THE MYXOSPORIDIA OF THE BEAUFORT REGION, A SYSTEMATIC AND BIOLOGIC STUDY

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By H. S. Davis University of Florida

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Contribution from the United States Fisheries Biological Station, Beaufort, N. C.

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INTRODUCTION.

For several years the writer has been engaged in a study of the Myxosporidia at the United States Fisheries Biological Station, Beaufort, N. C., and a part of the results of this work is included in the present paper.

When first undertaking the study of the Myxosporidia the author was impressed with'the fact that very little work had been done on the group in this country. With the exception of Gurley's paper (Gurley, 1894), which is largely a compilation, scarcely anything has been published on the Myxosporidia by American biologists. It is therefore not surprising to find that nearly all the Myxosporidia occurring at Beaufort belong to undescribed species and in a number of cases to new genera. Some of these are of great interest, since they differ widely from previously known forms and throw a new light on many problems connected with the group.

It became evident early in the work that many American species would not conform to any accepted system of classification, and it was hoped that it would be possible to work out a more satisfactory one. As the work progressed, however, it became more and more evident that a satisfactory classification could not be developed with our present limited knowledge of the Myxosporidia, and while some important modifications of the present classification are suggested, they are to be considered merely tentative and will undoubtedly require revision as knowledge of the group increases. They are suggested primarily with the object of showing the weak points in the present classification and, it is hoped, may serve as a basis for a more satisfactory one in the future.

This paper makes no pretense of including all the Myxosporidia of the Beaufort region. Indeed, it is very certain that many species are omitted, because the author has brief and fragmentary notes on a number of species to which no reference is made in the text. It has seemed better to omit these forms entirely until more complete data have been obtained. Then, too, the investigations have been confined to the months of June, July, and August, and there is evidence that some species of Myxosporidia are more or less seasonal in their occurrence, and there may very well be species which do not occur in the adult form during these months. Furthermore, it has not been possible to examine all the species of fish commonly found in the Beaufort region. For the most part the fish examined have been those which are abundant and easily taken. While only a limited number of fish belonging to rarer species have been examined the absence of Myxosporidia in these fish can not be considered of any particular significance, since it is comparatively rare to find all the fish of any species infected with the same parasite.

For these reasons it has not been considered advisable to include in this paper a list of the fish examined, since such a list might easily lead to very erroneous conclusions.

METHODS.

The description of the trophozoites and spores have been made almost entirely from living material. They were removed to the slide from the recently killed fish and studied at once. To prevent evaporation the cover glass was ringed with paraffin of a low melting point. In the case of the trophozoites it is essential that they be studied without delay, for they almost invariably undergo degenerative changes within a few hours after the death of the host.

Some efforts have been made to grow the trophozoites on culture media, but so far without success.

While the spores are naturally much more resistant to unfavorable conditions than the trophozoites, it is nevertheless important that all descriptions be based on perfectly fresh material. There is a marked tendency in most species of Myxosporidia for sporulation to take place much more rapidly under unfavorable conditions, and spores formed under such conditions are often smaller and may show other striking variations from the typical form.

Although the descriptions in all cases have been based primarily on living specimens, fixed and stained material has been utilized in many instances. For the study of the nuclei smears made by the Giemsa method have been of great value. They were prepared as follows:

A small amount of material was spread on the cover glass to form a very thin film, which was then exposed to osmic vapor for about one-half minute. The film was then allowed to dry, fixed in absolute alcohol about one-half hour, and after drying stained by Giemsa's method for several hours. After staining they were decolorized in acetone, dried, and mounted in damar. Damar has been found to be much more satisfactory than Canada balsam for this purpose. Films mounted in damar have faded very slightly, if at all, after several years, while those mounted in neutral balsam are so badly faded after a year or two as to be of no value.

The success of this method varies greatly in different species. In some cases the nuclei are brought out with great clearness, while in others the results are very unsatisfactory.

Wet smears were also used, but in general were not as valuable as those prepared by the Giemsa method. In making these the material was spread in a thin film over the cover glass, exposed to osmic vapor for one-half minute, and then placed at once in Worcester's formol-corrosive-acetic fixing fluid for one to three hours. They were stained with Delafield's hematoxylin, iron hematoxylin, or Mayer's acid hemalum.

In the preparation of sections the best results were obtained with material fixed in Worcester's formol-corrosive-acetic fluid and stained with iron hematoxylin and congo red.

GENERAL ACCOUNT OF THE MYXOSPORIDIA.

INTRODUCTORY.

The Myxosporidia are chiefly parasites of fishes, although several have been found in Amphibia and reptiles and possibly in a few invertebrates.^a So far as known they are all endoparasites, and no part of the fish, except possibly the digestive cavity, appears to be free from infestation. They are often described as being primarily tissue parasites, but this is a mistaken view, due to the fact that the tissue parasites often form conspicuous lesions and may be very injurious to the host. On this account they have attracted more attention, while the less injurious and less conspicuous forms occurring in various cavities in the body have largely been overlooked, although in reality much more common.

Not only are the Myxosporidia to be looked upon as occurring chiefly in various cavities of the host—at least for the greater part of their existence—but, as has been pointed out by Auerbach (1910), the "free" forms are more primitive and nearer the original ancestors of the group than the typical tissue parasites. The latter are highly specialized forms which have in all probability descended from species which lived free in cavities of the host. A number of recent writers have urged that the Sporozoa are a polyphyletic group—one division having been derived from the Mastigophora, the other from the Sarcodina. The sarcodine ancestry of the Myxosporidia is strongly indicated by the "free" forms, the trophozoites moving by means of well-developed pseudopodia and often, especially in the younger stages, appearing strikingly like Amebæ.

STRUCTURE AND ACTIVITIES OF THE TROPHOZOITES.

From what has been said it will be evident that the typical structure of the trophozoites is best shown by those forms which spend the greater part of their existence in cavities of the host. In these cases the trophozoites form plasmodia, which either float free in the fluids filling the cavities or are attached to the epithelial lining. Although the plasmodia of the same species often show considerable variations in form, it is nevertheless true that each species is characterized more or less definitely by peculiarities of form and structure, so that in many cases they can be recognized even in the absence of the spores. This is especially true when applied to the different genera, although even in this case there are numerous exceptions. For instance, in most species of *Ceratomyxa* the trophozoites are pyriform with a definite anterior end—in *Sphæromyxa* flat and leaflike, and in *Myxidium* Amebalike, with short, lobose pseudopodia. In practically all cases the trophozoites when irritated or subjected to unfavorable conditions tend to contract into a spherical mass.

Usually the trophozoites show a well-defined ectoplasm and endoplasm, but in some cases there is no distinct peripheral layer. When distinguishable, the ectoplasm may form a distinct layer all over the body or it may be recognizable only in certain regions. It is denser than the endoplasm, usually clear and homegeneous, and occasionally, as in *Myxidium lieberkühni*, *Sinuolinea dimorpha*, or *Chloromyxum leydigi*, may be covered with a layer of short hair or bristlelike processes. The more or less

^a The accounts of the occurrence of true Myxosporidia in the invertebrates are very few and fragmentary and badly in need of confirmation.

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characteristic shape of the trophozoite is undoubtedly due to the presence of this firm outer layer, which also serves for protection and furnishes the means of locomotion, the pseudopodia being always chiefly and in many cases exclusively of ectoplasm.

The endoplasm has a more fluid structure than the ectoplasm and can usually be easily distinguished by its distinctly granular appearance. It contains numerous nuclei, many of them inclosed in distinct cells, and in addition various metaplasmic products, such as fat globules, pigment granules, and crystals of various kinds. These may in some species be distributed uniformly throughout the endoplasm; in others their distribution is markedly irregular. In pyriform trophozoites they are usually much more abundant near the anterior end than elsewhere. In at least one instance (Sinuolinea dimorpha) the endoplasm also contains food particles in various stages of disintegration. It is often more or less distinctly vacuolated, the distinctness of the vacuoles varying greatly even in the same individual. The endoplasm, in several species of Ceratomyza and Leptotheca, has a very remarkable structure. In addition to granules and fat globules it contains large numbers of spherules, which are often so abundant as to entirely fill the endoplasm, with the exception of a short distance at the anterior end. These spherules vary greatly in size in different species and may be homogeneous throughout or inclose crystals or pigment granules. The consistency of the spherules may vary greatly in individuals of the same species. Sometimes they are so firm and dense as to retain their shape perfectly on drying; in other individuals they are much more fluid and easily distorted and in extreme cases may apparently break down into a watery substance. It is still a question whether adult trophozoites of the same species may occur in two extreme conditions---one with well-defined, dense spherules, the other with no trace whatever of spherules-and in all intermediate conditions. This is doubtless true in the case of very small trophozoites, the spherules only appearing as they grow larger, but there is also evidence that full-grown trophozoites may sometimes fail to develop spherules. In a few cases, notably in *Ceratomyxa attenuata*, rod-shaped bodies strikingly like bacteria were present in the endoplasm.

The sole method of locomotion is by means of pseudopodia, which vary greatly in shape and structure in different species. Sometimes they are long and filiform, in other cases short and conical, in still others branched or even arborescent, or, finally, they may be broad and coarsely lobate. In pyriform trophozoites, in addition to the numerous filiform pseudopodia, there is usually a rapid wavelike movement of the ectoplasm at the anterior end, very characteristic and very difficult to describe.

There is still considerable uncertainty in regard to the actual mode of progression among the Myxosporidia. In the case of those having lobose pseudopodia the problem is the same as in Ameba, and it is unnecessary to consider it further here. But in most species of *Ceratomyxa* and *Leptotheca* the trophozoites are pyriform and capable of comparatively rapid progressive movements in one direction only. Consequently a large, rounded, anterior end can be distinguished, while the posterior end usually terminates in a long, slender, tapering process, termed, by Doflein (1898) the "Stemmpseudopodien." At the anterior end are formed numerous, long, filiform pseudopodia. According to Doflein, locomotion in such cases is chiefly due to the "Stemmpseudopodien," which by elongation pushes the body forward. However, after a careful study of the movements of such trophozoites, the writer is convinced that locomotion is due entirely to the pseudopodia at the anterior end. Many times trophozoites have been seen moving rapidly forward when the posterior process extended out freely behind and was attached to neither the slide nor cover glass. This was conclusively shown when the trophozoite turned quickly to one side; the posterior process swept around and continued to extend straight out behind, which could not have been the case if it was

attached. In a few cases in turning the process hit an object and was then bent to one side. In one instance a sporulating trophozoite of Leptotheca scissura, which was entangled on the slide in a bit of débris was observed. In its efforts to escape the body became greatly elongated by the pulling of the pseudopodia at the anterior end until it finally became small enough to be pulled free. It is true that the posterior process can sometimes be seen to elongate as the trophozoite moves forward, but that only occurs when it adheres to some foreign object and is consequently pulled out into a long, slender process by the pseudopodia at the anterior end.

Under dark-ground illumination the long, filiform pseudopodia can be seen to be thrust forward and then sweep posteriorly toward the sides of the body, as in figures 3-7, plate xvi, and figure 43, plate xix. Movements of these pseudopodia in all directions can be plainly seen; in fact, they move about much like flagella. It is also certain that the filiform pseudopodia can be rapidly absorbed and reformed. Text figure 1 shows clearly the formation of these



FIG. 1.—Movements of pseudopodia in a vegetative trophozoite of either Leptotheca or Ceratomyza sp. from the gall bladder of Cestracion tiburo. A, trophozoite with two long pseudopodia, a and b, at anterior end. B, to minutes later, a has moved to posterior end of body, b has elongated and is also moving toward posterior end. C, r minute later, b has nearly reached posterior end and 2 new pseudopodia, c and c, have appeared at anterior end. D, 3 minutes later, c and b have nearly fused. E, to minutes later, a and b are completely fused and c has increased in length. \times tooo.

pseudopodia in a trophozoite which had been on the slide for some time, and consequently the movements were so slow that they could be easily followed.

In most cases, possibly in all, in addition to the filiform pseudopodia, short, conical, and branched pseudopodia are formed at the anterior end. These are difficult to see, as they are very transparent and continuously changing. They are simply an exaggerated form of the wavelike movements of the ectoplasm noted above, which are so characteristic of pyriform trophozoites. In the intense light of the dark-ground illuminator the filiform pseudopodia are often retracted after a short time, locomotion then being accomplished entirely by these short, conical, or branched pseudopodia. The same is usually true of trophozoites which have been on the slide for some time, locomotion in such cases becoming slower and the filiform pseudopodia entirely disappearing.

REPRODUCTION AND INFECTION OF NEW HOSTS.

In the case of parasitic protozoa Doflein (1898) has distinguished between multiplicative and propagative reproduction. The former results in the increase of the parasite within the host; the latter provides a means by which the parasite may gain entrance to a new host.

There is still a great deal of uncertainty regarding the methods of multiplicative reproduction in the Myxosporidia, and more knowledge on the subject is badly needed. It is, however, a problem which is peculiarly difficult of solution, especially in the "free" forms. According to Doflein, multiplicative reproduction in these forms is entirely by plasmotomy. In most cases the plasmodium separates into two equal or nearly equal parts. However, in Myxidium lieberkühni, according to Cohn (1896), multiplicative reproduction is accomplished by a method of exogenous budding. In a former paper (Davis 1916) reasons have been given for believing that the process observed by Cohn is abnormal and has nothing to do with reproduction. In Sinuolinea dimorpha (Davis 1916), a method of multiplicative reproduction by endogenous budding has been demonstrated. This method consists simply in the formation of gemmules from the generative cells within the mother trophozoite. These later emerge and give rise to new individ-There is evidence that a similar process occurs in several species of Ceratomyxa uals. and Sinuolinea, and the writer has no doubt that this is a common method of multiplication among the "free" forms. Possibly this method may be considered a form of plasmotomy, but if so it is certainly quite different from the process described by Doflein.

In the tissue parasites multiplicative reproduction by a process of multiple division or schizogony has been described by Doflein, Keysselitz (1908), Nemeczak (1911), and Hahn (1915).

Propagative reproduction, so far as known, is always by means of spores, which enables the organism to resist the unfavorable conditions which it may encounter in gaining entrance to a new host. The process of sporulation is a very complicated one and can not be gone into here. (See Auerbach (1910), Minchin (1912), and Davis (1916).) The spores are usually formed in pairs, each spore being composed of six cells (eight in *Chloromyxum*). Two of these cells form the outer protective covering, the sporocyst; two form the capsules; while the remaining two cells form the binucleate sporoplasm. The number of spores formed by a single trophozoite varies from one to a large number, and we often find considerable variations in this respect within the same species, for example, *Ceratomyxa multispora* and *Sinuolinea dimorpha*. In the case of the monosporous and disporous trophozoites the spores are set free by the death and disintegration of the mother trophozoites. In the polysporous trophozoites the spores may all be formed at the same time, as in the disporous forms, and liberated in the same way. More commonly, however, the formation of spores continues for some time, the mature spores being successively set free as new ones develop. In the case of species inhabiting the gall or urinary bladders it is, of course, a simple matter for the spores to reach the exterior. I have many times observed the spores of gall parasites in the intestine and rectum, whence they doubtless pass out with the feces. That the spores formed in the gall and urinary bladders pass out quickly after being set free is indicated by the fact that free spores are always comparatively rare in such cases, although there may be an abundance of sporulating trophozoites present.

It is interesting to note in this connection that, in many species at least, adverse conditions tend to hasten the process of sporulation. It has often been observed that after the death of the host or after being removed to the slide those trophozoites in which sporulation is well advanced mature their spores in a very short time. Such spores are often smaller than those formed under normal conditions.

It is obvious that in the tissue parasites the spores in many cases will not be able to reach the exterior until after the death and disintegration of the host. In other cases, however, the cysts may break open either on the outer surface or into some cavity of the body, whence they may easily make their escape. In a number of instances spores of Myxobolus in the gall bladder and intestine have been noted.

After reaching the exterior the spores are apparently dependent on chance in entering a new host. There is no evidence of secondary or intermediate hosts, although such a possibility is by no means excluded. On the theory of purely accidental infection it is somewhat difficult to account for the high percentage of infection met with in many instances. For example, although a large number of individuals of the squeteague, *Cynoscion regalis*, were examined, not one was found infected with *Sinuolinea dimorpha*. The gall bladder of the pipefish, *Siphostoma floridæ*, is always infected with *Sphæromyxa balbianii*, although in this case an infection of 100 per cent is not so difficult to explain on account of the feeding habits of the host. Similarly, the gall bladders of sharks and rays are, except in the case of very young individuals, practically always infested with one or more species of Myxosporidia. It has been suggested that the processes of various kinds with which so many of the spores are provided enable them to float for a much longer time and thus increase the chances of their being taken into the proper host.

So far as known the spores do not germinate until after reaching the stomach or intestine of the host. This would seem to decrease the chances of infection to a considerable extent, since most of the spores carried into the mouth in breathing would in all probability be swept out again through the gill openings. It would be interesting to follow the fate of the spores in case the host is devoured by another fish.

On reaching the intestine the valves of the spore separate under the influence of the digestive fluids, allowing the sporoplasm to creep out as a minute amœbula, which by actively crawling along the ducts or by entering the blood vessels finally gains access to the organ in which it is destined to undergo further development. The action of the digestive fluids has been shown by Auerbach, Thelohan, and others to cause the extrusion of the filaments from the capsules. It has been supposed that the function of these organs is to attach the spore to the wall of the gut, but it would seem much more probable that the filaments would simply become entangled in the partly digested food and swept along toward the rectum. Since in the few cases in which the germination of the spore has been investigated the sporoplasm emerged in a very short time after the spore

was brought in contact with the intestinal fluids, it is not obvious just what purpose would be served by the attachment of the spore to the intestinal wall. In short, it is believed that the function of the capsules which form such a conspicuous part of the spore has not been demonstrated.

According to Auerbach (1910) and Erdmann (1911) there is, even in the "free" forms, a short stage of intracellular existence, the newly emerged sporozoite entering an epithelial cell and multiplying by binary fission. In these species, however, the period of cell parasitism is comparatively brief, and the greater part of their existence is spent in the cavity of the infected organ, either attached to the epithelium or floating freely in the bile or urine. According to Auerbach, the intracellular phase is followed by the fusion of two planonts, the nuclei remaining separate, thus forming a binucleate trophozoite, which he believes develops into the spore-forming plasmodium. The sexual phenomena are, however, so complicated and the accounts of different writers so fundamentally diverse that it is not thought best to go into the subject at this time.

THE SPORE.

Since the spore is practically the only organ in the Myxosporidia having a welldefined structure the details of which are nearly constant for each species, it has always been the principal character used in distinguishing the different species. For this reason it is of the greatest importance to have a clear understanding of the fundamental structure of the spore. Unfortunately most writers have based their conception of the myxosporidian spore primarily on that of Myxobolus. It has already been pointed out that there are good reasons for believing that the Myxobolidæ represent the most highly specialized group of the Myxosporidia, and this specialization is as evident in the spore as in the plasmodium. A truer conception of the myxosporidian spore can be obtained from the study of those of less specialized genera, such as *Leptotheca* or *Ceratomyxa*. Text figure 2 shows diagrammatically the structure of the spore based primarily on *Leptotheca*. Surrounding the spore is a thin, tough, transparent membrane, the sporocyst (sp'c.), which is probably of a chitenoid nature. The sporocyst is composed of two valves, each valve being developed from a single parietal cell. The more or less degenerate nucleus of the parietal cell (par. n.) can often be distinguished in the mature spore. The valves of the sporocyst are united along the sutural line (s. l.), which usually forms a more or less distinct ridge around the spore. Within the sporocyst are the capsules and the sporoplasm or sporozoite.

The capsules a (cap.) are typically two in number, but in some cases there may be only one, as in a few species of *Myxobolus*, or four in *Chloromyxum*. Each capsule is formed within a capsulogenous cell (cap. c.), which can usually be more or less clearly distinguished even in the ripe spore. The capsules are usually pyriform, sometimes approximately spherical in shape, and drawn out at one side into a short, narrow duct opening to the exterior through a minute pore which is always situated along the sutural line. Surrounding the capsule is a tough, refractive envelope, probably chitenous. Coiled up within the capsule is a delicate filament (fil.), usually of comparatively great length, which is probably of the same material as the capsule, with which it is continuous

a The capsules have been termed "polar capsules" by most writers, but I agree with Gurley (1894) that it is better to refer to them simply as capsules, since they are often not in the position indicated by the term polar capsule.

at the end of the duct. In spores containing two capsules the filaments in each are always coiled in opposite directions—i. e., one is the mirror image of the other. Under suitable stimulation the filaments are everted through the pores in the sporocyst in much the same way as in the case of the coelenterate nematocyst. The sporoplasm (sp'p) is a finely granular, translucent mass of protoplasm, which commonly fills most of the space within the sporocyst not occupied by the capsulogenous cells. In the fully matured spore, expecially after it has left the body of the host, the sporoplasm may become rounded. It is usually situated in the middle of the spore, extending equally into both valves, but in some cases (many *Ceratomyxa*) may be unsymmetrically located,



FIG. 2.—Structure of myxosporidian spore based primarily on Leptotheca. A, viewed from above; B, viewed from the capsular side; C, viewed from one end; A-B, longitudinal axis; C-D, transverse axis; E-F, vertical axis; cap., capsule; cap. c., capsulogenous cell; fil., filament; par. n., nucleus of parietal cell; s. l., sutural line; sp'c., sporocyst; sp'p., sporoplasm; sp. n., nuclei of sporoplasm.

sometimes being confined entirely to one valve. Within the sporoplasm are two nuclei (sp. n.), which usually lie close together, often in contact. In addition to the nuclei there are often several small, fat globules present and in the Myxobolidæ the characteristic "iodinophilous vacuole," which is probably composed of glycogen. The liquid-filled space (pericystic space) within the spore not occupied by either the capsulogenous cells or sporoplasm often contains numerous granules and small fat globules.

The spore is usually bilaterally symmetrical, the sutural plane forming a plane of symmetry, but to this rule there are numerous exceptions, and in a few cases the shape of each value of the sporocyst is quite different. For descriptive purposes it is necessary to recognize certain topographic features in the spore. Unfortunately, somewhat ambiguous terms have been used by various writers which are likely to lead to confusion. The most objectionable are the terms "anterior" and "posterior" ends, first used in this connection by Thelohan. While the terms may not be open to serious objection in the case of Myxobolus, they are very inappropriate, to say the least, when applied to the spores of certain other genera. On this account it has seemed best to propose a slightly different terminology than that used by previous writers.

The position of the capsules in such genera as *Ceratomyxa*, *Leptotheca*, or *Myxobolus* indicate the capsular side or end (anterior end of Thelohan). The opposite side surrounding the sporoplasm is designated the postcapsular side. The plane extending between the capsules from the capsular to the postcapsular sides and dividing the spore into two symmetrical halves is the transverse plane (vertical plane of Gurley). The transverse plane is the plane of symmetry and may or may not coincide with the sutural plane. The longitudinal plane is the plane passing through the capsules and sporoplasm at right angles to the transverse plane and divides each valve into a superior and inferior half which are symmetrical with each other. The vertical plane (transverse plane of Gurley) is the plane passing approximately between the capsules and the sporoplasm at right angles to both the transverse and longitudinal planes, dividing the spore into unsymmetrical capsular and postcapsular regions.

The longitudinal axis (fig. 2, A-B) is the line formed by the intersection of the vertical and longitudinal planes. The transverse axis (fig. 2, C-D) is the line formed by the intersection of the transverse and longitudinal planes. The vertical axis (fig. 2, E-F) is the line formed by the intersection of the vertical and transverse planes.

The length of the spore is the distance along the longitudinal axis, and in case the valves are curved the axis is supposed to have a corresponding curvature. The width is the distance between the capsular and postcapsular sides along the transverse axis, while the thickness is the length of the vertical axis. In the case of the capsules when only one dimension is given it is always the longest diameter.

Although always constructed on the same fundamental plan, the modifications of form and structure which the spores may exhibit in different species are almost endless. However, even a cursory study shows that some modifications are much more fundamental than others. The former are of great value in differentiating between the different families and genera, while the latter are our main reliance in distinguishing the different species. Among the more important and deep-seated modifications may be mentioned the general form of the spore and the position and number of the capsules (cf. fig. 3-4). The principal specific differences consist of minor modifications of form, appendages of various kinds, markings on the exterior of the spore spore, position of the capsular pores, and size, including in the latter term not only the size of the spore as a whole but the relative sizes of the different parts.

Of course, as in all systematic work, there is the possibility of variations in these characters within the limits of the species. This is a problem regarding which there are very little data at present. There seems to be a general consensus of opinion among writers on the Myxosporidia that the spores show comparatively little variation, but so far as known there have been no special investigations in regard to this point. With this belief in the constancy of the spores the writer is in general agreement. The greatest intraspecific variation seems to be in size, and many writers have overestimated the importance of this as a specific character. As previously pointed out, there is a marked tendency in many species to form spores rapidly when subjected to unfavorable conditions, and such spores are very often undersized and also sometimes show considerable modifications in form. There is evidence that spores from different hosts may show greater differences than those from the same host, but special attention has not been given to this point, which is well worth extended investigation. There is, of course, a possibility that some of the forms described as distinct are simply different strains of the same species developed in different host species.

OCCURRENCE OF THE MYXOSPORIDIA.

The more the writer has studied the Myxosporidia the more confident he has become that no group of fishes are free from these parasites. It was only a few years ago that the Pleuronectidæ was pointed out as an example of a family which was immune to the attacks of Myxosporidia, but now a number of species are known from fishes of this family. The author has found several species of Myxosporidia in one species of flounder, *Paralichthys albiguttus*, and it is rare indeed to find a fish that is not infected by one or more of these parasites. The ganoids have also been believed to be free from Myxosporidia, but there has been found a species of *Sphærospora* abundant in the urinary bladder of the gar pike, *Lepisosteus platystomus*, at Gainesville, Fla.

Although numbers of species of fishes were examined without finding any evidence of Myxosporidia, it is doubtful if there are many fishes which are absolutely free from these parasites at all times. In most cases the species in which Myxosporidia were not found are those in which only a few fish or only young individuals have been available for examination. Probably the most striking exception is the menhaden, *Brevoortia tyrranus*, of which large numbers of adults were examined with uniformly negative results. But negative results in such cases are far from conclusive when it is considered that some species of Myxosporidia occur only at certain seasons and at other times no trace of them is to be found. A striking example of this is *Ceratomyxa streptospora*, new species, which is found in the gall bladder of *Chætodipterus faber*. This species was abundant in nearly every fish examined during the early part of June, but no trace of it was found in fish examined during July.

The occurrence of some species only at certain seasons is probably not a rare phenomenon, but to what extent this occurs can only be determined by investigations carried on throughout the year. In this connection it is interesting to find that in a number of species only the vegetative forms occur at certain seasons. The best-known example of this is Myxidium lieberkühni, a parasite in the urinary bladder of the European pike, *Esox lucius*. Although this species is very abundant, the spores are to be found only in summer, while during the winter the epithelium of the bladder is fairly covered with immense numbers of vegetative trophozoites. The writer has found a number of forms which are not included in this paper, since none but the vegetative trophozoites were discovered, and without the spores it was impossible to determine with certainty the genera to which they belong. In a number of other instances the vegetative trophozoites were very abundant, occurring in a large proportion of the fish examined, and yet the spores were so rare that they were found only after a long and arduous search. In the case of some species practically all stages in the development of the trophozoites can be

found in a single host; in others, all the trophozoites in the same fish are at about the same stage of development.

As has been previously noted by Auerbach (1910) and others, the susceptibility of fish to infection by Myxosporidia varies markedly with age. Auerbach found that in *Gadus virens*, which is parasitized by *Myxidium bergense*, the very young fish were practically free from parasites, while the half-grown fish were much more commonly infected than the adults. The author's experience has been that in general young fishes are rarely infected, while the maximum infection is to be found in nearly grown or adult fishes. This is strikingly shown in the sharks, the gall bladders of which are always infected with one or more species of Myxosporidia, except in the case of very young individuals. In young sharks less than 15 inches in length the author has very rarely found any trace of these parasites. It is also noteworthy that the young fish of any species when infected usually contain only a comparatively few trophozoites, which for the most part show no traces of spores.

Taking all the above facts into consideration, it is remarkable that such a large proportion of the fish examined should have been found to be infected.

As regards the site of the parasites within the host, the "free" forms appear to be confined for the most part to the gall and urinary bladders. A few species are found in the urinary tubules and ducts, but so far as known they are never found in the lumen of the digestive tract.

The tissue parasites are found chiefly in the connective and muscular tissues, although other classes of tissues are by no means immune, and a few species are known to attack the nervous system. The parasite may be concentrated at one spot in the tissues to form a distinct cyst, which is usually visible to the naked eye, or may spread in an irregular manner through the tissue, producing a condition known as "diffuse infiltration." The tissue parasites, although abundant in fresh-water fishes, are rare in marine fishes. They were only met with in a few species, and in every case these were fishes which also occur in brackish or fresh water.

It is often stated that parasites of the kidneys and urinary bladder are also confined chiefly to the fresh-water fishes, while parasites of the gall bladder are just as characteristic of marine species. With this the author is unable to agree. In marine fishes Myxosporidia were found to be fully as abundant in the urinary as in the gall bladder. On the other hand, such limited observations as were made on fresh-water fishes indicate that they are by no means rare in the gall bladders of these fishes.

As a general rule, each species of Myxosporidia is confined to one, or at most a few, closely related species of fishes, but to this rule there are numerous exceptions, and in some cases the parasites may occur in very different hosts, as, for example, *Sphæromyxa balbianii*, which occurs in the gall bladder of *Motella maculata* and *Siphostoma floridæ*.

Some genera appear to show a preference for certain organs of the host. The Geratomyxa are chiefly parasites of the gall bladder, although a few species are known from the urinary bladder. In the case of Sphæromyxa all the known species are from the gall bladder. On the other hand, the species of Sphærospora are, with one exception, all found in the urinary bladder. There is no known instance of the same species inhabiting both the gall and urinary bladders, but the tissue parasites do not seem to be so particular, many species being able to develop in quite different tissues.

It is very common to find two or more species of Myxosporidia living in the gall or urinary bladders at the same time. This is especially true of the sharks, where it is rare to find an adult whose gall bladder does not contain at least two species. For instance, the gall bladder of a sharp-nosed shark, *Scoliodon terræ-novæ*, 102 cm. long, taken on August 9, 1912, contained large numbers of trophozoites and spores of at least four species—*Chloromyxum leydigi*, *Ceratomyxa abbreviata*, *C. tænia*, and *C. sphairophora*.

PATHOGENESIS.

As a group the Myxosporidia are not particularly injurious. Considering their abundance this is indeed fortunate. Undoubtedly the same factors have been at work here as elsewhere to produce this result. As has often been pointed out, it is to the advantage of the parasite as well as the host to injure the latter as little as possible. Probably the majority of the most injurious species which have arisen have accomplished their own downfall along with that of the host.

As we would naturally expect, the tissue parasites are much more injurious than the "free" forms. Several of the former are known to be the cause of some of the most destructive diseases of European fresh-water fishes.

Apparently the "free" parasites for the most part cause but little injury to the host. Like so many other protozoan parasites which are common in the cavities of both vertebrates and invertebrates, they seem to have been able to establish relations with the host which admit of the minimum amount of injury. Still, it can hardly be claimed that they are perfectly harmless. Their brief period of intracellular existence must result in the destruction of the epithelial cells in which they develop. In many cases, when specially abundant, they cause the atrophy or hypertrophy of the epithelium lining the cavities in which they develop. Whether they may produce toxic products of any kind which when absorbed by the host may have far-reaching effects we have no certain knowledge. It has been noticed that the bile in gall bladders containing large numbers of parasites is often more watery and has a lighter color than normal, but whether this is of any significance the author is unable to say. Of course, any weakening effects of the parasites on marine fishes would in most cases be difficult to detect. It would, in all probability, simply result in their being quickly captured by some one of their numerous enemies, and the chance of finding fishes which were appreciably weakened would be slight indeed.

CLASSIFICATION.

The classification of the Myxosporidia is at present in a very unsatisfactory state. The most common classification is that of Doflein (1899 and 1901). This classification, slightly modified by Auerbach (1910), is as follows:

Suborder MYXOSPORIDIA Butschli.

Legion I. DISPOREA, Doflein.

Only one pansporoblast, forming two spores, developed in each trophozoite. Trophozoites disintegrate after the spores mature.

Family 1. CERATOMYXIDÆ Doflein.

Trophozoites floating freely in various cavities of the host; progressing by amœboid movements. Long diameter of spore at right angles to sutural plane.

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Genus 1. Leptotheca Thelohan.

Valves of the spore short and rounded.

Genus 2. Ceratomyxa Thelohan.

Valves of the spore produced into long, conical processes.

Legion II. POLYSPOREA Doflein.

Each trophozoite produces more than one, usually a number, of pansporoblasts.

Spores with two capsules.Family 1. MYXIDIDÆ Thelohan.Spores spherical.Genus 1. Sphærospora Thelohan.Genus 2. Myxidium Butschli.

Spores more or less fusiform with capsules at each end. Longest diameter along the sutural plane.

Genus 3. Sphæromyxa Thelohan.

Spores fusiform with truncated ends. Capsules opening at the ends. Filaments short and thick; coiled parallel to the longitudinal axis. Trophozoites forming a flattened disk.

Genus 4. Zschokkella Auerbach.

Spores when viewed from above semicircular; pointed at the ends. Capsules spherical; situated at ends of spore but opening on the flattened surface. Sutural line curved.

Genus 5. Myxosoma Thelohan.

Spores ovoid, flattened, elongated, and pointed at the capsular end.

Genus 6. Myxoproteus Doflein.

Spores somewhat pyramidal, with spinous processes from the base of the pyramid.

Genus 7. Lentospora Plehn.

Spore lenticular; resembling Myxobolus but without iodinophilous vacuole in the sporoplasm.

Family 2. CHLOROMYXIDÆ Thelohan.

Spores with four capsules.

Genus 1. Chloromyxum Mingazzini.

With the characters of the family.

Family 3. MYXOBOLIDÆ Thelohan.

Spores with one or two capsules and with an iodinophilous vacuole in the sporoplasm.

Genus 1. Myxobolus Butschli.

Spores ovoid, flattened, with one or two capsules. Sporocyst not prolonged into a postcapsular process.

Genus 2. Henneguya Thelohan.

Spores ovoid, with one or two median taillike processes at the postcapsular end.

Genus 3. Hoferellus Berg.

Spores pyramidal, compressed, with two lateral, taillike processes at the postcapsular end.

The classification here given is unsatisfactory in several respects. In some cases it groups together in the same family species which are obviously not at all closely related. But the most serious fault is its separation of the Myxosporidia into two divisions based on the number of spores formed by a trophozoite. Nothing can be more certain than that this is a relatively unimportant character. Even within the same species we find considerable variation in this respect. According to Auerbach (1910), Myxidium bergense is both monosporous and polysporous, and the same is true of a species of Myxidium from the gall bladder of Cottus scorpius, according to Amerinzew (1911), while Myxidium incurvatum is both monosporous and disporous. Ceratomyxa has always been looked upon as the very type of the disporous group, and yet Ceratomyxa recurvata is both disporous and polysporous.^a On the other hand, Ceratomyxa monospora is both monosporous and disporous. In other genera we find similar examples. Sinuolinea dimorpha occurs in two well-defined forms—one disporous, the other polysporous. Sphærospora polymorpha and Chloromyxum granulosum are also both disporous and polysporous.

The family Myxidiidæ, as defined by Doflein, includes a number of very diverse genera which have few characters in common. Such genera as *Sphærospora* and *Myxidium* are very unlike and should undoubtedly be assigned to different families.

On account of the obviously artificial character of Doflein's classification a new arrangement of the genera has been attempted, which, it is believed, much more nearly expresses their natural relationships. It is fully realized that the classification proposed here can be considered only tentative and no doubt will require considerable modification as knowledge of the group increases. It is, however, hoped that it will serve as a basis for a final classification of the group which will express their real relationships.

The author fully agrees with Amerinzew (1907) on the importance of other characters in addition to the spores, but with the present limited knowledge of the group any system of classification must of necessity be based, primarily, on the spores. Moreover, it is felt that this must always be the case. The plasmodia show few definite characteristics and oftentimes so closely resemble each other as to render it impossible to distinguish between certain species in the absence of the spores. Furthermore, the trophozoites are often very variable and may have a quite different appearance in different hosts.

It will be noticed that the proposed division of the Myxosporidia into two suborders, based on the structure of the spore separates the "free" forms from those which develop only in the tissues. Practically all the tissue parasites are characterized by the flattening of the spore parallel to the transverse plane, which in these forms always coincides with the sutural plane. As previously pointed out, there is good reason to believe that the tissue parasites are more highly specialized and further removed from the primitive forms than are the "free" species. This division, then, represents something more than a purely arbitrary distinction.

The only other important change in Doflein's classification is the rearrangement of the genera formerly included in the Myxidiidæ. It is believed that the proposed arrangement better indicates the relationships of the different genera, although it is felt that there are several inconsistencies here which can only be cleared up with increased knowledge of the group.

^a At least two other species of *Ceratomyxa* were found, which, judging from the number of sporoblast cells in the trophozoites, are in all probability polysporous. They are, however, not included in this paper, since only vegetative forms have been available for study.



Fig. 3.—Spore of Ceratomyxa. A, from above; B, from capsular side; A-B, longitudinal axis; C-D, transverse axis; E-F, vertical axis.



FIG. 4.-Spores. A and B, Leptotheca; C and D, Sphærospora. Lettering as in figure 3.

Proposed classification of the Myxosporidia:

Order MYXOSPORIDIA Butschli.

Suborder I. MYXOSPOREA. New suborder.

Spores not compressed parallel to the transverse plane; no iodinophilous vacuole in the sporoplasm. Trophozoites with few exceptions living free in the gall or urinary bladders. Monosporous, disporous, and polysporous.

Family 1. CERATOMYXIDÆ Doflein.

Spores longer than wide; valves rounded or pointed at the ends. Trophozoites usually pyriform. Commonly disporous.

Genus 1. Leptotheca Thelohan.

Values of spore short and rounded. Sutural plane usually slightly oblique to longitudinal axis. Sporoplasm filling most of both values. (See fig. 4, A and B.)

Genus 2. Ceratomyxa Thelohan.

Valves of spores produced into long, conical, or flattened processes, with rounded or pointed ends. Sutural plane usually perpendicular to transverse axis. Sporoplasm usually unsymmetrically located, extending only a short distance into one valve. (See fig. 3.)

Family 2. SPHÆROSPORIDÆ. New family.

Spores pyramidal or approximately spherical; not distinctly longer than wide; with or without lateral processes.

Genus 1. Myxoproteus Doflein.

Spores roughly pyramidal; with or without distinct processes from the base of the pyramid. Disporous.

Genus 2. Sphærospora Thelohan.

Spores spherical; without processes of any kind. Capsules distinctly pyriform; convergent when viewed from above (i. e., at right angles to the longitudinal plane). Sutural line forming a straight ridge around spore. (See fig. 4, C and D.) Disporous and polysporous.

Genus 3. Sinuolinea, new genus.

Spores approximately spherical; with or without lateral processes. Capsules rounded, not convergent when seen from above; capsular pores some distance apart, sometimes on nearly opposite sides of the spore. Sutural line forming a prominent ridge, which takes a sinuous course around the spore. Sutural plane usually distinctly twisted on its axis. (See fig. 5, A and B.) Disporous and polysporous.

Family 3. MYXIDIIDÆ Thelohan.

Spores distinctly elongated along longitudinal axis. Capsules at or near ends of spore and opening in opposite directions. Sutural plane more or less twisted on its axis.

Genus 1. Myxidium Butschli.

Spores more or less fusiform, pointed or rounded at ends, not truncated. Capsules may or may not open at ends of spore. (See fig. 5, C and D.) Monosporous, disporous, and polysporous.

Genus 2. Sphæromyxa Thelohan.

Spores fusiform, with truncated ends. Capsules opening at ends of spore. Filaments short and thick, coiled parallel to the longitudinal axis. (See fig. 6, A and B.) Trophozoites flattened and leaflike. Polysporous.

Genus 3. Zschokkella Auerbach.

Spores, when viewed from above, semicircular; pointed at ends. Capsules spherical; situated at ends of spore, but opening on the flattened surface.

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Family 4. CHLOROMYXIDÆ Thelohan.

Spores rounded, with four capsules. Polysporous.

Genus 1. Chloromyxum Mingazzini.

With the characters of the family.

Suborder II. CYSTOSPOREA. New suborder.

Spores more or less compressed parallel to the transverse plane; usually with two capsules, rarely one. Trophozoites with few exceptions are tissue parasites. Polysporous.



FIG. 5.-Spores. A and B, Sinuolinea; C and D, Myzidium. Lettering as in figure 3.

Family I. MYXOSOMIDÆ. New family.

Spores without iodinophilous vacuole in the sporoplasm. Two capsules always present.

Genus 1. Myxosoma Thelohan.

Spores ovoid, flattened, elongated along transverse axis and pointed at capsular end.

Genus 2. Lentospora Plehn.

Spores lenticular, resembling Myxobolus, but without iodinophilous vacuole.

Family 2. MYXOBOLIDÆ Thelohan.

Spores with one or two capsules and with an iodinophilous vacuole in the sporoplasm.

Genus 1. Myxobolus Butschli.

Spores ovoid, flattened, with one or two capsules. Sporocyst not prolonged into a postcapsular process. (See fig. 6, C and D.)



FIG. 6.-Spores. A and B, Sphæromyza; C and D, Myzobolus. Lettering as in figure 3.

Genus 2. Henneguya Thelohan.

Spores ovoid, with one or two median taillike processes at the postcapsular end.

Genus 3. Hoferellus Berg.

Spores pyramidal, compressed, with two lateral taillike processes at the postcapsular end. 69571°---18------15

DESCRIPTION OF SPECIES TAKEN AT BEAUFORT.

Suborder MYXOSPOREA. New suborder.

Family CERATOMYXIDÆ Doflein.

Genus Leptotheca Thelohan.

Leptotheca fusiformis, n. sp. (Pl. xvi, fig. 1-2.)

Trophozoite.—Vegetative trophozoites pyriform, tapering gradually toward the posterior end, which usually terminates in a long, slender process; colorless, transparent. Progressive movements rapid. Endoplasm distinctly granular, the granules being more abundant at the anterior end.

Length of full-grown trophozoites about 50μ , width about 13μ .

Disporous.

Spore.—Elliptical when viewed from above (fig. 1); base of valves circular in cross section but distally are somewhat compressed parallel to the longitudinal plane, so that when viewed from the capsular side the spore appears distinctly fusiform (fig. 2). Sutural plane slightly oblique to longitudinal axis; sutural line forming a distinct ridge. Capsules distinct, located at some distance from the capsular side and opening on opposite sides of the spore. Sporoplasm finely granular; confined to the central part of spore and not extending into the ends of the valves.

Length of spore 16μ , width 9μ ; diameter of capsules 4.5μ ; length of filament 30μ .

Habitat.—Both vegetative and sporulating trophozoites abundant in the bile of a hammer-head shark, Cestracion zygæna, taken July 11, 1914.

Leptotheca scissura, n. sp. (Pl. xvi, fig. 3-8.)

Trophozoite.-Young vegetative trophozoites elongate, with long attenuated posterior process; usually slightly constricted just posterior to rounded anterior end, which bears numerous long, filiform pseudopodia (fig. 3). Progressive movements rapid. Ectoplasm distinguishable only at anterior end, where it gives rise to pseudopodia. Endoplasm usually filled, except in very young individuals, with small, clear, colorless spherules, which are only visable under high magnification. As trophozoites increase in size, spherules become larger and more prominent. Later the spherules become distinctly yellow and contain one to several dark-brown granules, which increase in size and numbers and finally collect in an irregular clump at the center of spherules (fig. 4-6). In some cases the spherules may finally become almost entirely filled with these granules. Size of spherules in same trophozoite uniform but increases as trophozoites grow older. In rare cases the brown granules may be entirely absent even in sporulating trophozites (fig. 7). The spherules are arranged in definite rows, embedded in a clear, homogeneous matrix, giving a very characteristic appearance to the trophozoite. For a short distance at the anterior end spherules are entirely absent, the endoplasm being distinctly granular and containing numerous small fat globules. The larger trophozoites are usually distinctly flattened dorsoventrally. The posterior end may present a characteristic ragged or frayed appearance (fig. 4, 5), due to the endoplasm being drawn out into a number of long, slender processes, which when large enough contain one or more rows of spherules. Occasionally two or more adjacent processes may fuse (fig. 5), so that the posterior region of the trophozoite has the appearance of a network composed of anastomosing strands of protoplasm.

Average length of full-grown trophozoite about $125-150\mu$, width about $20-25\mu$. The longest trophozoite observed was 195μ long, with an extreme width of 16μ .

Disporous.

Spore.—Elliptical from above, somewhat flattened along postcapsular side (fig. 8). Sutural line distinct; sutural plane at right angles to longitudinal axis. Capsules and capsulogenous cells distinct, the capsules opening at some distance from the capsular margin. Sporoplasm distinct, finely granular, nearly filling both valves.

Length of spore 22μ , width 11μ ; diameter of capsules 4μ .

Habitat.—Abundant in the gall bladders of Dasybatis hastatus and Pteroplatea maclura during July and August. Lives floating freely in the bile or attached to wall of gall bladder.

Remarks.—The structure of the spherules is very similar to that of C. sphærulesa Thelohan, but in other respects this species is very different.

THE MYXOSPORIDIA OF THE BEAUFORT REGION.

Leptotheca lobosa, n. sp. (Pl. xvi, fig. 9, 10; pl. xvii, fig. 11.)

Trophozoite.—Vegetative trophozoites colorless, transparent to translucent; usually spherical (fig. 9), but may form a large rounded pseudopodium of ectoplasm (fig. 10). Amœboid movements very slow. Ectoplasm remarkable for its coarsely granular structure, the granules being very distinct and of uniform size. In rounded individuals the ectoplasm forms a distinct peripheral layer around the entire trophozoite (fig. 9). Endoplasm less granular and more transparent than extoplasm, containing numerous large, yellow, fat globules, which average larger and are more abundant in large trophozoites. Several denser sporoblast cells can often be indistinctly seen.

Diameter of rounded vegetative trophozoites up to 24μ .

Disporous.

Spore.—Elliptical when viewed from above; valves slightly tapering but rarely exactly alike. Sutural line forming a prominent, sinuous ridge around spore. Capsules and capsulogenous cells distinct; capsules opening at some distance from the capsular margin. Sporoplasm distinct, nearly filling both valves. Even after being freed from the mother trophozoite the postcapsular sides of the spores remain united at the sutural line. This seems to be very characteristic and has been observed in no other species.

Length of spores 16–18 μ , width 9–10 μ ; diameter of capsules 3μ .

Habitat.—Found in the urinary bladder of one individual of Paralichthys dentatus taken July 25, 1914.

Remarks.—In its general form the spore approaches that of *Ceratomyxa*, but on the whole seems to show more of the characteristics of *Leptotheca*. The trophozoites exhibit characteristics which are much more common in *Leptotheca* than in *Ceratomyxa*.

Leptotheca glomerosa, n. sp. (Pl. XVII, fig. 12, 13.)

Trophozoite.—Vegetative trophozoites colorless, transparent; rounded or somewhat irregular in shape, with short, lobose pseudopodia. Amœboid movements slow. Ectoplasm forming a distinct, hyaline layer around entire trophozoite. Endoplasm finely granular, with numerous small fat globules varying greatly in size. Almost the entire trophozoite is used up in the formation of the spores (fig. 12) and the ectoplasmic layer is no longer distinguishable.

Diameter of rounded sporulating trophozoites about 11µ.

Disporous.

Spore.—Approximately cylindrical; valves rounded at ends (fig. 13). Capsules distinct. Coiled filaments not visible. Sutural plane at right angles to longitudinal axis. Sporoplasm finely granular and completely filling cavity of sporocyst.

Length of spore 9μ , width 4.5 μ ; diameter of capsules 2μ .

Habitat.—Occurs occasionally in the urinary bladder of Paralichthys albiguttus. Not common.

Genus CERATOMYXA Thelohan.

Ceratomyxa mesospora, n. sp. (Pl. xvII, fig. 14-16.)

Trophozoite.—Vegetative trophozoites colorless, pyriform, elongate, with long, slender posterior process. Numerous filiform pseudopodia at anterior end. Progressive movements rapid. No sharp demarcation between ectoplasm and endoplasm. Endoplasm finely granular and filled with small, colorless, homogeneous spherules (fig. 14). Spherules absent at extreme anterior end, where the endoplasm is denser and more granular. After being on the slide for a short time vegetative trophozoites become rounded and motionless, but on account of the great length of the spores trophozoites containing well-developed spores are unable to contract in this manner.

Length of full-grown trophozoites varies within wide limits according to degree of attenuation. Total length of trophozoites about 70–85 μ , length exclusive of posterior process about 50–75 μ , width about 20–25 μ .

Disporous.

Spore.—Greatly elongate along longitudinal axis, each valve forming a slightly tapering cone more or less rounded at the apex (fig. 15, 16). Valves not compressed. Sutural plane forming an acute angle with longitudinal axis. Capsules conspicuous. Coiled filaments very distinct. Capsules are remarkable in that they are unsymmetrically situated, one capsule being always located in the widest part of the spore, the other being a little to one side (fig. 15). The capsule situated in the middle of spore is usually slightly larger than the other. Sporoplasm unsymmetrically situated, sometimes being entirely confined to the larger valve. Length of spore $50-65\mu$, width about 8μ ; diameter of capsules 4.5μ ; length of filament 90μ .

Habitat.—Both vegetative and sporulating trophozoites abundant in the gall bladder of Cestracion zygæna taken July 11, 1914, and in two individuals of Cestracion tiburo taken in July.

Remarks.—In many respects this species is similar to C. spharulosa Thelohan, but the spore is much smaller and the sutural plane is not perpendicular to the longitudinal axis as in spharulosa according to Thelohan's figures. According to Thelohan the spherules in spharulosa often contain minute colored granules, but this seems never to be the case in mesospora. This species occurs along with C. recurvata, new species, but can be easily distinguished by the much smaller size of the spherules.

Ceratomyxa sphairophora, n. sp. (Pl. XVII, fig. 17-21, 23; pl. XVIII, fig. 22.)

Trophozoite.--Vegetative trophozoites colorless, transparent, pyriform, elongate. Numerous fine filiform pseudopodia at anterior end. Progressive movements rapid. Ectoplasm clear, homogeneous, forming a distinct layer at anterior end and often a short distance back along the sides. Structure of endoplasm extremely variable, in majority of trophozoites filled with transparent, homogeneous spherules crowded closely together (fig. 17). These spherules never extend to extreme anterior end, the endoplasm in this region being filled with a closely crowded mass of small fat globules. In other cases the spherules are much less distinct (fig. 18) and sometimes, especially in sporulating individuals, the endoplasm may show only a very indistinct vacuolated structure (fig. 22). Usually, however, even sporulating trophozoites exhibit well-defined spherules. Apparently the indistinctness of the spherules in many cases is due to their becoming less dense until they form more or less distinct polygonal areas separated by a thin layer of granular endoplasm (fig. 18). Another variation is the extreme development of the spherules. In a small percentage of trophozoites the spherules are much larger than usual, sometimes reaching a diameter of $7-8 \mu$. These large spherules, unlike the smaller, contain numerous minute yellowish or brown granules collected into a mass at the center (fig. 19-20). The spherules or vacuoles, as the case may be, are separated by a thin layer of distinctly granular endoplasm containing numerous rod-shaped or rounded, colorless bodies, which in their appearance are strikingly like small bacteria. That they are, however, not bacteria is shown by the fact that they fail to take the Giemsa stain. Small vegetative trophozoites are usually much like the larger, except that the spherules are not so numerous and there is no distinct mass of fat globules at the anterior end. Occasionally small trophozoites are seen which contain large, clear, irregularly shaped vacuoles varying greatly in size (fig. 21). In the sporulating trophozoites the spores are always situated with the large central portion of the spore at the anterior end, the long attenuated ends of the valves being bent backwards at right angles and extending toward the posterior end of the trophozoite (fig. 22).

Length of sporulating trophozoites about 100-110µ width about 25µ.

Disporous.

Spore.—Valves greatly elongated, tapering gradually toward the ends but abruptly enlarged toward the base (fig. 22, 23). Long, attenuated ends of valves hollow and so fragile that it is almost impossible to find an example in which they are not more or less distorted. As shown in the figures, even after being set free the ends are usually sharply bent toward the postcapsular side a short distance from the sutural plane. Attenuated ends of valves are often twisted or bent in various ways, and there is a marked tendency for the membrane to collapse, especially near the extreme end. Sutural plane perpendicular or only slightly oblique to longitudinal axis. Capsules large, spherical, close together, and slightly convergent, opening some distance apart on the capsular side. Coiled filaments distinct. Sporoplasm confined to large, central part of spores, but extending farther into one valve than the other.

Total length of spore (i. e., from tip to tip of valves along longitudinal axis) $115-140\mu$, width about 12μ ; diameter of capsules 6μ ; length of filament 75μ .

Habitat.-Abundant in the gall bladder of the sharp-nosed shark, Scoliodon terræ-novæ.

Remarks.—This species appears to be a remarkably variable one. The assumption that the different types of trophozoites described above belong to one and the same species is based on the fact that similar spores have been seen in all the different types, and that all types of trophozoites with intermediate gradations can be found in the same host. It is remarkable that in the case of C. tania, which occurs along with this species, the trophozoites have practically the same structure, even exhibiting the same variations, while the spores are entirely different.

Ceratomyxa tænia, n. sp. (Pl. XVII, fig. 24; pl. XVIII, fig. 25, 26.)

Trophozoite.—Vegetative trophozoites similar to those of C. sphairophora, and no character has been found by which they may be distinguished. Spores of this species have been observed in the different

types of trophozoites described under *sphairophora*. The sporulating trophozoites, however, can be easily distinguished on account of the very different appearance of the spores and their different arrangement within the trophozoite (fig. 24). The spores of this species are situated, as is usually the case in *Ceratomyxa*, with the greater part of the spore parallel to the long axis of the trophozoite, only a part of one valve being bent back along the rest of the spore.

The sporulating trophozoites are somewhat smaller than in C. sphairophora, the average length being about 80μ , average width about 25μ .

Disporous.

Spore.—Very characteristic. Valves greatly elongated; sporocyst very thin, the membrance on opposite sides of each valve being in contact for about two-thirds of its length, forming a thin, ribbonlike structure; basal third of each valve only slightly compressed parallel to the longitudinal plane (fig. 25, 26); terminal ribbonlike portion of each valve usually twisted so that plane of ribbon is at right angles to longitudinal plane. Capsules small, pryiform to spherical, convergent, opening on capsular margin. Coiled filaments indistinct. Sporocyst slightly constricted along sutural line. Sutural plane perpendicular to longitudinal axis. Sporoplasm finely granular, filling the basal third of each valve, usually extending an equal distance into each (fig. 25), but sometimes extending farther into one valve than the other (fig. 26).

Length of spore about 140–150 μ ; length of central portion about 45μ , width about 6μ ; diameter of capsules 3μ .

Habitat.-Found in the gall bladder of Scoliodon terræ-novæ along with C. sphairophora.

Remarks.—Although the vegetative trophozoites are indistinguishable from C. sphairophora, the spores are so different that there seems to be no doubt that it should be regarded as a distinct species. No spores have been observed which could be considered as in any way intermediate between the two.

Ceratomyxa attenuata, n. sp. (Pl. xvIII, fig. 27, 28.)

Trophozoite.—Vegetative trophozoites elongate, pyriform, with long, tapering posterior process; at anterior end numerous long, filiform pseudopodia. Progressive movements rapid. Ectoplasm distinct only at anterior end. Endoplasm filled with small, refractive, yellow or brownish granules, which are uniformly distributed throughout the trophozoite (fig. 27); these granules are always some distance apart and are probably inclosed in very transparent spherules, which can only be distinguished with great difficulty. While no spherules can be made out in many instances, the fact that the granules are always situated at approximately equal distances from each other indicate that they must be inclosed in something of the kind. Between the brownish granules the endoplasm is clear and colorless, not granular, except at extreme anterior end where it contains a clump of small fat globules. In small trophozoites granules are much less abundant and in very small individuals entirely absent.

During development the spores are arranged side by side with their long axes parallel to the long axis of the trophozoite, the valves being bent on themselves near the middle so that the attenuated ends lie along the larger basal portion.

Length of full-grown trophozoites about $100-120\mu$, width about 27μ . The total length varies greatly, depending on the elongation of the posterior process, which in extreme cases may be drawn out to great length.

Disporous.

Spore.—Valves greatly elongated; unsymmetrical, one valve being about 15μ shorter than the other and ending abruptly; the longer valve tapering gradually to a point (fig. 28). About midway between the ends of each valve (i. e., where they were bent during development) is a thin septum, on the inner side of which the remains of the parietal cell nucleus can sometimes be distinguished; external to the septum the valves are empty, and in the more attenuated valve the walls collapse so that they are in contact along the longitudinal plane. Capsules large and conspicuous, opening on the capsular margin. Coiled filaments distinct. Sutural plane oblique to longitudinal axis; sutural line forming a distinct, sinuous ridge around spore. Sporoplasm distinct, asymmetrically situated in central part of sporocyst.

Length of spore about 115μ , width 9μ ; diameter of capsules 4.5μ ; length of filament 60μ .

Habitat.—Both vegetative and sporulating trophozoites abundant in the gall bladder of a sharpnosed shark, Scoliodon terræ-novæ, taken July 8, 1915.

Ceratomyza recurvata, n. sp. (Pl. XVIII, fig. 29-33.)

Trophozoite.—Vegetative trophozoites colorless, pyriform, with long, slender posterior process. Actively motile, forming numerous filiform pseudopodia of ectoplasm at anterior end. Endoplasm filled with large, homogeneous spherules (fig. 29, 31) which average about 10μ in diameter. In some cases spherules are crowded so closely together as to be considerably distorted from original spherical shape. Apparently the density of the spherules may vary considerably in different individuals, since there is great variation in their appearance in stained material. This is especially evident in Giemsa preparations, where in some individuals the spherules are well defined (fig. 30); in others they have so disintegrated in drying as to be practically indistinguishable. This is particularly noticeable in individuals with the largest spherules. Surrounding the spherules is a colorless, distinctly granular endoplasm, the granules being especially abundant at the anterior end, where for a short distance the spherules are absent.

Total length of full-grown trophozoites about $130-175\mu$, length exclusive of posterior process about 100μ .

Disporous and polysporous; trophozoites containing from 2 to 10 spores observed. Usually more than 2 spores formed, 6 and 8 being the most common numbers observed. It is of interest to note that the number of spores formed is not always an even one, 3, 5, and 7 spores being observed in some cases, indicating that the spores are developed singly from distinct sporoblasts and not necessarily in pairs. Fig. 30 shows a portion of a trophozoite containing 3 distinct sporoblasts and 3 generative cells which doubtless give rise to sporoblasts. Figure 31 also shows a trophozoite containing several sporoblasts, although the individual sporoblasts can not be distinguished in every case.

Spore.—Very characteristic; valves greatly curved toward the postcapsular side (fig. 32), usually symmetrical, but occasionally one may be much more incurved than the other (fig. 33); in some cases the ends may even overlap. Valves circular in cross section at the base but toward the ends greatly flattened parallel to the longitudinal plane the walls of the opposite sides being in contact. Ends of valves sharply pointed. Sutural line distinct. Capsules large and conspicuous, opening at some distance from the capsular margin. Coiled filaments distinct. Sporoplasm distinct, finely granular, usually extending farther into one valve than the other.

Length of spore between points of greatest curvature about 16μ , width 8-9 μ ; diameter of capsules 4.5 μ .

Habitat.—Both vegetative and sporulating trophozoites abundant in the gall bladder of a hammerhead shark, Cestracion zygæna, taken July 11, 1914.

Remarks.—In addition to this species the gall bladder contained large numbers of Leptotheca fusiformis, n. sp.; Ceratomyxa mesospora n. sp.; and Chloromyxum leydigi Mingazzini, so that the bile was fairly swarming with Myxosporidia.

Ceratomyxa lunata, n. sp. (Pl. xvIII, fig. 34, 35; pl. xIX, fig. 36, 37.)

Trophozoite.—Vegetative trophozoites pyriform, becoming rounded after being on the slide for a short time. Progressive movements slow. Endoplasm filled with large, homogeneous spherules, which are usually colorless, sometimes light yellow; spherules occasionally inclose several small, brownish granules. At extreme anterior end the endoplasm contains numerous small fat globules.

Disporous.

Spore.—The spores show considerable variation in size and form. The larger and more typical are more or less crescent-shaped; symmetrical; valves distinctly curved toward postcapsular side (fig. 34–36), the curvature often being more marked than in the figure; ends of valves more or less rounded. Sutural plane slightly oblique to longitudinal axis (fig. 37); satural line forming a distinct ridge. Capsules large, distinct, situated a short distance from capsular margin and opening on opposite sides of spore. Coiled filaments distinct. Sporoplasm distinct, finely granular, symmetrically situated, extending an equal distance into each valve and filling entire space around capsules. Smaller spores (fig. 36, 37) differ from larger chiefly in size; the valves are much shortened and have a greater curvature toward the postcapsular side, while the ends are more distinctly rounded.

Length of largest spore observed 38μ , width 9μ ; diameter of capsules 4μ ; length of filaments 37μ . This was an exceptionally large spore, the average length of the larger spores being about 30μ , the other dimensions being same as given above. Length of smallest spore observed 15μ , width 7μ ; diameter capsules 3μ .

Habitat.—Large numbers of vegetative and a few sporulating trophozoites were present in the gall bladder of a tiger shark, Galeocerda tigrinus, taken August 8, 1914.

Remarks.—The great variability in the spores was probably due to the fact that they were for the most part formed under abnormal conditions after the trophozoites had been placed on a slide and cov-

ered with a cover glass sealed with paraffin. The spores were not studied until 36 hours after being removed from the host, and by that time large numbers of trophozoites had sporulated, although very few spores were present when they were placed on the slide. Spores formed under such conditions are often smaller than those produced under more favorable circumstances.

Ceratomyxa abbreviata, n. sp. (Pl. XIX, fig. 38-41.)

Trophozoite.—Vegetative trophozoites colorless, elongate, pyriform, with usually a very long, slender posterior process (fig. 38, 39). Progressive movements rapid. Ectoplasm abundant, very distinct from endoplasm, thickened at anterior end, and usually forming a distinct layer over entire trophozoite; attenuated posterior process usually composed entirely of ectoplasm, but rarely the endoplasm may extend into it for a short distance; at anterior end the ectoplasm forms short, tapering or filiform pseudopodia. Endoplasm has a very characteristic longitudinally striate appearance; a few small fat globules present at anterior end.

Length of vegetative trophozoites up to about 90μ , width $10-12\mu$; diameter of rounded sporulating trophozoites about 27μ .

Disporous.

Spore.—Very characteristic. Roughly crescent-shaped; diameter of valves exceptionally great in comparison with their length (fig. 41). Base of valves circular in cross section or slightly compressed parallel to longitudinal plane (fig. 40); ends of valves rounded, slightly unsymmetrical. Sporocyst exceptionally tough and resistant to reagents. Capsules large, prominent, located at some distance from capsular side and opening on opposite sides of spore midway between capsular and postcapsular margins. Sporoplasm distinct, finely granular, confined entirely to one valve.

Length of spore 17μ , width 14μ ; diameter of capsules 4.5μ .

Habitat.—Found in the gall bladder of a sharp-nosed shark, Scoliodon terræ-novæ, taken August 2, 1912. Although a number of these sharks have been examined, this is the only time this species has been found.

Ceratomyxa flagellifera, n. sp. (Pl. XIX, fig. 42, 43.)

Trophozoite.—Vegetative trophozoites colorless, short, pyriform, tapering rapidly toward the posterior end (fig. 43). In some cases the posterior end may be divided into a number of long, slender, transparent processes. The anterior end bears large numbers of extremely long, filiform pseudopodia, which can be seen to sweep slowly back like a whiplash until they come to lie by the side of the body, sometimes extending some distance beyond the posterior end. Progressive movements slow. Ectoplasm clear, transparent, forming a distinct layer at anterior end, which gives rise to the pseudopodia and often extends for some distance along the sides. Endoplasm has a very peculiar structure; in large trophozoites filled with large numbers of rod-shaped, bacterialike bodies, which usually lie with their long axes parallel to the long axis of the trophozoite. These bodies are much more abundant in the anterior half of the trophozoite, gradually decreasing in number toward the posterior end, which is very transparent, its limits being often very difficult to make out. In smaller trophozoites the bacterialike bodies are much less numerous and may be entirely absent, the endoplasm then showing a distinctly vacuolated structure. In the larger trophozoites there can usually be seen one to several rounded bodies composed of clear, transparent, protoplasm containing a few small, refringent granules (fig. 43).

Length of vegetative trophozoites up to about 115-120µ; width about 40-45µ.

Disporous.

Spore.—Valves greatly elongated, conical, with rounded ends (fig. 42). Sutural line forming a prominent ridge around spore. Capsules large, conspicuous, opening on opposite sides of spore at some distance from capsular margin. Coiled filaments very distinct. Sporoplasm distinct, granular, symmetrically situated, but extending only a short distance into each valve.

Length of spore 118 μ , width 12 μ ; diameter of capsules 6 μ .

Habitat.-Found in the gall bladder of a shark, Carcharhinus sp?, taken July 27, 1911.

Remarks.—The rounded protoplasmic bodies which occur in the larger trophozoites are probably gemmules. They appear strikingly like the gemmules of *Sinuolinea dimorpha*. (See Davis, 1916.) In a number of cases these bodies were seen to escape from the mother trophozoite, breaking through near the posterior end, where the outer covering appears to be much weakened. I have considerable evidence that this method of reproduction is not rare in *Ceratomyza*.

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Ceratomyxa agglomerata, n. sp. (Pl. XIX, fig. 44, 45.)

Trophozoite.—Vegetative trophozoites colorless, transparent, pyriform, usually with long, slender, posterior process (fig. 44). Actively motile, moving by means of characteristic wavelike movements of the ectoplasm, from which are projected numerous short, conical to filiform pseudopodia. The pseudopodia travel back along sides of body for about one-third its length and then disappear, new ones being continually formed at the anterior end. Ectoplasm, distinguishable only at anterior end, where it forms a distinct layer extending back along the sides for a short distance. Endoplasm clear, very transparent, usually homogeneous, sometimes finely granular. In some cases homogeneous spherules can be indistinctly seen in the endoplasm. Large numbers of fat globules usually present, which are for the most part collected into one to several clumps in a characteristic manner (fig. 44).

Length of sporulating trophozoites about 38μ , width about 12μ . Disporous.

Spore.—Asymmetrical, one valve being somewhat smaller and more attenuated than the other (fig. 45); larger valve compressed parallel to longitudinal plane. In some instances the difference between the valves is not as great as figured. Capsules spherical. Coiled filaments indistinct. Sporoplasm filling nearly entire smaller valve, but only extending a short distance into the larger.

Length of spore $24-28\mu$, width 5μ ; diameter of capsules 3μ .

Habitat.—Abundant in the gall bladder of Synodus factans. Nearly all the fish of this species taken in the harbor, except in the case of very young individuals, were found to be infected.

Ceratomyxa amorpha, n. sp. (Pl. XIX, fig. 46, 47.)

Trophozoite.—Vegetative trophozoites colorless, rounded or irregular in shape, with short, lobose pseudopodia (fig. 46); not pyriform; slowly amœboid. Ectoplasm well developed, forming a distinct layer around entire trophozoite; transparent, finely granular. Endoplasm granular, with large numbers of small fat globules scattered through it or aggregated into one or two large clumps, not collected into a number of small clumps as in *C. agglomerata* which occurs in the same host.

Disporous.

Spore.—Asymmetrical; crescent-shaped; valves short, conical, somewhat compressed parallel to longitudinal plane; one valve distinctly smaller and more conical than the other (fig. 47). Sutural plane perpendicular to longitudinal axis; sutural line distinct. Capsules large, conspicuous, opening at some distance from the capsular side. Coiled filaments distinct. Sporoplasm distinctly granular, unsymmetrically situated, being chiefly confined to smaller valve.

Length of spore 27μ , width 11μ ; diameter of capsules 4μ .

Habitat.-Occurs in the gall bladder of Synodus fatans.

Remarks.—This species has been found only in fish collected outside the inlet and was present in two out of three fish examined. A number of these fish collected inside the harbor have been examined, but in no case did they show any traces of this species, although *C. agglomerata* were abundant. This would seem to indicate that the lizardfish from outside rarely enter the harbor.

Ceratomyxa monospora, n. sp. (Pl. x1x, fig. 48-51; pl. xx, fig. 52-57.)

Trophozoite.—Vegetative trophozoites colorless, very transparent, pyriform, usually with a short slender posterior process and one to several filiform pseudopodia at the anterior end (fig. 48–50). Trophozoites usually become rounded and motionless a short time after being removed to the slide. Progressive movements very slow. Ectoplasm not clearly distinguished from endoplasm, the entire trophozoite being composed of a clear, finely granular protoplasm. Larger trophozoites contain numerous small fat globules, which are usually aggregated into small clumps (fig. 48); fat globules much less abundant in small individuals (fig. 49). In some cases after having been on the slide for a short time small, homogeneous, spherical bodies could with difficulty be distinguished in the endoplasm. They were not observed in trophozoites which had just been removed from the host and are possibly to be regarded as the result of degenerative changes.

Length of vegetative trophozoites up to about 24μ , width up to about 15μ . Monosporous trophozoites much smaller than disporous.

Monosporous and disporous. Monosporous trophozoites are considerably more abundant than the disporous. The spores are relatively large, nearly the entire substance of the trophozoite being used up in their formation (fig. 51-54).

Spore.—Crescent-shaped; valves cylindrical, tapering gradually toward the end, which is rounded and compressed parallel to longitudinal plane (fig. 55–57); valves show considerable variation in amount

of curvature (cf., fig. 52, 55, 56). Spores usually slightly asymmetrical, one valve being more attenuated than the other; in rare cases the valves may be practically alike. Sutural plane perpendicular to longitudinal axis. Capsules large and distinct. Sporoplasm usually unsymmetrically situated.

Length of spore $18-25\mu$, width $5-6\mu$; diameter of capsules 3μ .

Habitat.—Very abundant in the gall bladder of *Peprilus alepidotus*. Is usually present in large numbers during June, being absent in only 5 out of 17 fish examined during this month. In July the parasites were much less frequently met with. Only 3 out of 6 fishes examined during the first week in July were infected, and later in the month nearly every fish examined showed no trace of this parasites. It is interesting to note that during June only vegetative trophozoites were found in a number of fishes, while in July the great majority were in late stages of sporulation and no case was found where only vegetative trophozoites were present.

This species also occurs in the gall bladder of *Prionotus evolans*. Both trophozoites and spores are somewhat larger in *P. evolans* than in *P. alepidotus*, but in other respects there is very little difference.

Remarks.—This species is evidently very close to C. *pallida* Thelohan, but unfortunately his description is very incomplete. According to Thelohan the trophozoites of *pallida* are rounded, with short, lobed pseudopodia, and he makes no mention of the presence of fat globules, which are characteristic for *monospora*. Thelohan was also uncertain as to whether *pallida* is monosporous. It has, therefore, seemed best to consider the species distinct, at least provisionally.

Ceratomyxa streptospora, n. sp. (Pl. xx, fig. 58-60.)

Trophozoite.—Vegetative trophozoites colorless, transparent, pyriform; a few conical, filiform, and wavelike pseudopodia at anterior end (fig. 58). Ectoplasm distinguishable only at anterior end, where it forms a clear, transparent layer. Endoplasm finely granular; filled with transparent, homogeneous spherules, which show only indistinctly; a few small, fat globules usually present but never abundant.

Measurements of full-grown vegetative and sporulating trophozoites vary from 48μ long by 12μ wide to 60μ long by 9μ wide.

Disporous.

Spore.—Valves greatly elongated, compressed parallel to longitudinal plane, ends rounded (fig. 59). Valves usually somewhat twisted on the longitudinal axis, so that the spores appear unsymmetrical (fig. 60), due to the valves being viewed at different angles to the plane of flattening. Sutural line forming a distinct ridge. Capsules large, spherical. Coiled filament not distinguishable. Sporoplasm finely granular, entirely filling both valves.

Length of spore $34-39\mu$, width 4μ ; diameter of capsules 3μ .

Habitat.—Common in the gall bladder of *Chætodipterus faber*. Large numbers were found in 8 out of 9 fishes examined June 9-17. None were found in 4 fishes examined July 11-13. Very few trophozoites were found floating freely in the bile, most of them being attached to the epithelium.

Ceratomyxa aggregata, n. sp. (Pl. xx, fig. 61-64.)

Trophozoite.—Vegetative trophozoites colorless, transparent, rounded to somewhat irregular in shape, rarely pyriform; slowly amœboid. Ectoplasm not distinct from endoplasm. Endoplasm finely granular, containing numbers of small fat globules, which are usually aggregated into irregular clumps. Sporulating trophozoites move very slowly if at all; show a marked tendency to collect in groups composed of a large number of individuals so closely associated that it is often impossible to make out the limits of individual trophozoites. During development the distal half of each valve is so closely applied to the postcapsular side of the proximal half as to be indistinguishable in the living trophozoite (fig. 62).

Length of sporulating trophozoites about 18μ , width about 14μ . Disporous.

Spore.—Crescent-shaped; valves much elongated, tapering gradually toward the ends, which are strongly compressed parallel to longitudinal plane, the opposite walls often being in contact (fig. 63, 64). Capsules large, approximately spherical, opaque, opening close together on the capsular side. Sporoplasm distinctly granular, usually symmetrically situated.

Length of spore about 50μ , width $6-7\mu$; diameter capsules 3.5μ .

Habitat.—Common in the gall bladder of Leiostomus xanthurus. Present in 14 out of 18 individuals examined during July.

Also occurs in the gall bladder of Micropogon undulatus.

Ceratomyxa undulata, n. sp. (Pl. xx, fig. 65, 66.)

Trophozoite.—Vegetative trophozoites colorless, pyriform, sometimes fusiform, tapering rapidly toward posterior end (fig. 65). Progressive movements rapid. Ectoplasm distinct at anterior end and extending along the sides from one-third to one-half distance to posterior end, gradually growing thinner until it entirely disappears. Ectoplasm constantly undergoing rapid, wavelike undulating movements and extruding fine conical or filiform pseudopodia. Pseudopodia form very rapidly and vary greatly in length. After reaching a considerable length the pseudopodia usually travel posteriorly along sides of body for some distance and then disappear. Endoplasm very transparent, often indistinctly vacuolated; containing numerous small fat globules distributed at random. Sporulating trophozoites usually fusiform, sometimes pyriform.

Average length of full-grown trophozoites about 25μ , width $10-12\mu$.

Disporous.

Spore.—Crescent-shaped, slightly asymmetrical, one valve being somewhat longer and more conical than the other; valves cylindrical, not compressed, ends rounded (fig. 66). Capsules convergent, opening on capsular side. Coiled filaments distinct. Sporoplasm distinctly granular, unsymmetrically situated, sometimes being almost confined to more conical valve; in such cases the other valve collapses so that walls of opposite sides are in contact.

Length of spore $22-24\mu$, width about 6μ ; diameter capsules 3μ .

Habitat.-Common in the gall bladder of Ancylopsetta quadrocellata during June, July, and August.

Ceratomyxa navicularia, n. sp. (Pl. xx, fig. 67-70.)

Trophozoite.—Vegetative trophozoites colorless, rounded or slightly irregular in shape, never pyriform. Very slowly amœboid. No distinct ectoplasm. Entire trophozoite finely granular; a few small fat globules present. Nearly entire trophozoite is used up in the formation of spores (fig. 67).

Diameter of sporulating trophozoite about 17μ .

Disporous.

Spore.—Shows considerable variation in shape and size. Symmetrical or asymmetrical, often boatshaped, slightly compressed parallel to longitudinal plane, ends of valves rounded. Capsules small, convergent, opening on capsular side. Coiled filaments very indistinct. Sporoplasm finely granular, extending into both valves, but usually somewhat farther into one than the other.

Average length of spore about 16μ ; extreme variations observed $14-22\mu$; average width about 6μ ; extreme variations observed $5-7.5\mu$; diameter of capsules 2μ .

Habitat.—Not uncommon in the urinary bladder of *Paralichthys dentatus* and *P. albiguttus* during June, July, and August. Also found in the urinary bladder of *Sphæroides maculatus* taken August 2, 1915.

Ceratomyxa spinosa, n. sp. (Pl. xx, fig. 71; pl. xx1, fig. 72.)

Trophozoite.—Vegetative trophozoites colorless, transparent, rounded or slightly irregular in shape, with short, lobose pseudopodia; slowly amœboid. Ectoplasm forming a distinct hyaline layer around entire trophozoite. Endoplasm indistinctly granular, with numerous small fat globules. During development ends of spores are folded along postcapsular side (fig. 71).

Monosporous and disporous.

Spore.—Very characteristic. Central portion greatly enlarged; ovoid, with very long tapering processes extending out from each end (fig. 72). Sutural plane perpendicular to longitudinal axis. Capsules large and conspicuous; spherical, opening at some distance from capsular margin. Coiled filaments indistinct. Sporoplasm finely granular, chiefly located in one valve, extending into the other only a short distance beyond the capsule.

Length of spore about 80μ ; length of enlarged central portion about 13μ ; width 7μ ; diameter of capsules 4μ .

Habitat.-Found in the urinary bladder of Paralichthys albiguttus. Rare.

Family SPHÆROSPORIDÆ, new family.

Genus Myxoproteus Doflein.

Myxoproteus cordiformis, n. sp. (Pl. xx, fig. 73; pl. xxi, fig. 74-80.)

Trophozoite.—Vegetative trophozoites colorless, transparent, rounded; very slowly amœboid, usually forming a single, short, lobose pseudopodium (fig. 74-76). Ectoplasm not distinct. Entire trophozoite finely granular, with a few fat globules. In rare cases may show an indistinct vacuolar structure, and occasionally small individuals are found with a single large, central vacuole (fig. 76).

Diameter of rounded sporulating trophozoites about 18μ .

Disporous.

Spore.—Heart-shaped when seen from above, with peculiar winglike expansions on each side which contain remains of parietal cells (fig. 78–80). Sutural plane oblique to longitudinal axis (fig. 80). Capsules opening at some distance from capsular margin. Capsulogenous cells distinct. Sporoplasm finely granular, entirely filling cavity of sporocyst not occupied by capsulogenous cells.

Length of spore 12μ , width $10-11\mu$, thickness 6μ ; diameter of capsules $3-4\mu$.

Habitat.—Common in the urinary bladder of *Chætodipterus faber*; usually attached to the epithelial lining (fig. 77). The parasites were found in all 12 fishes examined during June and July.

Myxoproteus cornutus, n. sp. (Pl. XXI, fig. 81-85.)

Trophozoite.—Vegetative trophozoites, usually somewhat elongated or irregular in shape, with short lobose pseudopodia (fig. 81-83); slowly amœboid, quickly becoming contracted and motionless (fig. 84) after being placed on the slide. Ectoplasm well developed, hyaline; in rounded individuals forming a distinct layer around entire trophozoite (fig. 83, 84). When moving, entire ectoplasm may flow into pseudopodia (fig. 81). Endoplasm opaque, crowded with coarse, refringent granules varying in shape, and with usually a few fat globules. In contracted resting condition endoplasm becomes more opaque, with granules crowded more closely together, while the ectoplasm is more abundant (fig. 84). This is apparently due to the granules becoming more concentrated into a central mass, the surrounding hyaline portion being squeezed out to form the very conspicuous ectoplasmic layer.

Diameter of rounded trophozoites up to 27μ .

Disporous.

Spore.—Heart-shaped, with two very characteristic processes projecting from the capsular side (fig. 85). Sporocyst relatively thick. Capsules large and conspicuous, opening some distance apart. Coiled filaments distinct. Sporoplasm finely granular, with a few small fat globules; entirely filling cavity of sporocyst not occupied by capsules and capsulogenous cells.

Length of spore 12μ ; width exclusive of capsular processes 9μ ; length of capsular processes about 5μ ; diameter of capsules 3μ .

Habitat.—Common in the urinary bladder of Bairdiella chrysura. Vegetative trophozoites abundant during June and July, but only a few sporulating trophozoites and free spores observed.

Genus Sphærospora Thelohan.

Sphærospora polymorpha, n. sp. (Pl. xxi, fig. 86-92; pl. xxii, fig. 93, 94.)

Trophozoite.—Colorless, usually somewhat elongate (fig. 90), but never very irreguar in shape; slowly amœboid. After being on the slide for a short time become rounded and motionless (fig. 86-89). Ectoplasm distinct around younger trophozoites, hyaline, forming one to several large lobate pseudopodia, which in turn extrude several short, conical pseudopodia (fig. 88). In larger trophozoites ectoplasm often not distinguishable except at ends of pseudopodia, which in such cases are composed chiefly of endoplasm. Endoplasm distinctly granular, sometimes vacuolated in smaller trophozoites (fig. 88), but in larger individuals vacuoles are indistinct or absent; small fat globules usually abundant, especially in larger individuals; numbers of rounded sporoblast cells can be distinctly seen.

Dimensions of large vegetative trophozoites about 35 by 50µ.

Disporous and polysporous. Polysporous trophozoites rarely contain many spores at the same time. *Spore.*—Spherical, sometimes slightly compressed parallel to longitudinal plane. Sutural line forming a distinct ridge around spore in the plane of the capsules (fig. 91, 92); on each side are a number of concentric striations extending around each valve parallel to the sutural line. Capsules large, distinctly pyriform. Coiled filaments indistinct. Sporoplasm distinct, finely granular. Diameter of spores 7-10 μ , averaging about 8μ ; capsules 2-2.5 μ by 4-5 μ .

Habitat.—Common in the urinary bladder of Opsaus tau. Found in 9 out of 11 fishes examined during June and July. Both vegetative and sporulating trophozoites were abundant.

Remarks.—The trophozoites are usually attached to the urinary epithelium, which in badly infected fish may be almost entirely covered with them. The younger trophozoites are attached to the free ends of the epithelial cells (fig. 93), which, however, show no signs of injury. As the trophozoites increase in size they come to lie in depressions formed by the destruction of the ends of epithelial cells (fig. 94). Often the injury to the epithelial cells is carried much farther than shown in the figure, in some cases the part of the cell immediately surrounding the nucleus being all that is left, but even in such cases the nucleus shows little signs of injury. In the larger trophozoites the basal portion becomes specially modified to form an organ of attachment. The protoplasm in sections appears much more densely granular in this region than elsewhere and stains more deeply with plasma stains. Sometimes the organ is drawn out into long processes which extend in between the epithelial cells.

Genus Sinuolinea, new genus.

Sinuolinea dimorpha (Davis.) (Pl. XXII, fig. 95-100.)

Sphærospora dimorpha Davis 1916.

Trophozoite.—Occurs in two very distinct forms, one disporous, the other polysporous.

Vegetative disporous trophozoites colorless, transparent, irregular in shape (fig. 95); progressing by slow amœboid movements. When attached to the epithelium of the urinary bladder they are usually rounded with one to several pseudopodia extending in between the free ends of the epithelial cells. Ectoplasm distinct, forming a homogeneous hyaline layer around entire trophozoite. Endoplasm filled with indistinct vacuoles separated by a denser homogeneous protoplasm similar to the ectoplasm in appearance. Endoplasm usually contains a few small fat globules, which are more abundant in older individuals. Occasionally the endoplasm contains one or more erythrocytes.

Average diameter of full-grown disporous trophozoites about 25-30µ.

The polysporous trophozoites have a very characteristic appearance and can be readily distinguished from the disporous form in all but the youngest stages. When attached to the urinary epithelium the free end of the body is drawn out into a long, cylindrical, taillike process (fig. 97). The body, except where attached to the epithelium, is covered with numerous short, hairlike ectoplasmic processes, which are longer and more abundant toward the free end. While not movable, these processes are readily absorbed and reformed. The trophozoites are attached to the epithelium by means of a specially modified part of the ectoplasm, which forms irregular, thin, platelike processes extending in between the outer ends of the epithelial cells. Owing to the viscid character of the ectoplasm, the trophozoites are often completely covered with extraneous particles of various kinds. After being placed on the slide the specially modified ectoplasm at the larger end gives rise to numerous conical or arborescent pseudopodia, by means of which the trophozoite progresses slowly, the enlarged basal part of the body becoming the anterior end. Pseudopodia are at first composed entirely of ectoplasm, but later as they increase in size the endoplasm may extend into the proximal portion. In extreme cases after being on the slide for some time almost the entire substance of the trophozoite may flow into the large branched pseudopodia (fig. 98). Endoplasm distinctly granular and usually more or less vacuolated; numerous small fat globules usually present and are often very abundant in enlarged basal or anterior part of body (fig. 96). In addition to fat globules, small, irregular, refractive granules and occasionally a few yellowish crystals (probably hematoidin) can be seen in the endoplasm. These crystals are never abundant and are entirely lacking in the majority of trophozoites. Endoplasm also sometimes contains erythrocytes in various stages of disintegration. Very often several comparatively large, rounded bodies can be plainly seen in the endoplasm. These are the gemmules, and each is composed of an outer hyaline layer surrounding a finely granular central portion containing numerous refractive granules.

The size of polysporous trophozoites varies within wide limits. The largest trophozoite measured was 575μ long with a maximum diameter of 90μ .

Spore.—Approximately spherical. Sutural line forming a prominent sinuous ridge around spore (fig. 99, 100). Capsules nearly spherical, large, conspicuous. Sporoplasm forming a distinct, rounded, granular mass.

Diameter of spore about 15µ; diameter of capsules 4.5µ; length of filaments 27-35µ.

Habitat.—Both disporous and polysporous forms abundant in the urinary bladder and ureters of *Cynoscion regalis*. Every one of a large number of fishes examined was found to be infected. In some individuals the polysporous forms were relatively rare, in others they were equally if not more abundant than the disporous.

Remarks.—For a detailed account of the structure and development of this species the reader is referred to a previous paper by the writer. (See Davis, 1916.)

Sinuolinea capsularis, n. sp. (Pl. XXII, fig. 101, 102; pl. XXIII, fig. 103-107.)

Trophozoites.---Vegetative trophozoites colorless or light yellow, rounded to very irregular in shape (fig. 101-103). Progressing by slow amoeboid movements. When first removed from the host are usually rounded, but after being placed on the slide soon become very irregular in shape, due to the formation of numerous large branched or arborescent pseudopodia formed entirely of ectoplasm Ectoplasm transparent, usually distinctly granular (sometimes homogeneous in younger trophozoites), merging gradually with the endoplasm. Often in rounded trophozoites no distinct ectoplasmic layer can be made out (fig. 102). Endoplasm differs from ectoplasm chiefly in the presence of large numbers of fat globules. In large trophozoites the endoplasm often contains one to several relatively large, rounded bodies which are sharply differentiated from the surrounding protoplasm (fig. 102, 103). These bodies are in all probability gemmules similar to those formed by S. dimorpha. They are more finely granular and more transparent than the surrounding protoplasm and are practically identical with the small, free trophozoites shown in figure 104, which are very abundant. Trophozoites containing several gemmules are usually rounded and motionless and appear to be more or less degenerate. Many such trophozoites were observed to disintegrate, thus liberating the gemmules. Whether the gemmules in this species are only liberated through the disintegration of the mother trophozoite was not decided. Sporulating trophozoites were rare and were never seen to contain gemmules.

Diameter of rounded vegetative trophozoites up to 40µ.

Disporous; possibly also polysporous. No sporulating trophozoites with more than two spores were observed, but many vegetative trophozoites were considerably larger than those containing spores, the latter measuring only about 27μ in diameter.

Spore.—Approximately spherical, sometimes slightly elongated along longitudinal axis (fig. 105-107). Sutural plane much twisted on its axis; sutural line forming a prominent ridge around spore. Capsules and capsulogenous cells large and conspicuous, occupying more than one-half cavity of sporcyst. Coiled filaments distinct. Sporoplasm distinct, granular, with numerous small fat globules. As is often the case in this genus, the spores are easily distorted by mechanical and osmotic pressure, and may then appear quite different from the normal form.

Diameter of spore $12-14\mu$, diameter of capsules 4.5μ , length of filament 50μ .

Habitat.—Common in the urinary bladder of Paralichthys albiguttus. Also found in the urinary bladder of Paralichthys dentatus and Spheroides maculatus. In all cases the vegetative trophozoites were abundant. Only a few sporulating trophozoites and free spores were observed and in most instances were entirely absent. Nine individuals of *P. albiguttus* were examined during July and August, of which eight were infected with this species, but in only one of the eight were sporulating trophozoites and spores found, and then only in small numbers. Most of the larger trophozoites contained gem, mules, and apparently this is the common method of reproduction at this season.

Sinuolinea arborescens, n. sp. (Pl. XXIII, fig. 108-110.)

Trophozoite.—Vegetative trophozoites colorless to light yellow; rounded to very irregular in shape; actively amœboid, forming large arborescent pseudopodia of ectoplasm (fig. 108). Ectoplasm well developed, very distinct, hyaline, homogeneous. Endoplasm coarsely granular, sometimes containing a few fat globules; several denser sporoblast cells can usually be distinguished. Older trophozoites are less active and the ectoplasm is less distinct. In sporulating trophozoites the ectoplasm may entirely disappear, the entire trophozoite consisting of a coarsely granular mass.

Diameter of rounded sporulating trophozoites about 75μ .

Polysporous.

Spore.—Rounded, slightly clongated along longitudinal axis (fig. 109, 110). Capsules large and conspicuous, opening a short distance apart on the capsular side. Sutural line forming a prominent ridge, which makes a characteristic S-shaped turn on the capsular side (fig. 110). Coiled filaments very distinct.

Length of spore 15μ , width 12μ , diameter capsules, 5μ .

Habitat .-- Common in the urinary bladder of Siphostoma floridæ.

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Sinuolinea opacita, n. sp. (Pl. XXIII, fig. 111, 112.)

Trophozoite.—Vegetative trophozoites colorless, opaque; rounded or slightly irregular in shape; slowly ameeboid, forming a number of short, lobose pseudopodia (fig. III). Ectoplasm not distinct, except around ends of pseudopodia, where it forms a thin, hyaline layer. Endoplasm opaque, finely granular, with numerous greenish-yellow fat globules varying greatly in size.

Diameter of rounded sporulating trophozoites about 22μ . Several exceptionally large trophozoites were seen with a diameter of about 100μ .

Disporous.

Spore.—Nearly spherical, with flattened, lateral appendages extending from the postcapsular side of each valve (fig. 112). Sutural plane slightly twisted on its axis; sutural line forming a prominent sinuous ridge around spore. Capsules large, opening at some distance from the capsular margin. Coiled filaments distinct. Capsulogenous cells large and easily distinguishable. Sporoplasm distinct, finely granular, containing several comparatively large fat globules.

Diameter of spores about $12-13\mu$, diameter of capsules 4μ .

Habitat.—Occurs in the urinary bladder of Paralichthys albiguttus. Found in only one fish taken August 4, 1914. Both vegetative and sporulating trophozoites were abundant.

Sinuolinea brachiophora, n. sp. (Pl. XXIII, fig. 113.)

Trophozoite.—Colorless; rounded to somewhat irregular in shape. Ectoplasm distinct, hyaline. Endoplasm granular, with numerous large fat globules. Spores arranged in the trophozoites with their postcapsular sides in contact and the armlike processes mutually interlocking.

Disporous.

Spore.—Presents a remarkable appearance. Central portion approximately spherical, with long, lateral, armlike appendages extending from each valve (fig. 113). These appendages are empty except at extreme distal ends, which contains a granular mass, probably the remains of the parietal cell. Sutural plane slightly oblique to longitudinal axis, not distinctly twisted on its axis, sutural line forming a prominent ridge around spore. Capsules large, conspicuous, opening at some distance from capsular margin; capsulogenous cells exceptionally large and distinct, the nuclei being plainly visible by the sides of the capsules. Capsulogenous cells and capsules occupying more than one-half cavity of sporocyst. Sporoplasm distinct, finely granular.

Length of spore exclusive of appendages $9-11\mu$, length of appendages $18-22\mu$, width of spore about 9μ , diameter of capsules 3.5μ .

Habitat.—Lives in the urinary bladder of Paralichthys albiguttus. Found only in one fish taken August 13, 1914. Vegetative trophozoites were abundant, but only a few spores were found.

Remarks.—Possibly this species should be made the type of a new genus, but the spore undoubtedly more closely resembles that of *Sinuolinea* than any other genus. In many respects this species is very similar to *S. opacita*, which occurs in the same host.

Family MYXIDIIDÆ Thelohan.

Genus Myxidium Butschli.

Myxidium incurvatum Thelohan. (Pl. XXIII, fig. 114-118; pl. XXIV, fig. 119-121.)

Myxidium incurvatum Thelohan, 1895, p. 341.

Trophozoite.—Vegetative trophozoites colorless, translucent, rounded to somewhat irregular in shape, with usually a single short, lobose pseudopodium of ectoplasm, which may extrude short conical or filiform pseudopodia along its margin (fig. 114, 115). Occasionally the pseudopodium may be drawn out into a long, slender process (fig. 117). In some cases after being on the slide for some time the trophozoites contract into a rounded mass covered with large numbers of short, hairlike pseudopodia. Ectoplasm clear, transparent, distinguishable only in the pseudopodia. Endoplasm distinctly, sometimes coarsely, granular, with a few comparatively large, greenish-yellow fat globules; usually several rounded, denser sporoblast cells can be distinctly seen. After having been on the slide for some time the endoplasm may be fairly riddled with large, clear vacuoles (fig. 117). The surface of the trophozoites is distinctly viscid, and it is common to find several individuals clinging closely together in an irregular mass, in which it is impossible to distinguish the outlines of individual trophozoites.

Diameter of rounded disporous trophozoites about 13-15µ, of monosporous about 10-11µ.

Monosporous and disporous, the majority being disporous.
Spore.—Cylindrical, valves rounded at ends (fig. 119–121). Capsules large, opening on each side of spore at right angles to the longitudinal plane. Sutural line can be faintly seen extending around spore just inside the capsular pores. Filaments when extruded in HCl remained tightly coiled

Length of spore $8-9\mu$, width $5-6\mu$; diameter of capsules about 3μ .

Habitat.—Common in the gall bladder of Fundulus majalis, Gambusia affinis, and Mugil cephalus. Found in 5 out of 16 adult F. majalis taken July 9 and 10.

Remarks.—Although the shape of the spore differs somewhat from Thelohan's figure, the difference is hardly sufficient to justify considering it specifically distinct. This species has a remarkably wide range of hosts. Thelohan has reported it from the gall bladder of Nerophis æquoreus, Sygnathus acus, Callionymus lyra, Blennius pholis, Trachinus draco, and Scorpæna scrofa.

Myxidium glutinosum, n. sp. (Pl. XXIV, fig. 122-124.)

Trophozoite.—Vegetative trophozoites colorless, elongated or somewhat irregular in shape; slowly amœboid, moving by means of a broad, lobose pseudopodium of hyaline ectoplasm (fig. 122). Ectoplasm not distinct except in pseudopodium. Endoplasm finely granular, with a number of rounded homogeneous spherules, which vary greatly in size and can be only indistinctly seen. The mature spores while still within the mother trophozoites are surrounded by a clear, refractive gelatinous envelope. This envelope only appears as the spore matures, and its absence around one spore in figure 123 is probably due to the fact that one spore matures a little before the other. A similar difference in the rate of development of the spores has been observed in other species.

Diameter of rounded sporulating trophozoites about 20µ.

Disporous.

Spore.—Cylindrical, ends of valves rounded except at one side, where the capsules open at the apex of a small, conical elevation (fig. 124). Spore characterized by the presence of a transparent, homogeneous gelatinous envelope, which under the microscope has the appearance of a bright ring or halo. This envelope is distinctly viscid, and small particles can often be seen adhering to it. Capsule large, distinctly pyriform, opening on each side nearly at right angles to the longitudinal plane.

Length of spore $10-11\mu$, width 6μ ; diameter of capsules 3μ .

Habitat.-Not uncommon in the gall bladder of Cynoscion regalis.

Myxidium phyllium, n. sp. (Pl. XXIV, fig. 125–127.)

Trophozoite.—Exceptionally large; flattened, leaflike, usually folded on itself; motionless, no signs of pseudopodia observed (fig. 125). Ectoplasm forming a distinct transparent layer around entire trophozoite. After being on slide for some time ectoplasm usually becomes covered with very numerous, short, indistinct, hairlike processes, which are not present when first removed from the host. Endoplasm finely granular, with numerous fat globules, not vacuolated.

Diameter of trophozoites up to 1.35 mm.

Polysporous.

Spore.—Fusiform, slightly truncated at each end where capsules open (fig. 126). Sporocyst with numerous longitudinal ridges. Capsules large, opening at ends of spore. Sporoplasm finely granular, with several small fat globules.

Length of spore 11 μ , width 8μ ; diameter of capsules 3μ .

Habitat.—Occurs in the gall bladder of Gambusia affinis. Only one trophozoite was found in each fish. A small percentage only of fish were infected, and in every case the infected fish were partly grown individuals about 2.5 cm. long.

Remarks.—This species has been found only in fish collected in one locality and was the only species found in these fish. Numbers of *Gambusia* from other localities have been examined and a considerable percentage found to be infected with *M. incurvatum* but never with *phyllium*.

Genus Sphæromyxa Thelohan.

Sphæromyxa balbianii Thelohan. (Pl. XXIV, fig. 128-131.)

Sphæromyza balbianii, Thelohan, 1895, p. 342.

Sphæromyza balbianii, Gurley, 1894, p. 282.

Trophozoite.—Small vegetative trophozoites colorless; slowly amœboid; surrounded by a homogeneous, hyaline layer of ectoplasm (fig. 128) from which arise short, lobose pseudopodia. In some instances when moving in the same direction for some time the ectoplasm may accumulate at the (temporarily) anterior end (fig. 120), the advancing edge of the ectoplasm forming small, conical pseudopodia, which are rapidly retracted and new ones formed in their place. Endoplasm not as transparent as ectoplasm, vacuolated, the vacuoles in some cases being very distinct, in others barely distinguishable. Endoplasm between the vacuoles distinctly granular. As trophozoites increase in size, vacuolated structure becomes more evident, the endoplasm often having the appearance of a foam or emulsion, as is well represented by Thelohan (1805, pl. VIII, fig. 55). The large sporulating trophozoites present a very different appearance. They are flattened, leaflike, with a very irregular contour and usually distinctly yellow, although large sporulating trophozoites were seen which were perfectly colorless. In medium-sized trophozoites the ectoplasm forms a transparent layer around the entire trophozoite, but in larger individuals ectoplasm becomes thinner until it can be distinguished only at the ends of the rounded, pseudopodialike projections. Larger trophozoites are often filled with spores arranged in pairs inclosed in a common membrane, as described by Schröder (1907) for S. sabrazesi. Such trophozoites often lose the characteristic vacuolated and granular structure and appear hyaline throughout. This is probably due to degenerative changes preparatory to the disintegration of the trophozoite. The trophozoites vary greatly in size and shape, the largest trophozoite measured having an extreme diameter of 900µ.

Polysporous.

Spore.—Fusiform, ends strongly truncated. Sporocyst comparatively thick and impervious to reagents; usually showing on its outer surface indistinct, parallel, longitudinal striations (fig. 130). Capsules large. Coiled filaments distinct, short, thick with rounded ends (fig. 131). Sporoplasm granular, the two nuclei distinctly visible as clear vacuolarlike structures.

Length of spore $17-20\mu$, width $5-6\mu$; length of filament 20μ .

Habitat.—Common in the gall bladder of Siphostoma floridæ and S. louisianæ. A large number of fishes were examined during June, July, and August and were all found to be infected. Usually the parasites were folded together in a mass which sometimes almost filled the gall bladder.

Remarks.—Both spores and trophozoites differ in some minor respects from Thelohan's description, the trophozoites being much more irregular and the spores larger, while the longitudinal striations are very faint or entirely absent.

Genus Zschokkella Auerbach.

Zschokkella globulosa, n. sp. (Pl. XXIV, fig. 132-135.)

Trophozoite.—Colorless, transparent; rounded; slowly amœboid, forming short, lobose pseudopodia (fig. 132, 134). Ectoplasm not distinct, trophozoites being distinctly granular throughout. Characterized by the presence of several large fat globules.

Diameter of rounded sporulating trophozoites about $15-16\mu$.

Monosporous and disporous.

Spore.—Capsular side flattened; postcapsular side rounded (fig. 134, 135). Sutural plane twisted on its axis and oblique to longitudinal axis; sutural line forming a distinct, sinuous ridge around spore. Capsules located at ends of spore and opening on capsular side. Sporoplasm finely granular, very transparent.

Length of spore 11 μ , width 7μ ; diameter of capsules 3μ .

Habitat.—Found in the urinary bladder of Spheroides maculatus taken August 2, 1915. Both vegetative trophozoites and spores were abundant.

Family CHLOROMYXIDÆ Thelohan.

Genus Chloromyxum Mingazzini,

Chloromyxum leydigi Mingazzini.

Chloromyxum leydigi, Mingazrini, 1890, p. 160. Chloromyxum leydigi, and C. incisum, Gurley, 1893, p. 259.

Chloromyzum leydigi and C. incisum, Guiley, 1995, p. 1391. Chloromyzum leydigi and C. incisum, Thelohan, 1895, p. 345.

Chloromyxum leydigi, Doflein, 1898, p. 160.

Chloromyxum leydigi, Erdmann, 1911.

This species is very widely distributed among elasmobranchs. It was found in the gall bladder of the following species: Dasybatis hastatus, Dasybatis sabina, Pteroplatea maclura, Socliodon terrænovæ, Cestracion zygæna, Cestracion tiburo, and Carcharhinus limbatus. Trophozoites were present in

practically every individual of these species examined except in very young sharks. Both vegetative and sporulating trophozoites were abundant. "Gregarinoid forms" similar to those described by Mingazzini and others were common.

Chloromyxum granulosum, n. sp. (Pl. XXIV, fig. 136-138.)

Trophozoite.—Colorless to light yellow; elongated when first placed on the slide (fig. 136) but soon became contracted and motionless; progressing by very slow amœboid movements. Ectoplasm usually not distinguishable, but in a few cases trophozoites were noticed which had formed one or two short, lobose pseudopodia of hyaline ectoplasm.

After having been on the slide for some time rounded trophozoites often became surrounded by a distinct ectoplasmic layer. Entire trophozoite usually distinctly and coarsely granular, the granules varying greatly in size and shape (fig. 136); sometimes indistinctly vacuolated. A few small fat globules present in younger trophozoites becoming more abundant with age.

Diameter of rounded polysporous trophozoites about 30µ.

Disporous and polysporous.

Spore.—Approximately spherical, with four distinct ridges on post-capsular half of each valve converging toward the capsular side (fig. 137, 138.) Sutural line forming a distinct ridge. Capsules distinctly pyriform, convergent.

Diameter of spore 7μ , diameter of capsules 2μ .

Habitat.--Common in the urinary bladder of Tylosurus marinus during July and August.

Remarks.—There is an interesting variation in the appearance of trophozoites from different fishes. In some fishes all were colorless, while in others the larger trophozoites were distinctly yellow.

Family MYXOBOLIDÆ Thelohan.

Genus Myxobolus Butschli.

Myxobolus capsulatus, n. sp. (Pl. XXIV, fig. 139.)

Trophozoite.—Very irregular in shape; distributed through connective tissues in state of "diffuse infiltration."

Polysporous.

Spore.—Distinctly pyriform, strongly compressed parallel to sutural plane. Characterized by exceptionally large pyriform capsules (fig. 139), which fill almost the entire cavity of sporocyst. Sporoplasm relatively small, limited to extreme postcapsular end of spore. Iodinophilous vacuole visible in living spore.

Length of spore (i. e., along longitudinal axis) $10-11\mu$, width (along transverse axis) 16μ , capsules 4μ wide by 11μ long, length of filament 84μ .

Habitat.-Not uncommon in the visceral connective tissues of Cyprinodon variegatus.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of the Abbe camera lucida at the level of the base of the microscope. Unless otherwise specified, all figures were drawn from living material.

PLATE XVI.

Leptotheca fusiformis.

FIG. 1. Spore from above. \times 1,500. FIG. 2. Spore from capsular side. \times 1,500.

Leptotheca scissura.

Fig. 3. Young trophozoite. \times 700.

FIG. 4, 5. Large vegetative trophozoites in which spherules are well developed. \times 700.

FIG. 6. Portion of trophozoite more highly magnified to show structure of spherules. \times 1,500.

FIG. 7. Sporulating trophozoite. \times 700.

FIG. 8. Spore from above. \times 1,500.

Leptotheca lobosa.

FIG. 9. Contracted vegetative trophozoite. \times 1,500.

FIG. 10. Vegetative trophozoite with large pseudopodium of ectoplasm. \times 1,500.

PLATE XVII.

FIG. 11. Leptotheca lobosa. Spores still united at the sutural line. \times 1,500.

Leptotheca glomerosa.

FIG. 12. Sporulating trophozoite. \times 1,500.

FIG. 13. Spore from above. \times 1,500.

Ceratomyxa mesospora.

FIG. 14. Trophozoite drawn from Giemsa smear. \times 1,500.

FIG. 15. Spore from above. \times 1,500.

FIG. 16. Spore from the capsular side. \times 1,500.

Ceratomyxa sphairophora.

FIG. 17–19. Vegetative trophozoites. \times 700.

FIG. 20. Portion of trophozoite showing structure of spherules. \times 1,500.

FIG. 21. Young vegetative trophozoite. \times 700.

FIG. 23. Spore from above. \times 950.

Ceratomyxa taenia.

FIG. 24. Sporulating trophozoite. \times 700.

PLATE XVIII.

Ceratomyxa sphairophora.

FIG. 22. Sporulating trophozoite. \times 700.

Ceratomyxa taenia.

FIG. 25, 26. Spores from above. \times 700.

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Ceratomyxa attenuata.

FIG. 27. Vegetative trophozoite. \times 700.

FIG. 28. Spore from above. \times 950.

Ceratomyxa recurvata.

- FIG. 29. Vegetative trophozoite. Drawn from a wet smear fixed in formol-corrosive-acetic and stained with acid hemalum. \times 950.
- FIG. 30. Portion of trophozoite showing several generative cells and sporoblasts. Drawn from a wet smear stained with iron hematoxylin. \times 1,500.
- FIG. 31. Large trophozoite showing several generative cells and sporoblasts. Drawn from a Giemsa smear. \times 950.

FIG. 32, 33. Spores from above. \times 1,500.

Ceratomyxa lunata.

FIG. 34, 35. Spores from above. \times 1,500.

PLATE XIX.

Ceratomyxa lunata.

FIG. 36. Spore from above. \times 1,500.

FIG. 37. Spore from capsular side. \times 1,500.

Ceratomyxa abbreviata.

FIG. 38. Vegetative trophozoite. \times 700.

- FIG. 39. Vegetative trophozoite containing two generative cells and one vegetative nucleus. Drawn from a wet smear stained with iron hematoxylin. \times 1,500.
- FIG. 40. Two spores viewed from the end. \times 640.

FIG. 41. Spore from above. \times 1,500.

Ceratomyxa flagellifera.

FIG. 42. Spore from above. \times 750.

FIG. 43. Vegetative trophozoite. \times 400.

Ceratomyxa agglomerata.

FIG. 44. Vegetative trophozoite. \times 1,500.

FIG. 45. Spore from above. \times 1,500.

Ceratomyxa amorpha.

FIG. 46. Vegetative trophozoite. \times 700.

FIG. 47. Spore from above. \times 1,500.

Ceratomyxa monospora.

FIG. 48. Vegetative trophozoite. \times 1,500.

FIG. 49. Vegetative trophozoite. Drawn from unstained specimen killed in osmic vapor. \times 1,500. FIG. 50. Vegetative trophozoite in which two sporoblasts can be faintly seen. \times 1,500.

FIG. 51. Disporous sporulating trophozoite. \times 1,500.

PLATE XX.

Ceratomyxa monospora.

FIG. 52-54. Monosporous sporulating trophozoites. \times 1,500. FIG. 55. Spore from above. From the gall bladder of *P. alepidotus*. \times 1,500. FIG. 56, 57. Spores form the gall bladder of *P. evolans*. \times 1,500.

Ceratomyxa streptospora.

FIG. 58. Sporulating trophozoite. \times 640. FIG. 59,60. Spores from above. \times 1,400.

THE MYXOSPORIDIA OF THE BEAUFORT REGION.

Ceratomyxa aggregata.

FIG. 61. Small vegetative trophozoite. X1,500.

FIG. 62. Sporulating trophozoite. \times 1,500.

FIG. 63. Spore from above. \times 1,400.

FIG. 64. Spore with filaments extruded. \times 1,400.

Ceratomyxa undulata.

FIG. 65. Vegetative trophozoite. \times 1,500. FIG. 66. Spore from above. \times 1,500.

Ceratomyxa navicularia

FIG. 67. Sporulating trophozoite. \times 1,400. FIG. 68-70. Spores from above. \times 1,500.

Ceratomyxa spinosa.

FIG. 71. Monosporous sporulating trophozoite. \times 1,500.

Myxoproteus cordiformis.

FIG. 73. Vegetative trophozoite. \times 1,500.

PLATE XXI.

Ceratomyxa spinosa.

FIG. 72. Spore from sporulating trophozoite (fig. 71). \times 1,500.

Myxoproteus cordiformis.

FIG. 74-76. Vegetative trophozoites. \times 1,500.

FIG. 77. Trophozoite attached to epithelium of urinary bladder. Drawn from a section stained with iron hematoxylin. \times 1,500.

FIG. 78. Spore from one end. \times 1,500.

FIG. 79. Spore from above. \times 1,500.

FIG. 80. Spore from capsular side. \times 1,500.

Myxoproteus cornutus.

FIG. 81-84. Vegetative trophozoites. \times 1,500.

FIG. 85. Spore from above. \times 1,400.

Sphærospora polymorpha.

FIG. 86. Disporous vegetative trophozoite. \times 1,500.

FIG. 87. Disporous sporulating trophozoite. \times 1,500.

FIG. 88. Polysporous sporulating trophozoite containing only two spores. \times 1,300.

FIG. 89. Polysporous sporulating trophozoite. \times 700.

FIG. 90. Vegetative trophozoite containing a large number of nuclei. Drawn from a Giemsa smear. \times 1,500.

FIG. 91. Spore viewed at right angles to the sutural plane. \times 1,500.

FIG. 92. Spore viewed at right angles to figure 91. \times 1,500.

PLATE XXII.

Sphærospora polymorpha.

FIG. 93, 94. Vegetative trophozoites attached to epithelium of urinary bladder. Drawn from a section. X 800.

Sinuolinea dimorpha.

FIG. 95. Vegetative disporous trophozoite. \times 1,500.

FIG. 96. Vegetative polysporous trophozoite. \times 640.

- FIG. 97. Vegetative polysporous trophozoite attached to epithelium of urinary bladder. Internal structure not shown. \times 640.
- FIG. 98. Vegetative polysporous trophozoite some time after being placed on the slide. \times 640.
- FIG. 99. Spore slightly compressed under the cover glass. The entire course of the sutural line is shown. \times 1,400.

FIG. 100. Mature spore. \times 1,400.

Sinuolinea capsularis.

FIG. 101. Vegetative trophozoite showing characteristic pseudopodia. \times 700. FIG. 102. Contracted trophozoite containing several rounded bodies, probably gemmules. \times 700.

PLATE XXIII.

Sinuolinea capsularis.

FIG. 103. Large vegetative trophozoite. \times 700.

FIG. 104. Small vegetative trophozoite. \times 700.

FIG. 105-107. Different views of spores. \times 1,500.

Sinuolinea arborescens.

FIG. 108. Large vegetative trophozoite. \times 640.

FIG. 109. Spore from above. \times 1,500.

FIG. 110. Spore from capsular side. Internal structure not shown. \times 1,500.

Sinuolinea opacita.

FIG. 111. Vegetative trophozoite. \times 1,500. FIG. 112. Spore from above. \times 1,500.

Sinuolinea brachiophora.

FIG. 113. Spore from above. \times 1,500.

Myxidium incurvatum.

FIG. 114-117. Vegetative trophozoites. \times 1,500. FIG. 118. Monosporus sporulating trophozoite. \times 1,500.

PLATE XXIV.

Myxidium incurvatum.

FIG. 119, 120. Two views of spore at right angles to each other. \times 1,500. FIG. 121. Spore drawn from a Giemsa smear. All the nuclei are shown. \times 1,500.

Myxidium glutinosum.

FIG. 122. Vegetative trophozoite. \times 1,400.

FIG. 123. Sporulating trophozoite. \times 1,400.

FIG. 124. Spore. \times 1,400.

Myxidium phyllium.

FIG. 125. Trophozoite just removed from gall bladder. Internal structure not shown. \times 33. FIG. 126. Spore. \times 1,400.

FIG. 127. Spore drawn from Giemsa smear. The parietal nuclei are not visible. \times 2,000.

THE MYXOSPORIDIA OF THE BEAUFORT REGION.

Sphæromyxa balbianii.

FIG. 128, 129. Small vegetative trophozoites. \times 640. FIG. 130. Spore. \times 2,100. FIG. 131. Spore with filaments extruded. \times 1,400.

Zschokkella globulosa.

FIG. 132. Vegetative trophozoite. \times 1,500.

FIG. 133. Sporulating trophozoite. \times 1,500.

FIG. 134. Spore from above. \times 1,500.

FIG. 135. Spore from postcapsular side. \times 1,500.

Chloromyxum granulosum.

FIG. 136. Trophozoite containing only a few spores. \times 720.

FIG. 137. Spore. \times 1,500.

FIG. 138. Spore from capsular side. \times 1,500.

Myxobolus capsulatus.

FIG. 139. Spore viewed at right angles to sutural plane. \times 1,500.





PLATE XVIII.





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PLATE XIX.















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PLATE XXII.





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