxidium sp.	Gurley, 1894	*Raja batis*	? Germany.

	Species	Author	Host	Country
*	M. danilewskyi	Laveran, 1897	Emys orbicularis	France.
*	M. gigantcum	Doflein, 1898	Raja asterias	Italy.
3	M. rhodei	Léger, 1905	Abbottina rivularis Carassius auratus gibelio Cobitis taenia Cyprinus carpio Leuciscus leuciscus Nemacheilus barbatulus Pseudorasbora parva Rhodeus amarus Rhodeus sericcus Rutilus rutilus Scardinius erythrophthalmus	France, U.S.S.R.
* 4	M. barbatulae	Cépède, 1906	Nemacheilus barbatulus	France.
*	M. giardi	Cépède, 1906	Anguilla anguilla Lampetra fluviatilis	France. U.S.S.R.
* 5	M. pfeifferi	Auerbach, 1908	Tinca tinca Aspius aspius Scardinius erythrophthalmus	Germany, U.S.S.R.
*	Myxidium sp.	Awerinzew, 1908	Cottus scorpius	U.S.S.R.
*	M. inflatum	Auerbach, 1909	Cyclopterus lumpus	Norway.
*	M. bergense	Auerbach, 1910	Boreogadus saida G. merlangus G. morhua Liparis liparis Melanogrammus aeglefinis Merlangius merlangus Myoxocephalus scorpius Pleuronectes platessa Pollachius virens Sebastes viviparus	Norway. U.S.S.R. Canada.
* 6	M. procerum	Auerbach, 1910	Argentina silus	Norway.
*	M. mackiei	Bosanquet, 1910	Trionyx gangeticus	India.
*	M. macrocapsulare	Auerbach, 1910	Scardinius erythrophthalmus Aplodinotus grunniens	Germany, U.S.S.R., U.S.A.

Species	Author	Host	Country
M. depressum	Parisi, 1912	Eucitharus linguatula	Italy.
M. oviforme	Parisi, 1912	Aposon imberbis	Italy.
		Coris julis	Norway.
		Gadus morhua callarias	U.S.S.R.
		Oncorhynchus keta	
		O. gorbuscha	
		O. nerka	
		O. tschawytscha	
		Salmo salar	
		Salvelinus sp.	
* M. anguillae	Ishii, 1915	Anguilla japonica	Japan.
* Myxidium sp.	Mavor, 1915	Pseudopleuronectes americanus	Canada.
Myxidium sp.	Ishii, 1916	Eel	Japan.
* M. gadi	Georgévitch, 1916	Gadus morhua	France,
		G. pollachius	U.S.S.R.,
		Melanogrammus aeglefinis	Canada.
		Pollachius virens	
		Solea vulgaris	
* M. striatum	Cunha & Fonseca, 1917	Menticirrhus americanus	Brazil.
		Bairdiella ronchus	
M. glutinosum	Davis, 1918	Cynoscion regalis	U.S.A.
M. phyllium	Davis , 1918	Gambusia affinis	U.S.A.
* M. theranon	Johnston & Bancroft, 1919	Theraponcarbo	Australia.
		T. hillii	
M. kagayamai	Kudo, 1919	Miscurnus anguilli-caudatus	Japan, U.S.S.R.
M. americanum	Kudo, 1919	Trionyx spiniferus	U.S.A.
Myxidium sp.	Fantham, 1919	Argyrozona argyrozona	South Africa
		Blenniosimus cottoides	
		B. taurus	
		Pachymetopon blochii	
M. intermedium	Dunkerly,, 1920	Pleuronectes flesus	U.K.

Species	Author	Host	Country
M. rhomboideum	Schuurmans-Stekhoven, 1920a	Gasterosteus pungitius G. aculeatus	Holland
M. noxostomatis	Kudo, 1921	Noxostoma sp.	U.S.A.
M. crassum	Fujita, 1923	Xystrias grigorjewi	Japan.
M. microcapsulare	Fujita, 1923	Atheresthes evermanni	Japan.
M. clidodermatis	Fujita, 1923	Clidoderma asperrimum	Japan.
M. oshorocnse	Fujita, 1923	Paralichthys olivaceus	Japan.
M. microstomi	Fujita, 1923	Microstomus achne	Japan.
M. oncorhynchi	Fujita, 1923	Oncorhynchus masou	Japan.
M. ochotcnse	Fujita, 1923	Gadus macrocephalus	Japan.
M. theragrae	Fujita, 1923	Theragra chalcogramma	Japan.
M. fusiforme	Fujita, 1923	Sebastes flameus	Japan.
M. tzudae	Fujita, 1923	Sebastes taczanowskii Sebastobolus macrochir	Japan.
M. cuneiforme	Fujita, 1924	Carassius carassius C. auratus gibelio Cyprinus carpio	Japan, U.S.S.R., China.
Myxidium sp.	Debaisieux, 1924	Anguilla anguilla	France.
M. ischikauiae	Fujita, 1927	Ischikauia steenackeri	Japan.
M. uchiyamae	Fujita, 1927	Anguilla japonica	Japan.
M. lentiforme	Fujita, 1929	Anguilla japonica	Japan.
M. cruzi	Penido, 1927	Chalcinus nematurum	Brazil.
M. fonsecai	Penido, 1927	Gymnotus fasciatus	Brazil.
M. furseli	Pinto, 1928b	Acestrorhynchus sp.	Brazil.
M. matsui	Fujita, 1929	Anguilla japonica	Japan.

Species	Author	Host	Country
M. contortum	Fantham, 1930	Blennius cornutus	South Africa.
M. pagelli	Fantham, 1930	Lithognathus lithognathus	South Africa.
M. parvoviforme	Fantham, 1930	Johnsonius hololepidotus	South Africa.
M. alosae	Léger, 1931	Alosa fallax Alosa fallax rhodenensis	France.
M. truttae	Léger, 1931	Brachymystax lenok Coregonus ussuriensis Salmo trutta	France. U.S.S.R.
M. umblae	Léger, 1931	Salvelinus alpinus	France.
M. lindoyense	Carini, 1932	Bufo marinus	Brazil.
M. aplodinoti	Kudo, 1934	Aplodinotus grunniens	U.S.A.
M. bellum	Meglitsch, 1937	Ictalurus punctatus	U.S.A.
M. illinoisense	Meglitsch, 1937	Anguilla rostrata	U.S.A.
M. kudoi	Meglitsch, 1937	Ictalurus furcatus	U.S.A.
M. folium	Bond, 1938	Fundulus heteroclitus	U.S.A.
M. glossogobi	Chakravarty, 1939	Glossogobius giuris	India.
M. percae	Fantham, Porter & Richardson, 1939	Perca flavescens	Canada.
M. myoxocephali	Fantham, Porter & Richardson, 1940	Myoxocephalus octodecemspinosus	Canada.
M. serotinum	Kudo & Sprague, 1940	Rana pipiens	U.S.A.
M. melum	Otto & Jahn, 1943	Ictalurus melas Pomoxis sparoides	U.S.A.
M. gasterosteï	Noble, 1943	Gasterosteus aculeatus	U.S.A., U.S.S.R.
M. heteropneustesi	Chakravarty, 1943	Heteropneustes fossilis	India.

	Species	Author	Host	Country
+	M. japonicum	Dogiel, 1948	Bero elegans Ceratocottus dicerraus Gymnocanthus galeatus Myoxocephalus brandti	Japan.
	M. enchelypterygii	Hoshina, 1952	Anguilla japonica	Japan.
	M. minteri	Yasutake & Wood, 1957	Oncorhynchus kisutch	U.S.A.
	Myxidium sp.	Yasutake & Wood, 1957	Oncorhynchus kisutch	U.S.A.
+	M. perniciosum	Dogiel & Bogolepova, 1957	Asprocottus herzensteini Abyssocottus pallidus A. werestschagini Comephorus dybowskii	U.S.S.R.
+	M. pseudomacrocapsulare	Gvozdev, 1950	Gobio gobio	U.S.S.R.
+	M. scorpii	Schulman - Albova, 1950	Myoxocephalus scorpius	U.S.S.R.
	M. ctenopharyngodonis	Akhmerov, 1960	Ctenopharyngodon idella	U.S.S.R.
	M. cyprini	Akhmerov, 1960	Cyprinus carpio	U.S.S.R.
	M. ophiocephali	Akhmerov, 1960	Ophicephalus argus warpachowskii	U.S.S.R.
3				
	M. pseudogobii	Akhmerov, 1960	Pseudogobio rivularis	U.S.S.R.
3				
+	Myxidium sp.	Schulman, 1962	Pseudobagrus fulvidraco	China.
+	Myxidium sp.	Schulman, 1962	Pseudorasbora parva	China.
+	M. arcticum	Zhukov, 1962	Cottus kapanowskii	U.S.S.R.
+	M. triangulum	Schulman, 1962	Lateolebrax japonicus	China.
+	M. monstruosum	Schulman, 1962	Hypseleotris swinchonis	China.
±	M. orientalis	Schulman, 1962	Gobio albipinnatus	U.S.S.R.

	Species	Author	Host	Country
‡	M. rimsky-korsakowi	Schulman, 1962	Percottus glehni	U.S.S.R.
‡	M. ventricosum	Schulman, 1962	Thymallus arcticus	U.S.S.R.
	M. rostowstschikowi	Schulman, 1962	Schizothorax intermedius	U.S.S.R.
9				
	M. laticurvum	Kabata, 1962	Trachinus draco T. vipera	North Sea.
	M. scardini	Qadri, 1962	Scardinius erythrophthalmus	U.K.
	M. bajacalifornium	Noble, 1966	Bajacalifornia burragei	U.S.A.
	M. coryphaenoidium	Noble, 1966	Coryphaenoides sp.	U.S.A.
	M. melanocetum	Noble, 1966	Melanocetus johnsoni	U.S.A.
	M. melanostigmum	Noble, 1966	Melanostigma pammelas Sagamichthys abei	U.S.A.

Notes

* Original references not seen. Descriptions of these species, together with references, are given by Kudo (1919).

\+ Original references not seen. Descriptions of these species, with references, are given by Bykhovskaya - Pavlovskaya et al. (1962).

\± These species are described as spp. nov. Schulman by Bykhovskaya - Pavlovskaya et al. (1962).

1. Dogiel and Bykhovskii (1934) described a variety of M. lieberkuehni from Esox lucius in the U.S.S.R. which they named M. lieberkuehni var. nanum. (See Bykhovskaya - Pavlovskaya, et al., 1962).

2. Originally described as Cystodiscus immersus by Lutz (1889). Kudo (1933) considered that the genus Cystodiscus should be synonymised with the genus Myxidium. M. lindoyense Carini, 1932 was later considered by Carini (1937), in a letter to Kudo, to be M. immersum. (See Kudo and Sprague, 1940).

3. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. cyprini Akhmerov, 1960 and M. pseudogobii Akhmerov, 1960 should be synonymised with M. rhodei Léger, 1905.

4. Bykhovskaya - Pavlovskaya et al. (1962) consider that M. barbatul Cépède, 1906 can probably be synonymised with M. rhodei Léger, 1905.

5. Bykhovskaya - Pavlovskaya et al. (1962) consider that some descriptions of M. pfeifferi Auerbach, 1903 refer to M. rhodei Léger, 1905, the spores of these two species having similar morphology.

6. Chakravarty (1943) described a variety of M. procerum Auerbach, 1910 from Lates calcarifer in India, which he named M. procerum var. calcariferi.

7. M. lentiforme was described by Fujita (1927) under the name M. fusiforme. In his paper of 1929, Fujita renamed the species M. lentiforme, having previously (1923) given the name M. fusiforme to a species from Sebastes flammeus.

8. Bykhovskaya - Pavlovskaya et al. (1962) also mention M. perniciosum subsp. omuli Zaika, 1961 from Coregonus lavaretus.

9. M. rostowstschikowi is a nomen novum for M. lieberkuehni Bütschli after Rostovshchikov, 1952 (See Bykhovskaya - Pavlovskaya et al., 1962)

(iii) Species of the genus Myxosoma Thélohan, 1892 not listed by Baker (1963).

Species	Author	Host	Country
M. percotti	Dogiel & Akhmerov, 1960	Percottus glehni	China.
Myxosoma sp.	Lom, 1961	Rutilus rutilus	Czechoslovakia.
M. neurophila	Guilford, 1963	Etheostomum nigrum Perca flavescens	U.S.A.
M. scleroperca	Guilford, 1963	Perca flavescens Percina caprodes	U.S.A.
M. hoffmani	Meglitsch, 1963	Pimephales notatus	U.S.A.
M. cartilaginis	Hoffman, Putz & Dunbar, 1965	Lepomis cyanellus L. macrochirus Micropterus salmoides	U.S.A.

SECTION 2.

OTHER PHOTOZOA

1. Introduction.

The Myxosporida have been the chief subject of the present study because of their high incidence in the fish examined. During these investigations, various other protozoan parasites have been found, and these are described in this section. They include two species of Microsporida, one coccidian species, and Rhabdospora thelohani Laguesse, 1895, a parasite of uncertain taxonomic position.

2. Description of Species

(1) Plistophora longifilis Schuberg, 1910 from the testis of Rutilus rutilus.

In one specimen of Rutilus rutilus collected in the Brocket Estate, white spots measuring up to 1 mm. in diameter were seen on the testis. Gentle pressure on these spots liberated a white fluid which was found to consist largely of microsporidan spores which, for the reason given below, could be identified as Plistophora longifilis (order Microsporida, sub - order Monocnidina, family Nosematidae, according to the classification of Léger and Hesse, 1922). Spores were drawn and measured from fresh preparations, and sporonts from Giemsa - stained sections.

Spores; Spores of two distinct sizes were present. Microspores (Fig: 23a) were oval in shape, with a clear region at one end. In Giemsa - stained smears, an irregular uptake of stain occurred, but in sections the sporoplasm appeared as a deep blue ring - like zone in

the middle of the spore. The polar filament was invisible in both fresh and stained spores. Macrospores (Fig: 23 b) were less numerous. They were also oval, but with a slightly expanded anterior end. In Giemsa - stained sections, the sporoplasm appeared as a deep blue ring. Spore measurements (in μ) were as follows;-

	Microspores (50 measured)		Macrospores (25 measured)	
	Length	Breadth	Length	Breadth
Average	4.28	2.40	10.49	5.01
Range	3.75 - 5.00	2.00 - 3.00	9.00 - 11.25	4.25 - 5.75

Trophozoites; Macroscopically, trophozoites were visible as white cyst - like bodies in the testis of the host. In sectioned preparations, large areas of the testis were seen to be destroyed and occupied by the parasite. No cyst wall was present, and therewas no evidence of host reaction against the invasion. Sporonts usually contained microspores or macrospores alone (Fig: 23 c,d), although some appeared to contain both (Fig: 23 c). The number of spores in each sporont varied considerably; sometimes as many as 40 microspores were visible in one section. Sporonts were irregularly spherical or oval in shape with a thin surrounding membrane, and measured up to 30 μ in diameter.

Identification; The species described here can be ascribed to the sub-order Monocnidina because the spores contained only one polar filament, to the family Nosematidae because of the spore shape, and to the genus Plistophora Gurley, 1893 because of the variable number of spores in each sporont.

Putz et al. (1965) give a synopsis of Microsporida from freshwater and euryhaline fish. Of the species of Plistophora listed by these authors, only P. mirandellae Vaney and Conte, 1901, P. ehrenbaumi Reichenow, 1929 and P. longifilis Schuberg, 1910 have macro - and micro-spores. P. mirandellae, from the ovary and ovum of Alburnus alburnus (L.) in France, differs from the present species in having larger spores of each type (macrospores 12.00 μ long by 6.00 μ broad, microspores 7.50 μ long by 4.00 μ broad). P. ehrenbaumi, from the musculature of catfish in Germany, has smaller spores than the present species (macrospores 7.50 μ long by 3.50 μ broad, microspores 3.00 μ long by 1.50 μ broad). P. longifilis, described from the testis of Barbus plebejus C. and V. in Germany, has identical features to the present species, with the exception of the microspore size which is smaller (3.00 μ long by 2.00 μ broad). In other respects, e.g. the trophozoites, sporonts and appearance of the spores in fresh and stained conditions, P. longifilis is so similar to the present material that this difference is not considered of importance.

Conclusion; The species of Plistophora from Rutilus rutilus in the Brocket Estate is identified as P. longifilis. This is believed to be the first record of the parasite in R. rutilus.

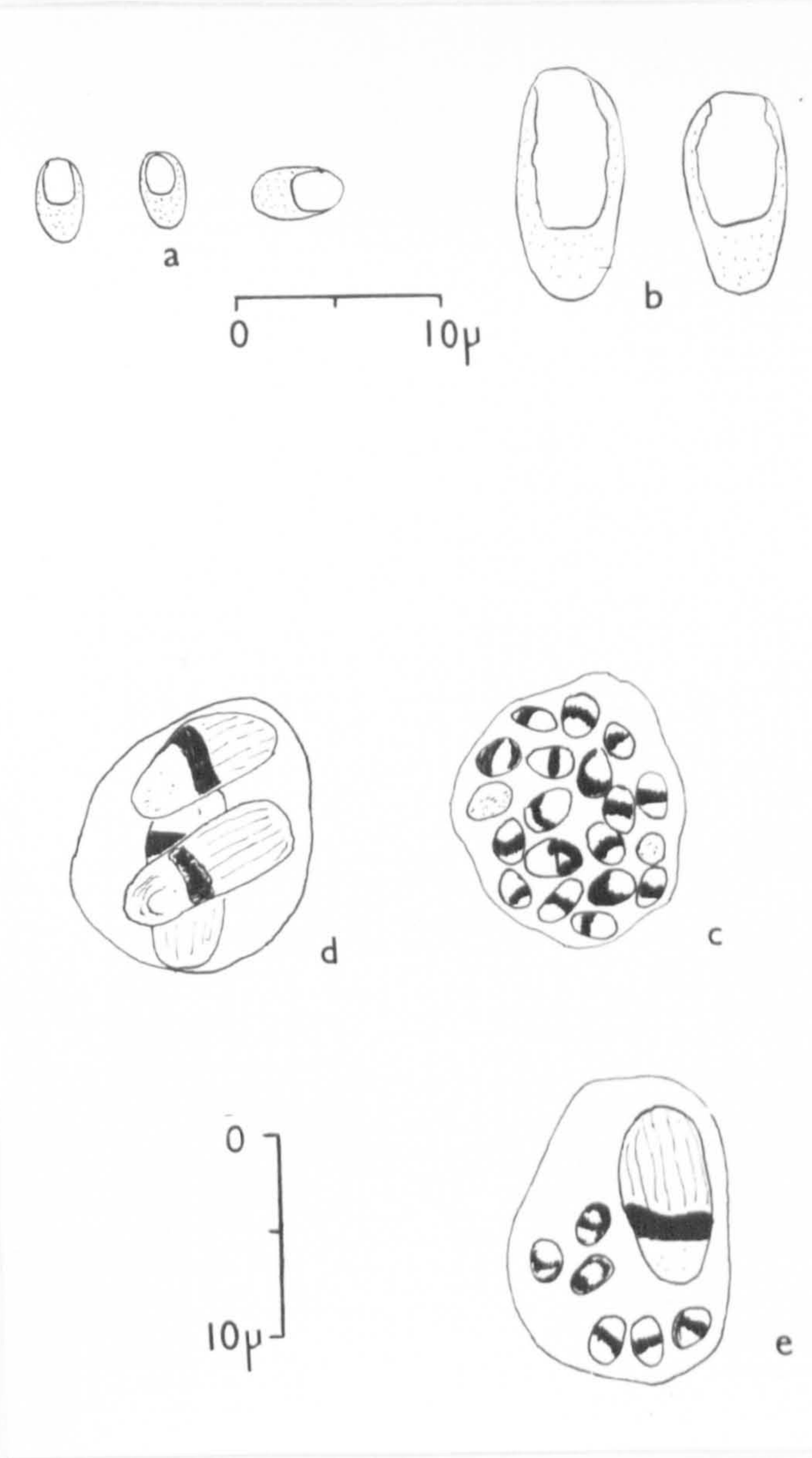


FIGURE 23.

Plistophora longifilis from Rutilus rutilus.

a. Microspores. Fresh preparations.

b. Macrospores. Fresh preparations.

c. Sporont containing microspores alone. Giemsa's sta

d. Sporont containing macrospores alone. Giemsa's sta

e. Sporont with microspores and macrospore.

(ii) A microsporidan parasite (? Glugea sp.) from the intestine of Rutilus rutilus.

In one specimen of Rutilus rutilus from St. James's Park, sectioned preparations of the intestine stained with Giemsa's stain and with Ehrlich's haematoxylin and eosin revealed round, schizont - like, bodies in the central lacteals of the villi (Fig: 24 a). At first, it was thought that these were coccidian schizonts, possibly belonging to Eimeria rutili Dogiel, 1939, as oocysts of this species had been found in the kidney of the same fish. Closer study (Fig:24 b), however, showed that the enclosed nucleated bodies were more rounded in shape and possessed a more prominent wall than typical coccidian merozoites. In Gram's stain (Fig: 24 c), the wall of these bodies took up a black stain, a reaction typical of microsporidan spores. It was concluded, therefore, that the "schizonts" were, in fact, microsporidan cysts filled with spores.

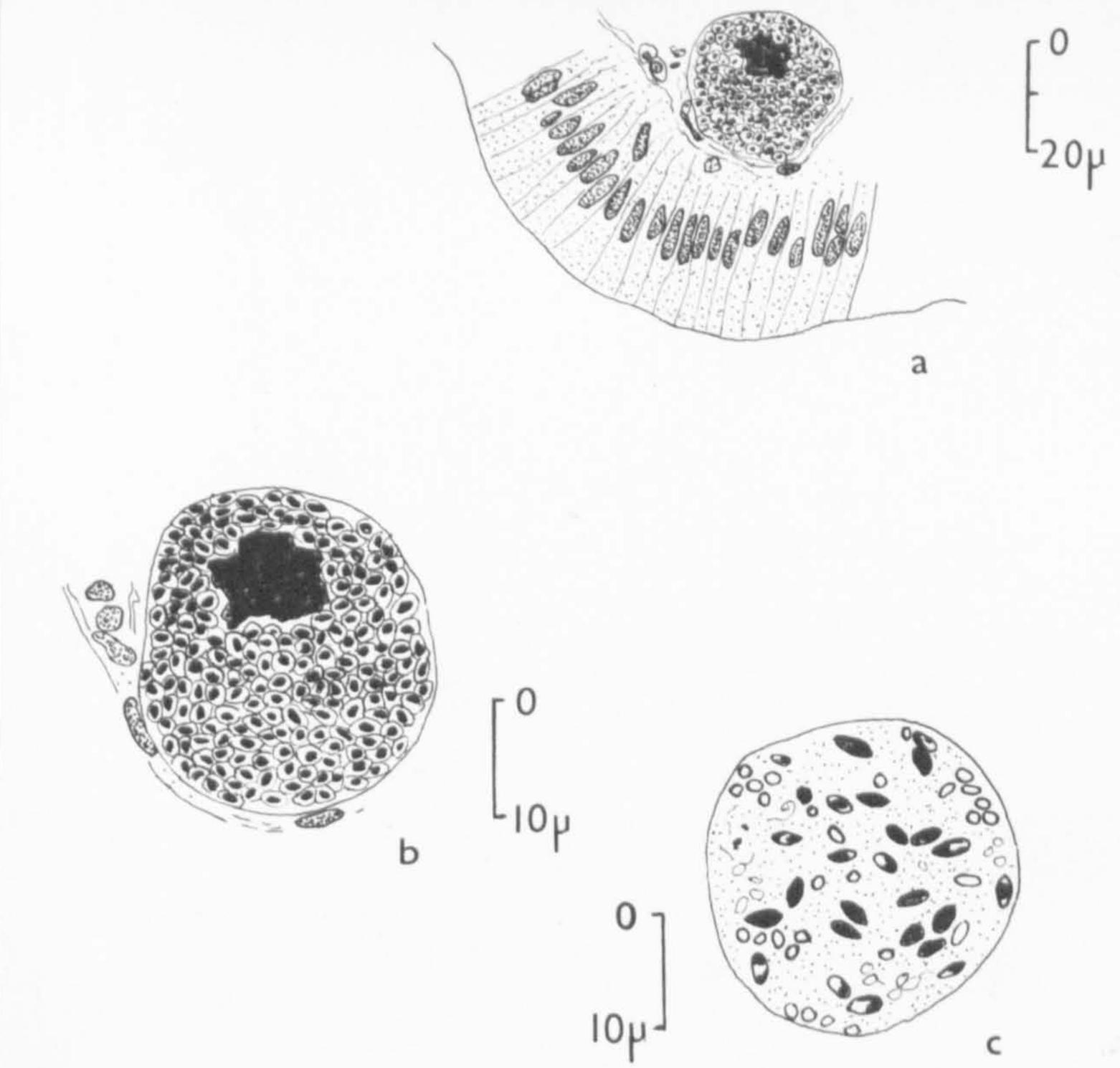
The cysts were spherical, with a thin wall, and measured up to 40 μ in diameter. Spores, in formol saline fixed material, measured approximately 2 μ long by 1 μ broad. A residual body of irregular shape, appearing blue in Giemsa stained sections, was present in each cyst.

Identification; From the appearance and shape of the spores, the species could be allocated to the sub - order Monocnidina and family Nosematidae. From the material available, however, it was not possible to ascertain the number of spores in a sporont and so to identify it to the genus level.

Putz et al. (1965) give five species of the genus Glugea Thélohan, 1891 which occur in the alimentary canal of freshwater and euryhaline fish, viz:- G. anomala (Moniez, 1887), G. hertwigi Weissenberg, 1911, G. intestinalis Chen, 1956, G. luciopercae (Dogiel, 1939) and G. stephani (Hagenmuller, 1899), and one of the genus Nosema Nägeli, 1857, viz:- N. girardini Lutz and Splendore, 1903. Of these, no information is availab on the trophozoites of N. girardini, although the spore dimensions are similar to those of the present species (2.00 - 2.50 μ long by 1.00 - 1.50 μ broad). Of the Glugea species, the most similar is G. stephani, described from Pleuronectes spp. and Pseudopleuronectes americanus (Walbaum) in Europe and the U.S.A. This species forms large cysts (up to 1 mm. in diameter) and may cause severe damage to the intestinal wall. G. stephani spores measure 3.00 μ long by 1.50 μ broad.

No microsporidan has been described from R. rutilus, although Plistophora elegans Auerbach, 1910 is described by Kudo (1924) as occurring in the "ovarium of Abramis brama x Leuciscus rutilus". The spores of P. elegans are much larger than those of the present species, however, measuring 10 μ long by 4 μ broad.

It would be clearly unwise to name this species until further study can be carried out, using fresh material. Because species of Glugea are common in the alimentary canal of fish, it may belong to this genus.



F I G U R E 24.

A microsporidan (? Glugea sp.) in an intestinal villus of Rutilus rutilus.

a. Low - power view. Giemsa's stain.

b. High - power view. Giemsa's stain.

c. High - power view. Gram's stain.

(iii) Eimeria rutili Dogiel, 1939 from Rutilus rutilus.

In specimens of Rutilus rutilus collected in St. James's Park and the river Colne, coccidian oocysts were found in the kidney tissue between the tubules, and occasionally in the spleen and musculature. For the reasons given below, they could be identified as Eimeria rutili (order Lucoccida, sub - order Eimerilina, according to the classification suggested by the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists, 1964, and the family Eimeriidae, sub - family Eimerilnae according to Hoare's classification, 1956). Portions of infected kidney were macerated and placed in a 2% solution of potassium bichromate for seven days to cause oocyst sporulation.

Oocysts (Fig: 25); In the kidney and spleen, oocysts were surrounded by regions of a homogeneous yellow material. They were of elongated oval shape, with a thin, fragile wall often closely applied to the four sporocysts. Each sporocyst was oval, with a small but distinct operculum at one end. In specimens kept in 2% potassium bichromate for seven days, a varying number of irregularly shaped inclusions was seen in each sporocyst. In general, two large inclusions and one or two small globular bodies were present. According to the description of E. rutili given by Markevich (1951), these inclusions represent the residual body; the sporozoites are described as elongated and very transparent. Such sporozoites were not visible in the present material.

Oocyst measurements (in μ) were as follows (15 measured);-

	Length	Breadth	Sporocyst length	Sporocyst breadth
Average	26.10	9.71	10.70	5.20
Range	24.00 - 30.00	8.00 - 12.00	9.00 - 12.00	4.00 - 6.00

Identification;- The appearance and dimensions of the oocysts correspond well with those given by Markevich (1951) for E. rutili. The species was first described by Dogiel in the kidney of R. rutilus in the Caspian Sea. This is believed to be the first record of its presence in Great Britain.

Discussion;- The life - cycles of fish coccidia are little - known. In higher vertebrates, species of the genus Eimeria are usually parasitic in the alimentary canal, and oocysts pass out with the faeces. In fish, oocysts are commonly found in other regions of the body, two of the best - known species of this type being E. sardinae (Thélohan, 1890), with oocysts in the testis of sardine and herring, and E. clupearum (Thelohan, 1894a), with oocysts in the liver of herring and mackerel. In E. brevoortiana Hardcastle, 1948, schizogony occurs in the epithelium of pyloric caeca of the host (Brevoortia tyrannus (Latrobe)), while sporogony occurs in the testis. According to Markevich (1951), Dogiel indicated that the kidney was probably not the primary site of infection of E. rutili. In the present study, examinations were made of sectioned preparations of kidney, liver, spleen and regions of the alimentary canal of infected host but no evidence was seen of other stages in the life - cycle.

In neither St. James's Park nor the river Colne were heavy infections of E. rutili found. In St. James's Park, two out of ten R. rutilus caught in May, 1965, and two out of twelve caught in September, 1965, had oocysts in the kidney. In the river Colne, one out of six fish examined in June, 1965, was infected.

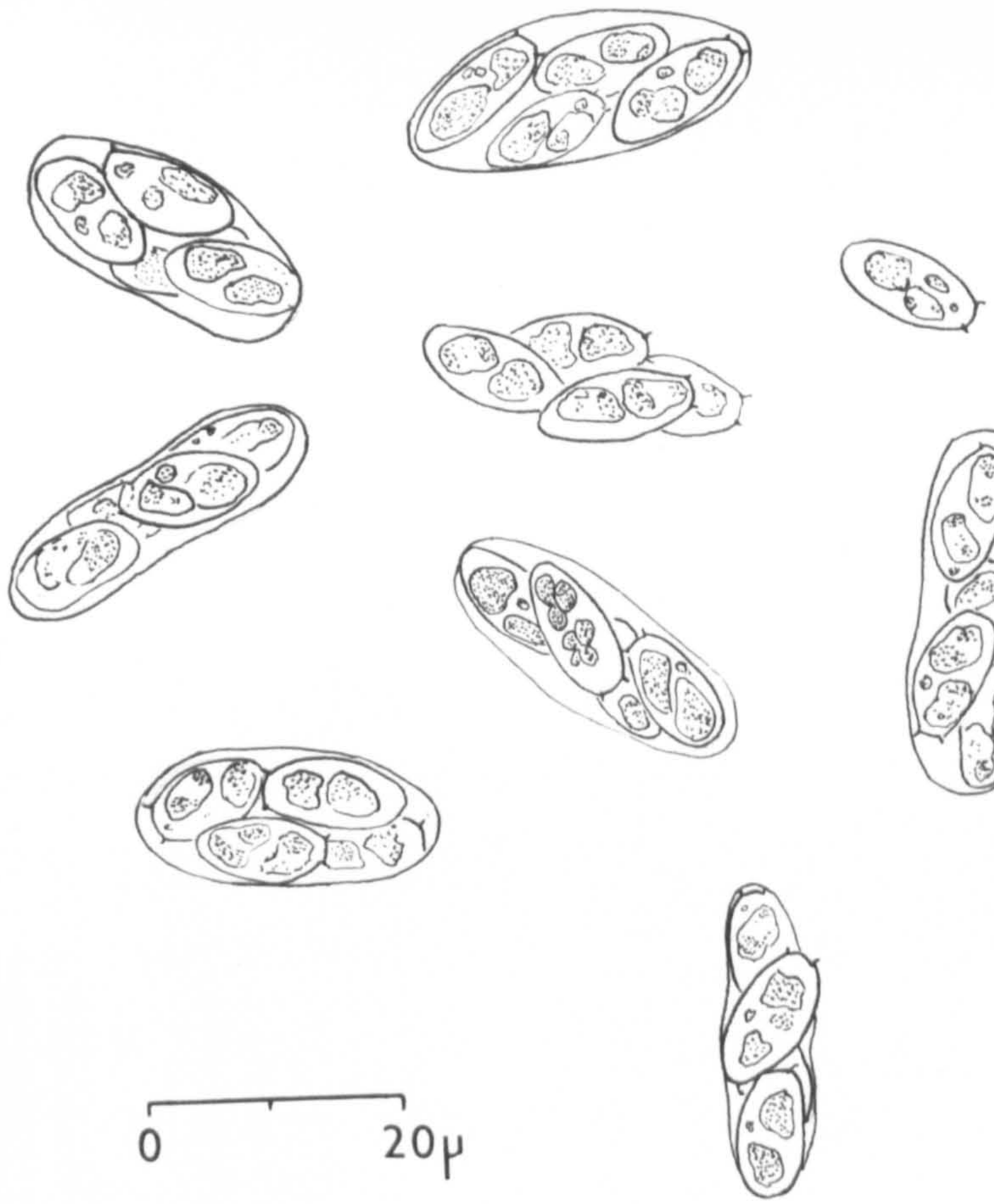


FIGURE 25.

Eimeria rutili oocysts from the kidney of *Rutilus rutilus*.

Fresh preparations.

(iv) Rhabdospora thelohani Laguesse, 1895.

The cell type named as Rhabdospora thelohani by Laguesse (1895) was found in various sites in the fish examined during the present study. It was particularly abundant in the epithelium of the duodenum and intestine of some specimens of Rutilus rutilus from St. James's Park (Fig:26a). In sectioned preparations, its appearance in this site was as follows.

The cell was ovoid in shape and lay between the epithelial cells, opening at its apex to the gut lumen (Fig: 26 b). It measured from 10 μ to 17 μ in length, and 5 μ to 10 μ in breadth. In Ehrlich's haematoxylin and eosin, Giemsa's stain and Feulgen's stain, a round, basal nucleus was visible. In the apical half of each cell, a cluster of rodlets, from 10 to 25 in number, was arranged in a conical shape. Each rodlet was rounded at the basal end and pointed at the apex. The rodlets were unaffected by the three stains mentioned above, but appeared red in P.A.S. and Bauer - Feulgen stains, and dark blue in Gram's stain.

R. thelohani cells, with the above appearance, were found in the following hosts;-

Rutilus rutilus, in the duodenum and intestinal epithelium, kidney, spleen and gall - bladder wall.

Salmo salar, in the duodenum and intestinal epithelium.

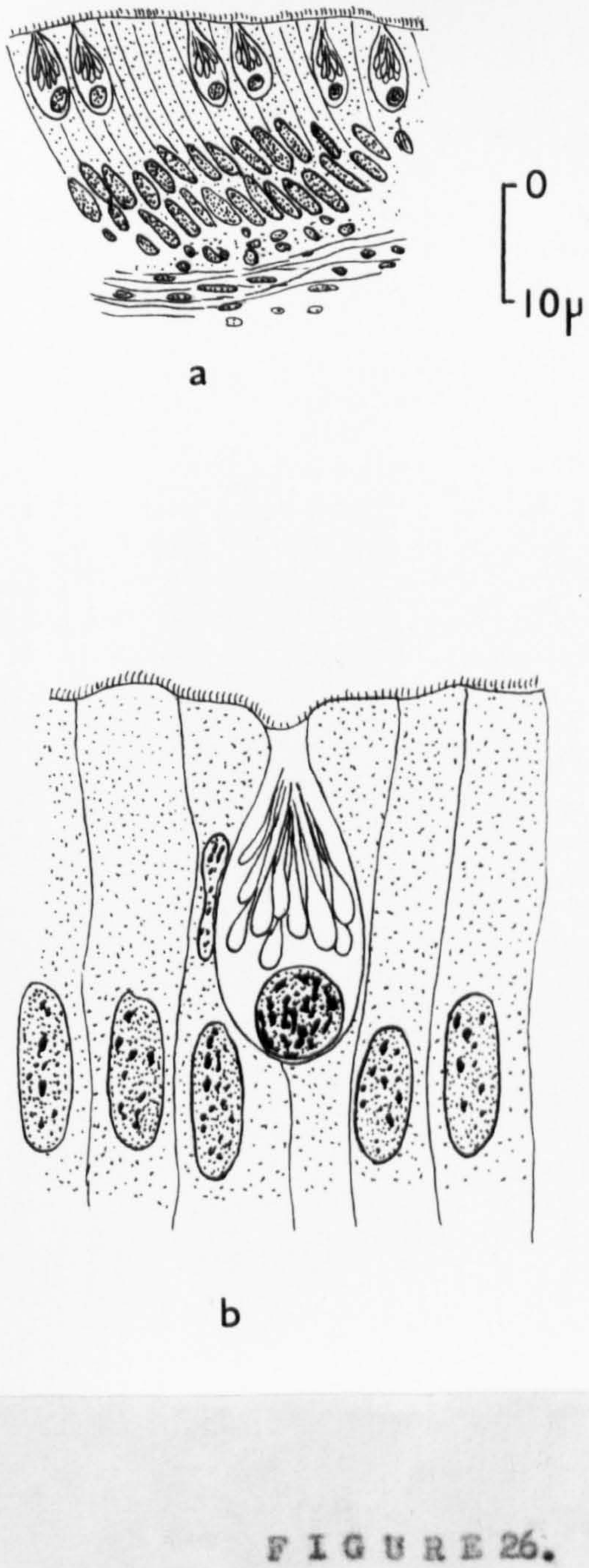
Serrasalmus sp.,in the kidney tubules.

Discussion; The nature of R. thelohani is not clear, even though its existence has been known for over seventy years. Some authors consider it to be a normal tissue cell of fishes, while others maintain that it

is a parasitic organism. Bannister (1966) reviewed the evidence for and against both theories and, after examining its ultrastructure, concluded that it was probably a protozoan parasite.

The present work supports the hypothesis that R. thelohani is foreign to the tissues in which it occurs. This may be deduced from the fact that it occurs in the same form in several sites in the body. Further, in the population of Rutilus rutilus in St. James's Park, some individuals contained dense aggregations of R. thelohani, while others were apparently free of it.

The unicellular nature and general appearance of the parasite have led several workers to classify R.thelohani as a sporozoan. As Bannister (1966) points out, however, this interpretation is based mainly on the lack of features such as cilia and flagella rather than on any positive characters. Much further work is needed to determine the true taxonomic position of R. thelohani.



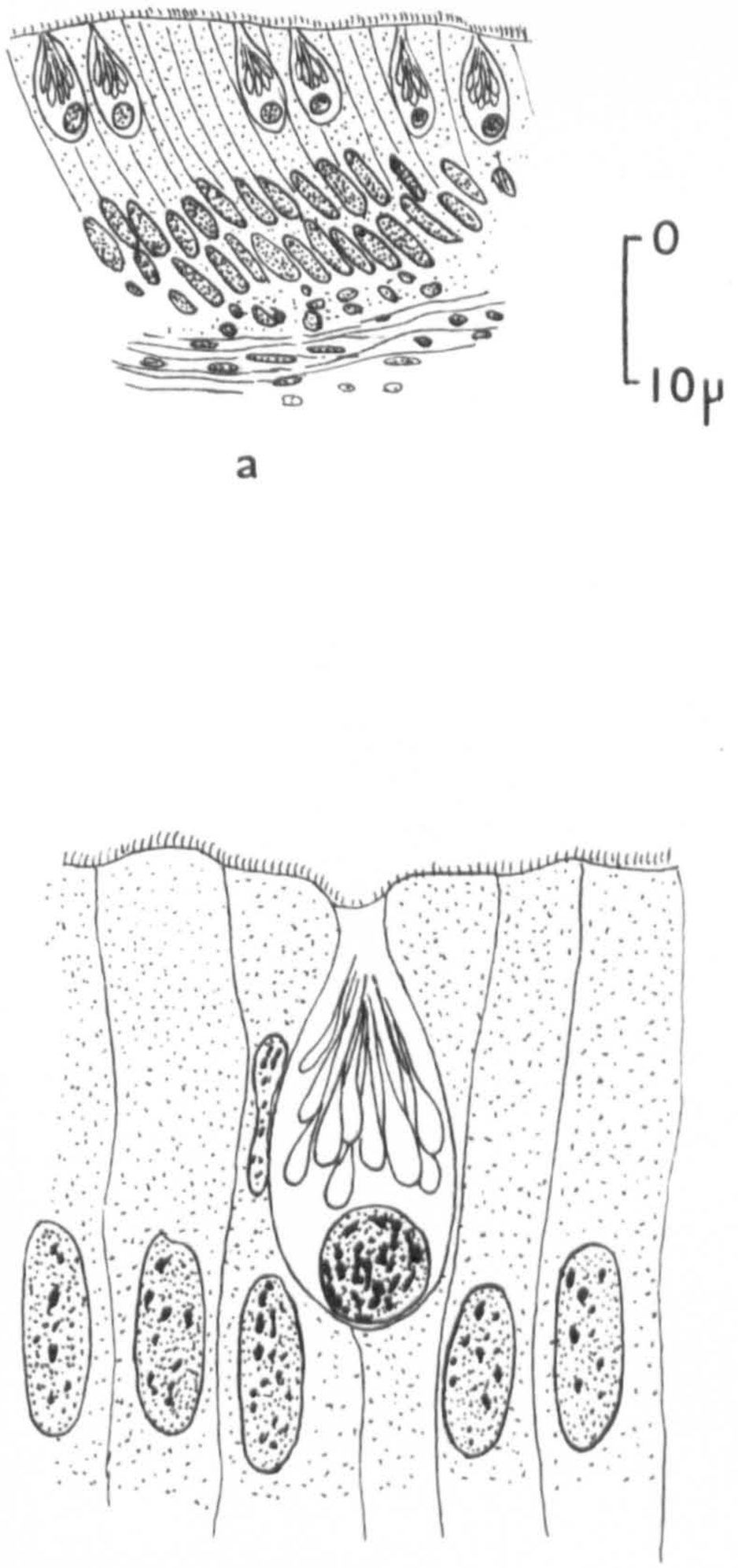
F I G U R E 26.

Rhabdospora thelohani from Rutilus rutilus.

Giemsa's stain.

a. Section of intestinal epithelium showing heavy infection.

b. Section of individual to show arrangement of ro and nucleus.



F I G U R E 26.

Rhabdospora thelohani from Rutilus rutilus.

Giemsa's stain.

a. Section of intestinal epithelium showing heavy infection.

b. Section of individual to show arrangement of ro and nucleus.

B I B L I O G R A P H Y

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WALLIKER (D.)

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Ph.D.

smallest in size of the three types, producing only 12-16 merozoites, and were mature by 96 hours. They were found throughout the small intestine and the lower half of the caeca which was a distribution similar to that of the sexual stages. The prepatent period was shown to vary between 112 and 124 hours (mean 117 hours).

The oöcysts were ellipsoidal in shape. The size was shown to vary significantly from infected chick to infected chick and there was a tendency to increase in size during the patent period.

The species was shown to be pathogenic to young poults, causing a loss of live-weight gain, and even death, depending on the size of the infective dose.

In each of two separate strains of *E. phasiani*, infections produced by doses of 25,000 oöcysts were completely suppressed by 0·006% w/w sulphaquinoxaline, but less so by 0·006% w/w amprolium and 0·0125% w/w zoalene.

Dr. Helen Hein, Dr. L. P. Joyner, Mr. C. C. Norton and Mr. D. D. Pout

Central Veterinary Laboratory, Weybridge

Gametocytes of some species of *Eimeria* in domestic animals

Gametocytes of *Eimeria ahsata* and *Eimeria arloingi* from lambs, *Eimeria bovis* from a calf, and *Eimeria maxima* and *Eimeria brunetti* from chickens were demonstrated in stained tissue sections and photomicrographs.

Mr. D. Walliker

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Some protozoan parasites of the roach (*Rutilus rutilus*)

In specimens of roach (*Rutilus rutilus*) collected from St. James's Park lake, London, and the river Colne. Rickmansworth, the following parasites were found:

1. *Myxosoma* sp. (Order Myxosporida, family Myxosomatidae). Trophozoites, in the form of multinucleate protoplasmic masses with no bounding cyst wall, are found in the liver, spleen, kidney and body musculature. Mature spores measure 10-12·5μ in length and 7-9μ in breadth. The polar capsules are unequal in size, the larger measuring 5·5-7·5μ × 3-4μ and the smaller 4·5-6μ × 2·25-3μ. They open to one side of the spore axis.

2. *Myxidium pfeifferi* Auerbach, 1908 (Order Myxosporida, family Myxidiidae). Spherical cysts, up to 0·15 mm. in diameter, are found in the kidney. Mature spores measure 11-13·5μ × 3·5-5·5μ, and the polar capsules 3·5-5·25μ × 2·0-3·0μ.

3. *Eimeria rutili* Dogiel, 1939 (Order Eucoccida, family Eimeriidae). Oöcysts, measuring 24-30μ × 8-12μ, are found in the kidney, and occasionally in the spleen and musculature.

4. *Rhabdospora thelohani* Laguesse. This parasite, which has an uncertain taxonomic position, occurs in the intestinal epithelium and kidney tubules. It has a widespread distribution in many species of fish. A full account of its fine structure is being prepared for publication by Dr. L. H. Bannister.

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Objects seen in the organs of *Gecko gecko*

1. An unidentified object in the lung of a gecko, *Gecko gecko*. The object seen in a section of lung is probably a mite.

2. Developmental stages of a haemogregarine in the lung and liver of *G. gecko*. All stages from single nucleated bodies to "cysts" containing (a) a few large forms, and (b) many small forms.

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The Management and Diseases of Fish—III

Protozoal Diseases of Fish with Special Reference to those Encountered in Aquaria

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PROTOZOA are the causative agents of a wide variety of diseases of fish, both under natural conditions and in aquaria. These diseases are undoubtedly of major economic importance but relatively little is known about them. Here, it is proposed to deal mainly with those protozoal infections normally encountered by amateur fish keepers. A brief description of each parasite is given and this is followed by a discussion on their pathogenicity and on their methods of control. The reader is referred to Reichenbach-Klinke and Elkan (1965), Dogiel *et al.* (1961) and Davis (1961) for more extensive accounts of the subject.

The two diseases most frequently encountered in aquaria are white-spot, caused by the ciliate *Ichthyophthirius multifiliis* Fouquet, and velvet, caused by the flagellate *Oodinium*, two common species being *O. limneticum* Jacobs and *O. pillularis* Schäperclaus. Other ectoparasites of importance include *Costia necatrix* (Henneguy), a flagellate which produces a condition known as infectious turbidity of skin and gills, *Chilodonella cyprini* Moroff, a ciliate having a similar effect to *C. necatrix* and *Trichodina domerguei* Wallengren, a parasite especially common in fish-farms. Other diseases of importance to the fish industry are caused by trypanosomes, trypanoplasm, myxosporidia, microsporidia and coccidia, although these rarely cause epizootics in aquaria.

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WHITE-SPOT DISEASE

White-spot affects a wide variety of freshwater fish and has been much studied, a comprehensive account of its symptoms and treatment being given by Ghadially (1956). *Ichthyophthirius multifiliis*, the causative agent, is a large, usually spherical, ciliate measuring up to 1 mm in dia. (Fig. 1). It can be recognized by its horse-shoe shaped macronucleus and by its continuous rotating motion. By this rotation, the organism burrows into the skin surface, causing a local irritation. The host's epithelium grows over and encloses the parasite thus producing a white spot. Each spot, therefore, represents a site of infection in which one or, occasionally, two parasites are found. In severe infections the whole body surface, fins and gills may be attacked. The organism feeds, through a small cytostome, on debris and dead epithelial cells.

The parasite matures in 1–3 weeks and then leaves its host coming to rest on a submerged object such as a stone or plant. Here a jelly-like cyst is formed within which a rapid series of cell divisions takes place, resulting in the formation of up to 1000 young swarmers. These are pear-shaped, measuring up to 40 μ in length. On reaching a new host they penetrate the epithelium with their pointed end. Swarmers perish if they fail to find a host within 3–4 days.

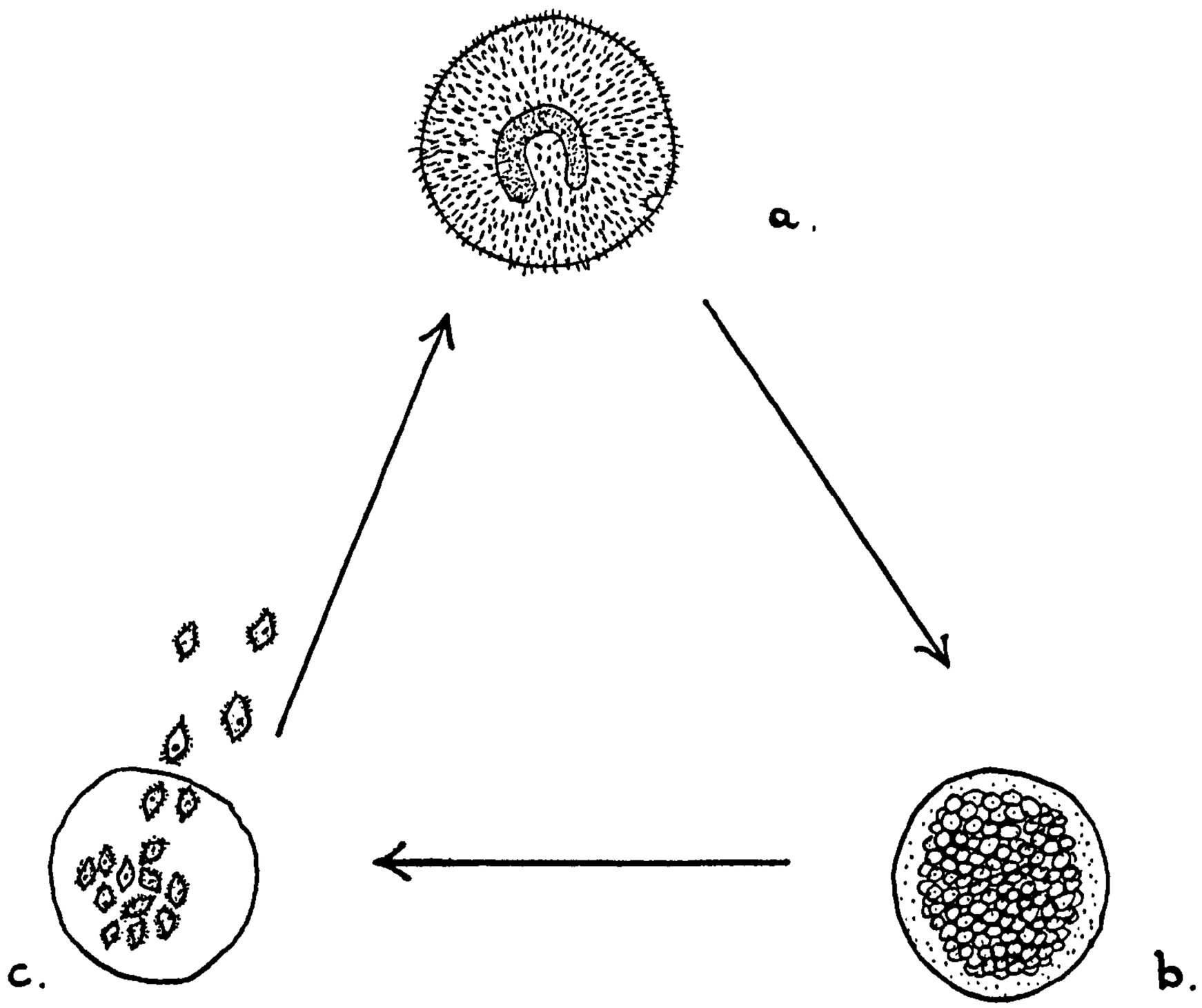

FIG. 1. *Ichthyophthirius multifiliis* ×100.
(a) Adult; (b) cyst containing maturing swarmers; (c) swarmers leaving cyst.

The life-cycle varies according to the conditions. For example, the time taken for a swarmer to grow to a mature adult varies with temperature from 3 days to 3 weeks. Mature forms may produce swarmers without encysting, and in certain cases parasites divide without leaving the host.

The chief symptoms of white-spot are damage to the epithelium, causing irritation and small wounds. In severe infections the host's mobility is affected and death may result. Diagnosis is by microscopic analysis of scrapings of mucus from the body surface, when the constantly rotating parasites can be seen.

The disease can attack many species of aquarium fish some of which are apparently more susceptible than others. In general, larger-scaled fish seem to be more resistant. Latent infections with only a few parasites present on the host are common, but a slight change in conditions may cause an epizootic. After an outbreak, a temporary immunity may be acquired (Ghadially, 1964).

The best method of preventing an attack of white-spot is to quarantine new stock in water at a temperature of 25°C. The average incubation period of the disease is 4 days so that fish which show no sign of infection after 1 week can usually be considered parasite-free. However, it is generally advisable to quarantine for several weeks because of the possibility of a latent infection. Similarly, new plants, etc. should be quarantined in case cysts are present.

Curative treatment must be directed against the free-swimming adults and swarmers as parasites on the host and within cysts are protected. In general it is possible to clear up an infection in 8–10 days. The following treatments are available:

(a) *Methylene blue*

This has the advantage of being cheap and relatively harmless to fish. However, it can damage plants so that treatment in bare, unfurnished tanks is advisable. The dose recommended by Ghadially is 15 mg/gal, with a further 5 mg/gal added each alternate day to maintain the concentration.

(b) *Acriflavine*

This widely used drug has the disadvantage of being insoluble in salt water. There is also some evidence that it can cause a temporary sterility in fish. The recommended dose in a bare tank is 2 mg/gal, with a further 1–2 mg/gal each alternate day.

(c) *Quinine*

This is not as reliable as the above drugs as it tends to be unstable in solution. There is also the danger of its toxicity to both fish and plants. The generally recommended dose is a 0·0003% solution.

(d) *Mercurochrome*

This is highly effective against the parasites, although it tends to be unstable and can cause kidney damage. A dose of 4 drops of a 2% solution/gal is said to be effective.

(e) *Chloramine*

This drug appears to kill swarmers by producing nascent oxygen. A recommended dose is a 0·01% solution for several days.

(f) *Outside aquaria*

A method for use in outside aquaria is to keep infected fish in a swift current of water, thereby washing away the parasites as they leave their host.

VELVET DISEASE

Velvet, rust, or gold-dust disease is caused by the flagellate *Oodinium* which, like *Ichthyophthirius*, affects many species of fish from both temperate and tropical regions. *O. limneticum* is especially common in North America and *O. pillularis* in Europe.

The adult parasite (Fig. 2) is pear-shaped, measuring up to 150 × 70μ and lacks a flagellum. By means of pseudopodia projecting through a funnel-shaped cytostome at its narrow end, it penetrates the host's epithelium and feeds on epidermal cells. The cytoplasm of the parasite contains numerous starch grains and light green chromatophores; its nucleus is spherical or egg-shaped.

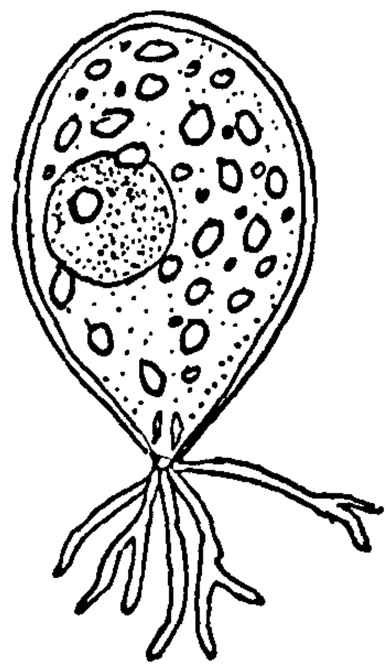

FIG. 2. *Oodinium limneticum* adult ×800.
(Redrawn from Kudo, 1954.)

Reproduction is similar to the type found in *Ichthyophthirius*, the mature parasite leaving its host after a few days and sinking to the bottom. Here a cyst is formed within which cell division takes place, resulting in the formation of 32 or 64 flagellated daughter cells, or dinospores. The latter perish if they fail to find a new host within 24 hr. On alighting on the host, the flagellum is lost.

The chief symptom of velvet is a discoloration of the skin, which becomes dusty brown in appearance. In a heavy infection the whole body surface is covered and sometimes the parasites burrow through the epidermis into subcutaneous connective tissue. The parasite spreads rapidly as an epizootic and in favourable conditions may be lethal within a few days.

The parasite can apparently survive for long periods of time in a non-parasitic form, by virtue of its chlorophyll content (Ghadially 1964); it is thus difficult to eradicate. Treatment with methylene blue and acriflavine is effective and to ensure

keeping an aquarium parasite-free, new stock should be quarantined for 2 weeks in either of these drugs. Other recommended treatments include a 0·15% solution of quinine dihydrochloride for 2–3 days and a 3–5% solution of sodium chloride for 1–3 min.

DISEASES CAUSED BY OTHER ECTOPARASITES

(*a*) Costia necatrix

The parasite (Fig. 3) affects many species of freshwater fish. It is characterized by two flagella and a kidney shape and lives on the body surface by feeding on mucus and epithelial cells.

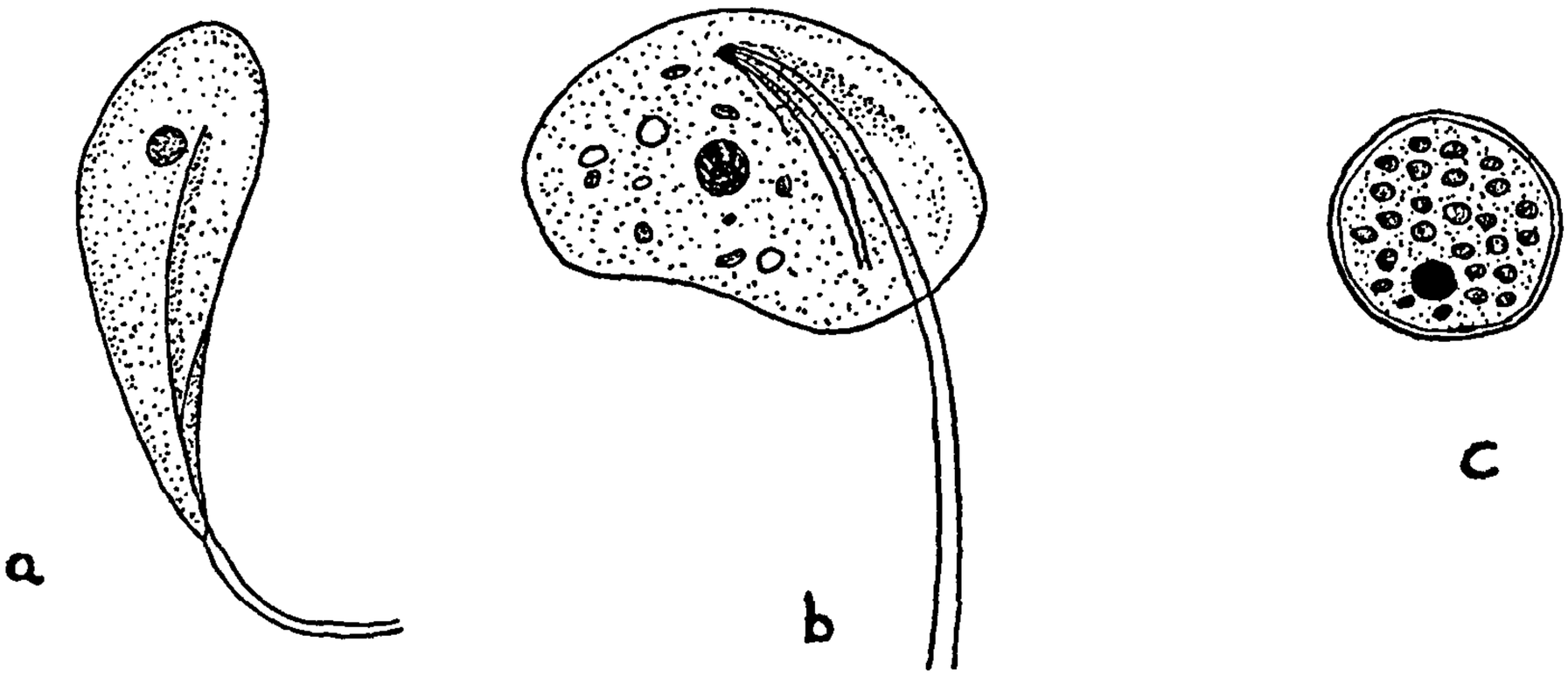

FIG. 3. *Costia necatrix* ×2000.
(a) Lateral view; (b) division stage; (c) encysted form.
(Redrawn from Wenyon, 1926.)

Unlike *Ichthyophthirius* and *Oodinium*, *Costia necatrix* divides on the host and is unable to survive longer than 1 hr if detached, although encystment may occur in unfavourable conditions. Division is normally very rapid, dividing forms with four flagella being commonly seen. The parasite is transmitted either directly or by transport of cysts.

The disease, sometimes called infectious turbidity of skin and gills, is characterized by epithelial damage, accompanied by secretion by the host of large quantities of mucus which gives the skin a bluish-white appearance. Respiration and mobility may become affected and death can result.

Among recommended treatments are the following:

(*i*) sodium chloride — a 5% solution for 5 min. The treatment is repeated at intervals of 5–8 days;
(*ii*) quinine — a 0·02% solution for 2 days;
(*iii*) acriflavine — a 0·01% solution for 2 days, and
(*iv*) formalin — a 0·025% solution for 1 hr.

(*b*) Chilodonella cyprini

This parasite is a ciliate (Fig. 4) characterized by a dorso-ventrally flattened shape with 8–15 rows of cilia on the ventral surface. It measures up to 70μ in length, 40μ in breadth and 18μ in height. The macronucleus is oval. Two contractile vacuoles are present. Around the cytostome there is an oral basket consisting of 14–26 cuticular rods.

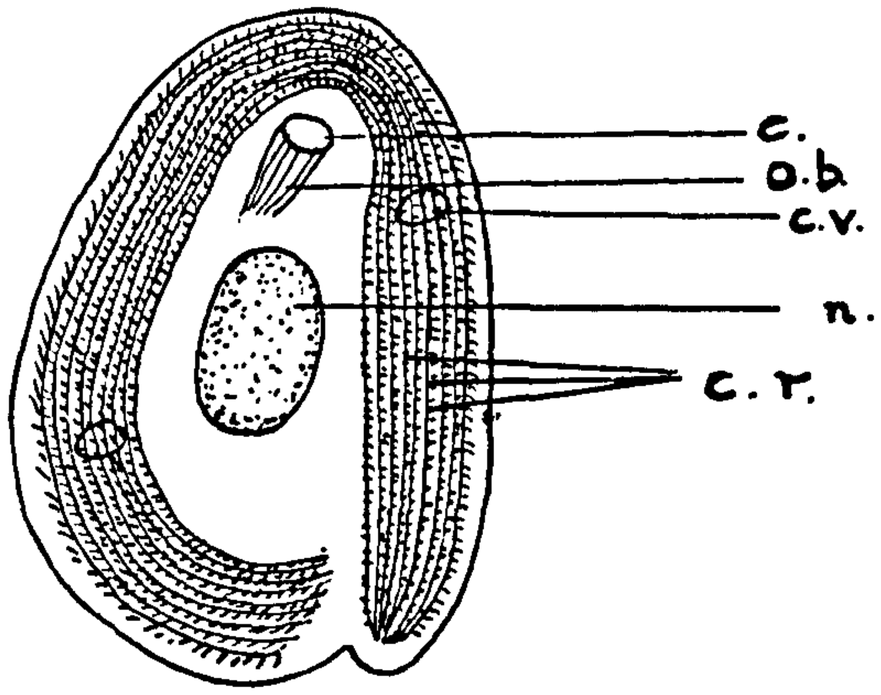

FIG. 4. *Chilodonella cyprini* ×1000.
c—Cytostome; c.r.—rows of cilia; c.v.—contractile vacuole;
n.—macronucleus; o.b.—oral basket.
(Redrawn from Markevich, 1951.)

Like *Costia* this parasite can affect many freshwater fishes. It feeds on debris and epithelial cells and divides on the host. The optimum temperatue for division is between 5 and 10°C, reproduction ceasing completely at 20°C. Under natural conditions, therefore, *Chilodonella* is most numerous during spring and autumn. Like *Costia* it perishes within a few hours if separated from its host, although encystment can occur.

The parasite causes a blue–grey film on the skin surface; microscopic examination of scrapings is necessary to determine whether the causative agent is *Chilodonella* or *Costia*. The host's respiration is affected and death may result. The treatments used against *Costia* are also effective against *Chilodonella*, sodium chloride, quinine, acriflavine and formalin being commonly used.

(*c*) Trichodina domerguei

T. domerguei (Fig. 5) is perhaps the most common of several *Trichodina* species which affect freshwater fish; it has a wide distribution among European fish. The parasite is disk-like, measuring up to 66μ in dia. and is characterized by several rows of cilia, an adhesive attachment ring and a circular ring of up to 29 chitinous hooks, lying within the body.

The parasite attacks the gills and skin of many species of freshwater fish, and by its rotation causes irritation and epithelial damage. It is apparently omnivorous, being able to live away from its host.

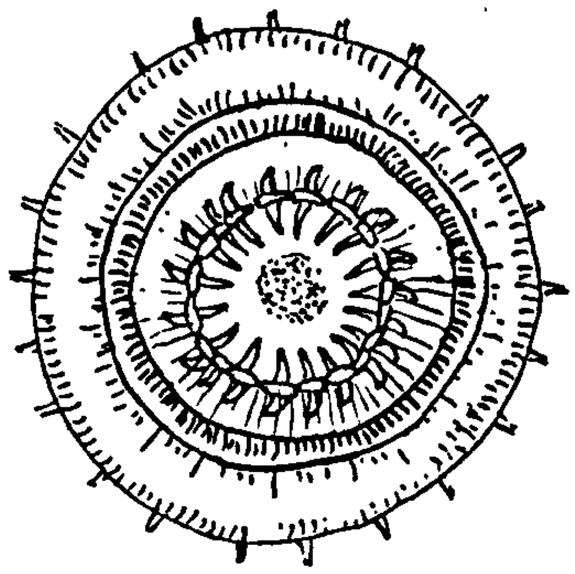

FIG. 5. *Trichodina domerguei* ventral view ×535.
(Redrawn from Kudo, 1954.)

The most common treatments are those described above for *Costia* and *Chilodonella*, viz: sodium chloride, acriflavine and quinine.

OTHER DISEASES CAUSED BY PROTOZOA WHICH MAY BE SEEN IN AQUARIA

There are numerous other protozoal diseases encountered under natural conditions, some of which are of economic importance to the fish industry. They are widespread and occasionally they may be found in aquaria, especially when attempts are made to establish new colonies of fish. Because of their relative rarity in aquaria, they are dealt with only briefly here.

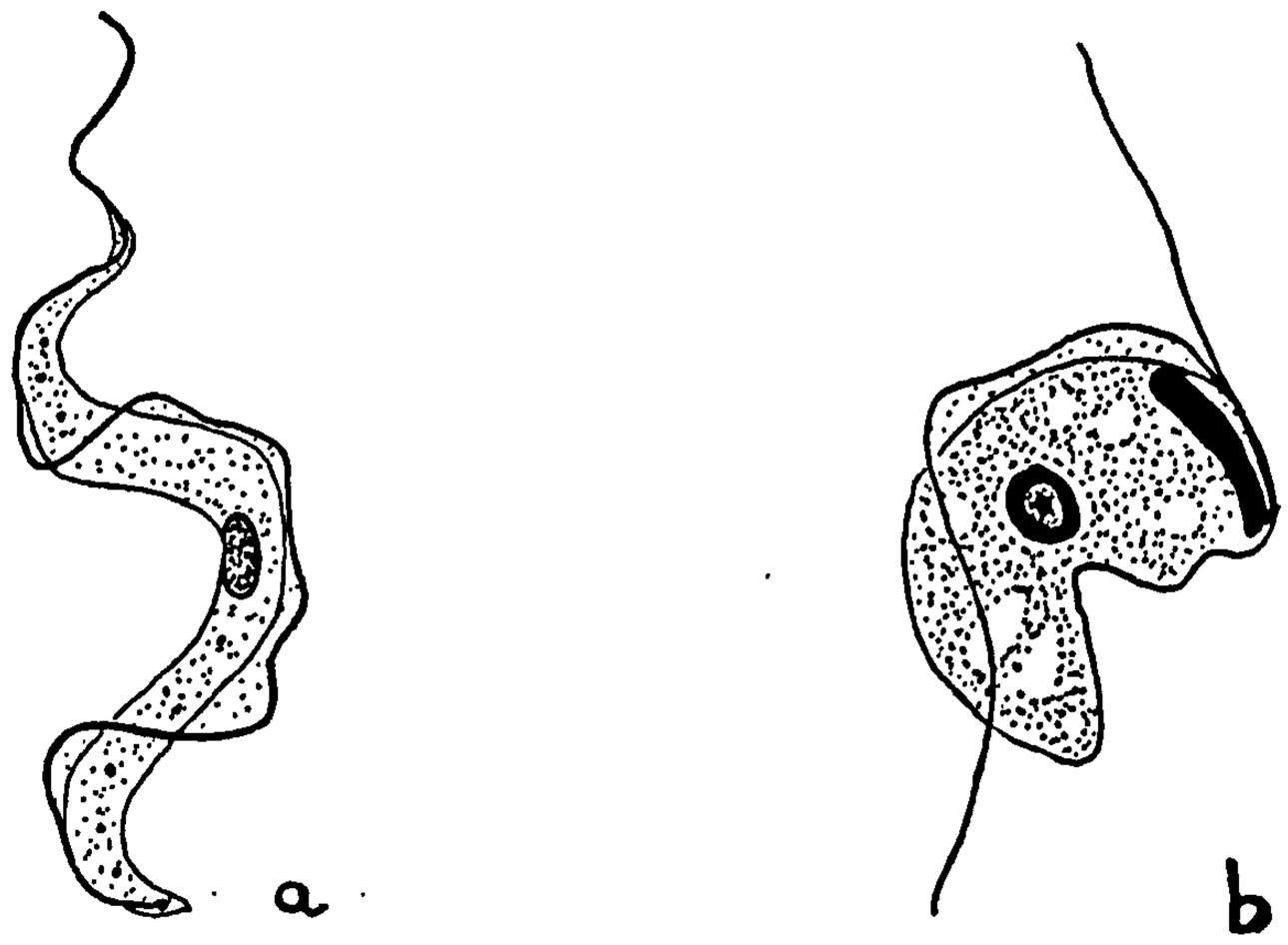

FIG. 6. (a) *Trypanosoma remaki* of the pike ×2000; (b) *Cryptobia cyprini* of the goldfish ×4000.
(Redrawn from Wenyon, 1926.)

(*a*) *Trypanosomes and trypanoplasms*

These organisms have a wide distribution in freshwater and marine fish. They live in the blood, trypanosomes possessing one flagellum and trypanoplasms two (Fig. 6). They are transmitted by leeches. Of interest to the aquarist is the trypanoplasm, *Cryptobia cyprini*, which occurs in goldfish and is thought to cause anaemia and loss of weight when present in large numbers.

(*b*) *Myxosporidia*

These are possibly the most common of all fish protozoa with a wide distribution in marine and freshwater hosts. They are found in almost every tissue and organ of the body, the gills and gall-bladder being commonly infected. The infective stage is the spore (Fig. 7) which consists of a bivalve membrane enclosing a sporoplasm and

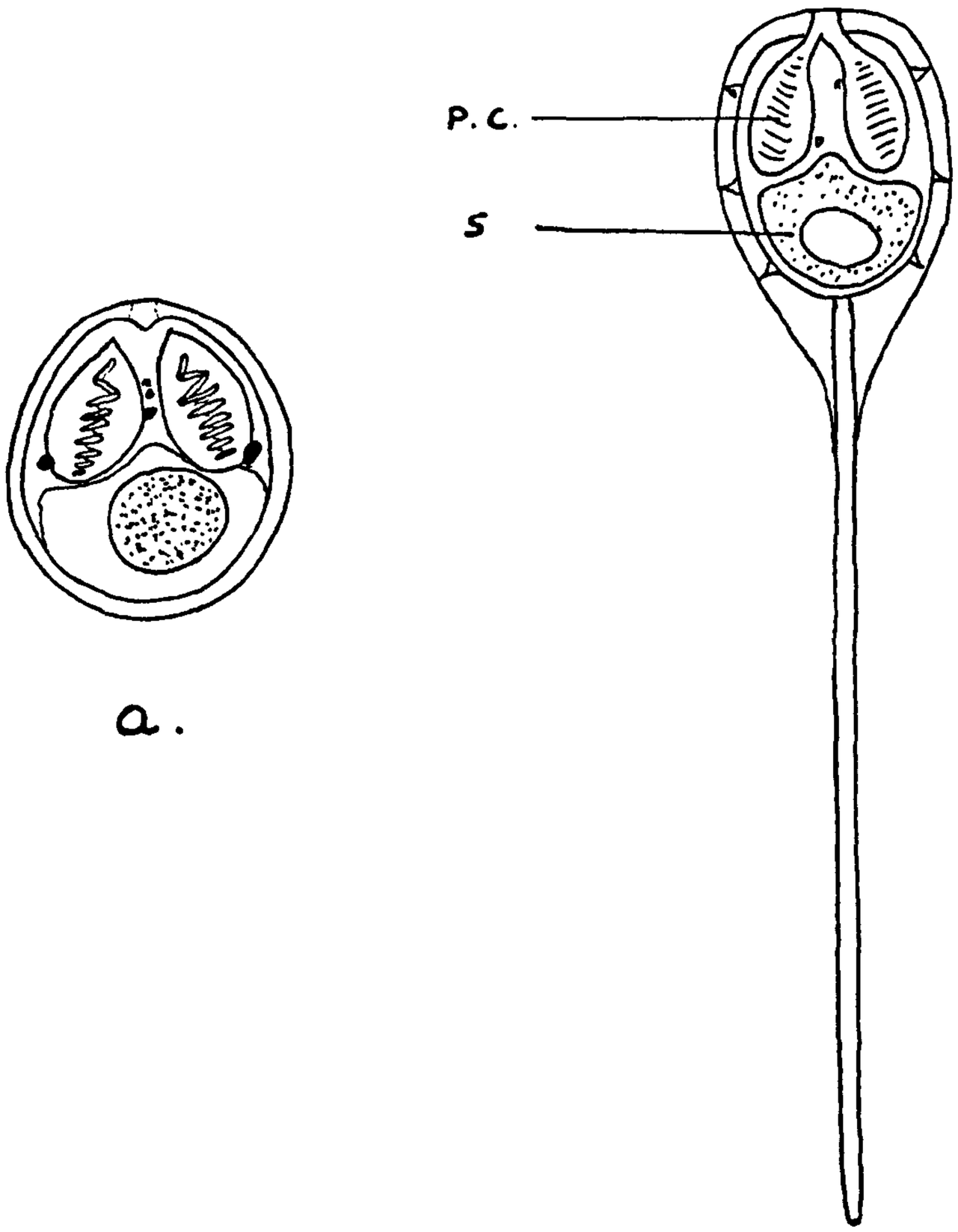

FIG. 7. Myxosporidian spores ×2150.
(a) *Myxobolus pfeifferi;* (b) *Henneguya salminicola.*
p.c.—polar capsule; s.—sporoplasm.
(Redrawn from Kudo, 1920.)

polar capsules. Spore characters are use for species identification. After being taken into the body the sporoplasm leaves the spore, makes its way to the specific organ and grows into a trophozoite within which spores are differentiated.

Although Myxosporidia affect a wide variety of hosts, the majority appear to be non-pathogenic. Some, however, can cause fatal epizootics. These include *Myxosoma cerebralis* (Hofer) of Salmonidae, which parasitizes cartilage and the semi-circular canals causing "whirling" disease; *Myxobolus pfeifferi* Thélohan, living in the muscles of the barbel (*Barbus barbus*) and causing "boil" disease; and *Henneguya salminicola* Ward, which invades the body musculature of some Salmonidae to produce white cysts, the condition being known as "tapioca" disease. Full accounts of Myxosporidia are given by Kudo (1920, 1954), Markevich (1951) and Bikhovskaya-Pavlovskaya *et al.* (1962).

(*c*) *Microsporidia*

These are similar to Myxosporidia, the infective stage being a spore consisting of a sporoplasm and polar filament enclosed by a spore membrane. An important species is *Glugea hertwigi* Weissenberg, which infects the intestine of smelt (*Osmerus* spp.) and causes epizootics. More information on Microsporidia is given by Kudo (1954), Markevich (1951) and Bikhovskaya-Pavlovskaya *et al.* (1962).

(*d*) *Coccidia*

All coccidia so far described from fish belong to the genus *Eimeria* and can infect diverse body organs. Examples are *E. cyprini* Plehn infecting carp fry (*Cpryinus carpio*) and causing severe damage to the intestinal mucosa, and *E. sardinae* which parasitises the testis of Clupeidae, a heavy infection causing parasitic castration.

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