ON SOME PROTOZOA PARASITIC IN FRESH-WATER FISHES OF NEW YORK*

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The Protozoa upon which the present communication is based, were found parasitic in fresh-water fishes collected in the vicinity of New York City, during August, 1920. Although no extensive research could be undertaken due to the small number of fishes obtained, yet new species of Myxosporidia as well as new host species were noticed. Furthermore, it became known that some of the common protozoan parasites of European fresh-water fishes of which little has been recorded up to date, occur also in North American waters. Therefore, I shall here briefly state my observations upon them.

Six species of fish were examined. *Abramis crysoleucus* (two individuals), *Ameiurus nebulosus* (four), *Anguilla chrysypa* (ten) and *Lepomis humilis* (three) showed no myxosporidian parasites. The blood of none of these fishes was examined so that I cannot make any statement regarding the blood-inhabiting Protozoa. The results of examination of *Lucius reticulatus* and *Moxostoma* sp. are as follows:

LUCIUS RETICULATUS

The host fish, thirteen in all, varied from 6.5 to 15 centimeters in length. On examination of internal organs, it was found that they were infected by three different protozoan parasites.

Wardia lucii nov. spec. (Figs. 1 to 9)

Habitat. In the uriniferous tubule, the space in the Malpighian body and connective tissue of the kidney. Two out of thirteen fish examined were found to be infected by the present Myxosporidian. In both cases the infection was light. A number of young vegetative forms and a few mature spores were observed in smears as well as section preparations.

Vegetative form. Form variable. It is usually rounded, viewed in fresh hanging drop preparations. The cytoplasm is distinctly differentiated into the homogeneous hyaline ectoplasm and highly vacuolated endoplasm (Fig. 3). Young forms in fixed preparations usually do not show the differentiation; the entire body is filled with granulated endoplasm (Figs. 1 and 2). Youngest forms are apparently uninucleate. The nucleus divides as the body becomes larger, producing a vegetative and a generative nucleus (Fig. 1); the latter divides soon afterwards into two (Figs. 2 and 3). Further changes seem to take

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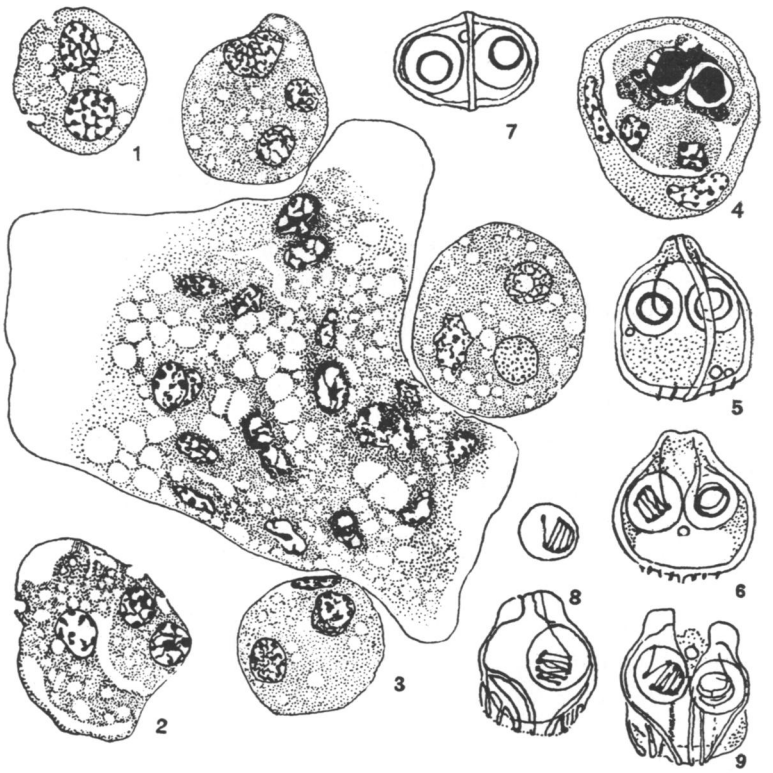
place in the ways similar to those of *Leptotheca ohlmacheri* which will be published elsewhere, including the formation of trinucleate gemmae which however are formed more than one in number in the present species (Fig. 3). Contrary to the above mentioned species, large forms (Fig. 3) which may produce a number of spores are also encountered, although no large cyst formation takes place. Lobose pseudopodia which vary in size according to the size of the trophozoite from which they are protruded, are slowly formed at unlocalized parts of the body. Polysporous and disporous.



Figs. 1 to 9, *Wardia lucii* nov. spec. Fig. 1, a binucleate trophozoite. Fig. 2, a trinucleate trophozoite. Fig. 3, a large trophozoite with three extruded gemmae (?). Fig. 4, a young spore. Fig. 5, the front view of a spore. Fig. 6, an optical section. Fig. 7, the end view of a spore. Figs. 8 and 9, spores slightly pressed under the cover glass. Figs. 1 to 4, Schaudinn, Giemsa, smears; × 2100. Figs. 5 to 9, fresh spores in physiological solution; × 2350.

Spore. Studied in hanging drop preparations, the spores show the following characters: Rounded triangular with convex sides in front view; oval in end views; and oblong in profile. The shell valves are thickened at the anterior tip. The sutural ridge prominent, is at right or acute angles to the line connecting the two polar capsules. Each

shell valve has irregularly marked ridges on its posterior portion. Two polar capsules spherical and usually equal in one and the same spore, occupy the anterior portion of the spore. The polar filament, coiled 4 to 5 times, is distinctly visible, and shows the similar condition as was shown by me (Kudo, 1920: 86, Figs. 620 and 621) in the case of *Mitraspora elongata*. The sporoplasm is homogeneous or finely granulated, and seems to fill the posterior half of the spore. Dimensions of fresh spores: sutural diameter 8 to 9μ, breadth 8 to 8.5μ, thickness 5 to 6μ, diameter of polar capsules 2.5 to 3.5μ, length of extruded polar filament (by pressure or potassium hydrate solution) 50 to 70μ. When stained, the sporoplasm appears distinctly to be a single mass containing two nuclei as is the case in the great majority of Myxosporidia.

The above description leads one to place the Myxosporidian in the genus Wardia (Kudo, 1920: 56) which I established (Kudo, 1920: 82-83) for *Wardia ovinocua,* the ovarian parasite of *Lepomis humilis*. The spores of both species were studied and measured in the fresh state, and show marked difference in every respect. The vegetative forms and the modes of spore formation are also entirely different one from the other. Hence, I consider that the present form is an unrecorded species, and have named it *Wardia lucii* nov. spec.

Myxidium lieberkühni Bütschli (Figs. 10 to 18)

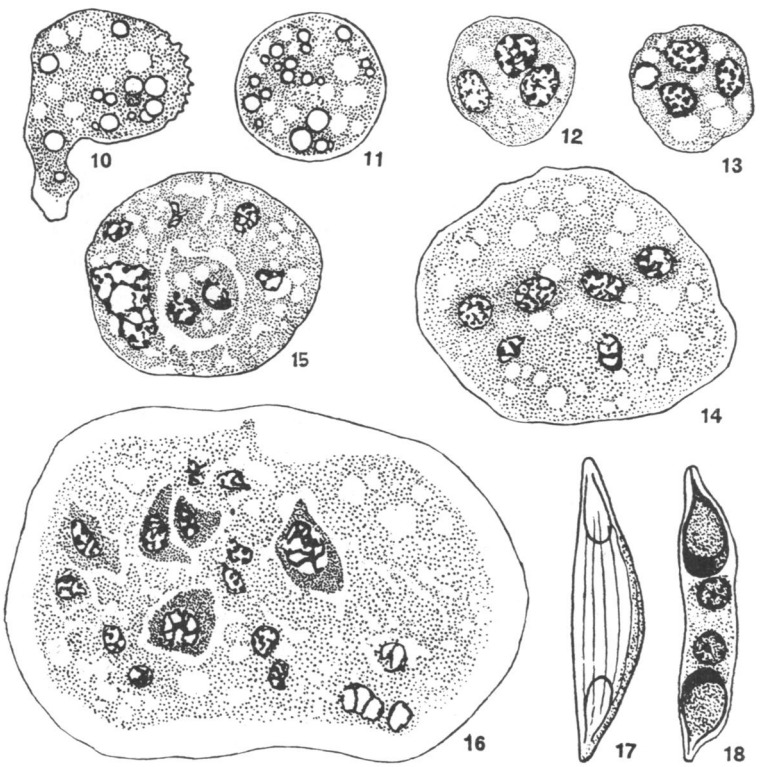
All the fish harbored the trophozoites and four also the spores of this well known Myxosporidian in their urinary bladders. *Myxidium lieberkühni* has been found by several European authors in the urinary bladder of *Lucius lucius* and *Lota lota* from various parts of Europe, and also by Mavor in the first named host species of Canada and the United States (Kudo, 1920: 107). Recently Debaisieux (1918) states that the Myxosporidian not only invades the bladder, but also the ureters, uriniferous tubules and further produces conspicuous cysts in the glomeruli of the Malpighian bodies of the kidney of *Lucius lucius*.

Lucius reticulatus is the third host species thus far found. Careful examination of the sections of the kidneys of the four host fish failed to confirm Debaisieux's observation upon the infected *Lucius lucius*.

Smaller fish showed apparently comparatively heavier infection than larger ones. Two fish, 6.5 and 6.8 centimeters long respectively, contained a large number of trophozoites and mature spores in their bladders. On the other hand, two large fish, 13.5 and 15 centimeters long respectively, showed a number of trophozoites and a few spores. The convincing evidence to explain this circumstance is missing. But the difference in the size of urinary bladders among small and large host fishes may hold some relation between the size of host fish and the sporulation of the Myxosporidian. The lack of space along the lining epithelium of the bladder in smaller fish, to which the tropho-

zoites attach themselves, may cause the sporulation in much shorter time than in larger fish where more space is found than the former.

Vegetative form. In every case, the trophozoites were small, the largest not exceeding 40μ in largest diameter. Form rounded or amoeboid (Figs. 10 and 11). Pseudopodia lobose or bristle-like (Fig. 10). Some individuals do not show any pseudopodium at all, examined even soon after the urine was made into hanging drop preparations (Fig. 11). The cytoplasm is distinctly differentiated into ectoplasm



Figs. 10 to 18, *Myxidium lieberkühni* Bütschli. Figs. 10 and 11, trophozoites viewed in hanging drop preparations. Figs. 12 to 16, trophozoites at various stages of development. Smear, Schaudinn, Giemsa, cedar oil; Fig. 16, the trophozoite is extremely thinly spread out. Fig. 17, a fresh spore. Fig. 18, a stained spore. Schaudinn, Giemsa. Figs. 10 and 11, $\times$1500; Figs. 12 to 18, $\times$2100.

and endoplasm (Figs. 10 and 11). The endoplasm is highly vacuolated and granular in structure, and contains droplets of oil. In contrast with the larger forms observed by European investigators, the trophozoites present in my preparations were strikingly uniformly small. I had an opportunity of comparing my smears and sections with a beautiful section preparation of the infected bladder of *Lucius lucius* pre-

pared by Professor A. Prenant of the University of Paris. Besides containing small trophozoites similar to those found in my preparations, the section showed numerous large individuals reaching 130μ in largest diameter. In the section preparations of the infected bladder of *Lucius reticulatus,* the small trophozoites are always seen attached to the epithelium, particularly, at the deepest part of the folds of the bladder. The changes that take place during the development of the trophozoites could not be studied in the fresh condition due to the pressure of other work conducted at that time. Uninucleate, binucleate and trinucleate trophozoites, particularly the latter, were found in a large number in stained preparations. In the trinucleate form, one can easily distinguish one vegetative and two generative nuclei, although the condition is in some individuals apparently reversed. Contrary to *Leptotheca ohlmacheri,* the vegetative nucleus divides repeatedly as the generative nuclei multiply. There is further seen that the division of the vegetative nucleus is far more active than that of generative nucleus. Consequently, as previous authors noticed, the trophozoites contain numerous nuclei of two kinds, one large and the other small. The distinction between the two kinds of nuclei is easily done in Giemsa stained smears, especially in very thinly spread smears, where one sees larger nuclei in dense islands of cytoplasm which stain in beautiful blue color, and the smaller nuclei simply scattered in the vacuolated endoplasm (Fig. 16). Some of the vegetative nuclei seem to undergo degeneration when the spore formation takes place. Viewed from the large number of uniformly small parasites, there most probably occurs active multiplication of the vegetative forms. Since I have not studied the fresh material thoroughly, I am unable to make any definite statement. In the stained preparations, I have not seen any indication that gemmation may take place in the present form as in *Leptotheca ohlmacheri.* On the other hand, I am inclined to think that plasmotomy most probably occurs. In sections, many trophozoites are found to form groups. In smears, I have quite frequently seen many small individuals connected with a somewhat larger one, the number reaching up to eight. In such young attached forms, the nuclei of two kinds were recognized, varying from 3 to 12 in number. Two spores are formed in one pansporoblast. These spores remain attached to each other even after being fully matured. Polysporous and disporous.

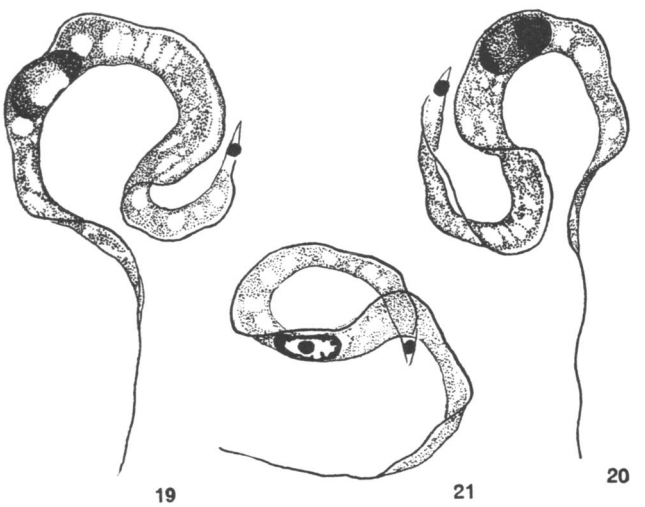
Spore. Studied in fresh state, the spores show the following characters: Form exactly as was figured by Bütschli (Kudo, 1920, Fig. 238). Fusiform bent to one side (Fig. 17). The spore membrane is comparatively thin. Sutural ridge hardly recognizable. The spore membrane is longitudinally striated. Polar capsules are pyriform and equal in one and the same spore. Contrary to the observations of

Thélohan and Mavor, the coiled polar filament is only faintly visible. Dimensions of fresh spores: length 18 to 20μ, breadth 4.5 to 5.5μ, polar capsules 5.5μ by 2μ.

When stained, the nuclei of the sporoplasm are seen rather widely separated from each other.

Trypanosoma remaki Laveran et Mesnil (Figs. 19 to 21)

Three fish were found to harbor in the blood a species of Trypanosoma, whose characters agree with those of *Trypanosoma remaki* described by Laveran and Mesnil (1902) and Minchin (1909). According to Laveran and Mesnil, the flagellate seems to have been observed by several authors in the blood of *Lucius lucius* from various



Figs. 19 to 21, *Trypanosoma remaki* Laveran et Mesnil. Figs. 19 and 20, var. *magna.* Schaudinn, Giemsa, cedar oil. Fig. 21, var. *parva.* Schaudinn, Heidenhain's iron hematoxylin. All $\times$ 3300.

parts of Europe. I have failed to find any North American record regarding this Protozoan.

According to the above mentioned three European authors, the number of the flagellates found in blood of the host fish is usually small. In the case of *Lucius reticulatus,* this was also true. The blood smears of two host fish showed one trypanosome in about every tenth field under the combination of compensation ocular 12 and apochromatic objective 16 mm., while that of the third host, one or two in every field under the same combination. In fresh as well as fixed and stained smears, active animals were only recognized. Viewed in hanging drop preparations, the flagellate undergoes very active movements by means of its undulating membrane and flagellum.

As were noted by the three previous investigators, two types of the animals which differ mainly in the dimensions of the body were also recognized in my preparations, although the distinction is not so sharply marked as Minchin stated (1909: 22). The larger type, var. *magna* (Figs. 19 and 20), measured after smears, fixed in Schaudinn, stained with Giemsa and mounted in cedar oil, varied from 30 to 33μ in length excluding the flagellum, and showed an average width of 2μ. The length of the flagellum seemed to vary, but could not be measured accurately due to its poor staining reaction.

The smaller type, var. *parva* (Fig. 21), measured in similarly prepared smears as above, showed the following dimensions: length of the body 24 to 27μ, average breadth 1.5μ.

The difference in the nature of the cytoplasm in the two subspecies observed by previous authors, was also noted. I have, however, not noticed in my preparations the "coarse granules staining reddish with Giemsa stain," observed by Minchin (1909: 22). Although not much difference in the size of blepharoplasts of two subspecies was noticed, they appeared larger in size than those figured by Laveran and Mesnil. The nucleus is usually located close to the anterior extremity. It is rounded oblong. In smears stained with Heidenhain's iron hematoxylin, a centrally located karyosome was surrounded by chromatic granules which were attached closely to the nuclear membrane. In Giemsa stained specimens, I have frequently noticed that the nucleus has two deeply staining masses located at opposite ends, the central part being apparently filled with a fluid which stained poorly (Figs. 19 and 20). Whether this is a stage in the division of the nucleus, I cannot decide at present. I have not seen any individual with two nuclei or two blepharoplasts so that I have no datum concerning the multiplication of the flagellate.

PARASITES FOUND IN MOXOSTOMA SP.

Myxidium moxostomatis nov. spec.

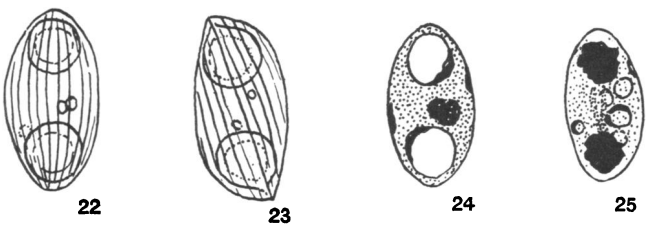
(Figs. 22 to 25)

Habitat. In the gall bladder of *Moxostoma* sp. Two fish were examined. One female, 30 centimeters long, showed a light infection by the present parasite, while the other male, 34 centimeters long, was found uninfected. Only a few spores and trophozoites were seen floating in the bile when examined.

Vegetative form. Large trophozoite is a rounded leaf-like body, the margin being frequently folded up in the bile. The cytoplasm is distinctly differentiated into ectoplasm and endoplasm. Further cytological study could not be carried out. No active amoeboid movements

of the body were noticed. The largest trophozoite observed reached a size of 2 by 1.5 millimeters. Each pansporoblast produces two spores. Polysporous.

Spore. Studied in fresh conditions, the spores show the following characters. Broad fusiform. Both ends are equally rounded in view at right angles to the sutural line; while they are pointed toward the diagonally opposite directions seen from a point on the sutural plane (Fig. 23). The spore membrane is comparatively thin. The sutural plane makes an acute angle with the longitudinal axis of the spore. The sutural line is straight, but is faintly marked. Fine striae, about ten in number on each shell-valve, run longitudinally. The polar capsules are spherical, and each is slightly drawn out toward its foramen. Coiled polar filament is invisible. Dimensions of fresh spores: length 8.5 to 10.5μ, breadth and thickness 5 to 6μ, diameter of polar capsules 3μ, length of polar filament 30 to 35μ.



Figs. 22 to 25, *Myxidium moxostomatis* nov. spec. Figs. 22 and 23, different views of fresh spores. Figs. 24 and 25, Giemsa stained spores. All ×2350.

When stained the sporoplasm shows occasionally a single but usually two nuclei. Dimensions of stained spores (smears): length 8 to 9μ, breadth and thickness 4.5 to 5μ, diameter of polar capsule 2.5 to 3μ.

From the above description, it is clear that the Myxosporidian is a species of the genus Myxidium. Compared with 27 species of the genus (Kudo, 1920), one finds that the dimensions of the spore do not agree with any one of them. In the shape of the spore, it is somewhat similar to *Myxidium macrocapsulare* (Kudo, 1920: 113), but is different in dimensions. Further comparison cannot be made, as the vegetative form of the latter species is unknown. Hence, I believe this Myxosporidian is a new species, and name it *Myxidium moxostomatis* nov. spec.

2 *Myxobolus* (?) sp.

In the smears of the kidney of a small fish, 9.5 centimeters long, a few isolated spores apparently belonging to the genus Myxobolus, were noticed. As the preparations were misplaced, detailed study could not be made.

In any case, no pathological changes in the external characters or internal organs, were recognized.

SUMMARY

1. Two new species of Myxosporidia, *Wardia lucii* and *Myxidium moxostomatis,* parasite in *Lucius reticulatus* and *Moxostoma* sp. respectively, of New York, are described.

2. A new host fish for *Myxidium lieberkühni* Bütschli is observed.

3. *Trypanosoma remaki* Laveran et Mesnil occurring in the blood of *Lucius reticulatus,* is studied.

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