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Title	STUDIES ON Myxosporidia of Japan
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Citation	Journal of the College of Agriculture, Hokkaido Imperial University, Sapporo, Japan, 16(5), 229-247
Issue Date	1927-06-25
Doc URL	https://hdl.handle.net/2115/12591
Type	departmental bulletin paper
File Information	16(5)_p229-247.pdf



Studies on Myxosporidia of Japan

By

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With Plate XVII

5. On Myxosporidia in Fishes of Lake Biwa

The present work embodies the studies on the Myxosporidian parasites of fish, which were undertaken with the intent to continue previous investigations on a similar subject already published in this Journal, vol. X, 1923. This time the research was confined to the fresh water fishes of Lake Biwa, Shiga Prefecture. This lake which is the largest in Japan covers 68,624 hectares in surface extent and is 36.6m to 96.4m in depth. As it is located at the middle part of Honshu in the region of mild climate, it teems with different sorts of fishes, which are known at present to comprise 46 genera and 60 species. These include almost all our fresh water forms except some northern types of the salmonid family. During the past two years, i.e. 1922 and 1923 these lake-fishes were collected for the present purpose from the north-eastern section of the lake. The specimens then at hand were 210 in number from 19 genera and 21 species.

The fishes when delivered to the author were fairly well preserved in formaline. Soon afterwards they were thoroughly examined for the Myxosporidian parasites. Ultimately it was found that the majority of the fishes were totally immune. Nevertheless in some of them the parasites were detected to lodge mostly in their kidney or in their gall bladder. In no case were these two organs damaged by one and the same parasite. At any rate out of 19 genera, 21 species and 210 fishes examined, 10 genera, 10 species and 38 fishes were notably affected by the parasites. These were represented by 7 genera and 13 species of Myxosporidia. Thus the infection rate in number was comparatively

low being barely 20 per cent. Such a condition will be fairly made clear by examining the annexed table. In this connection it is worth mentioning that although the parasites have affected the fishes to a small extent, yet they appeared in quite a number of different forms. They belong to the following genera: *Leptotheca*, *Mitraspora*, *Mixidium*, *Zschokkella*, *Lentospora*, *Chloromyxum* or *Myxobolus*.

List of fishes from Lake Biwa and the numbers infected.

Host.	Number of fish		infected part		
	examined	infected	skin	gall bladder	kidney
<i>Acheilognathus lanceolatum</i> ...	12	1	1	0	0
<i>Hemibarbus barbus</i>	7	0	0	0	0
<i>Leucogobio mayedae</i>	10	0	0	0	0
<i>Leucogobio biwae</i>	10	4	0	0	4
<i>Pseudogobio esocinus</i>	10	9	0	1?	9
<i>Sarcocheilichthys variegatus</i> ...	10	0	0	0	0
<i>Pseudorasbora parva</i>	10	0	0	0	0
<i>Richardsonius hakuensis</i>	10	0	0	0	0
<i>Zacco platypus</i>	20	3	0	3	0
<i>Zacco temminckii</i>	10	0	0	0	0
<i>Opsarichthys uncirostris</i>	10	0	0	0	0
<i>Ischikania steenackeri</i>	10	3	0	3	0
<i>Cyrinus carpio</i>	10	3	0	2	2
<i>Chloea castanea</i>	8	0	0	0	0
<i>Misgurnus anguillicaudatus</i> ...	9	0	0	0	0
<i>Cobitis biwae</i>	5	0	0	0	0
<i>Parasilurus asotus</i>	10	4	0	3	2?
<i>Fluvidraco nudiceps</i>	10	7	0	0	7
<i>Anguilla japonica</i>	10	2	0	0	2
<i>Salvelinus pluvius</i>	9	0	0	0	0
<i>Plecoglossus altivelis</i>	10	2	0	0	2
Total	210	38	1	12	28

LEPTOTHECA

L. acheilognathi nov. sp.

Figs. 1-7.

Cyst. Numbers of very minute spherical warts were sometimes perceived diffusely scattered on the forehead of an acheilognathoid fish (Fig. 1). In section it was soon recognizable that this is a cyst

of the Myxosporidian parasite. This cyst lies just under the body epithelium and is enveloped with a thin fibrous membrane similar in nature to that forming the basement membrane of the epithelium (*Fig. 2*). A moderately large cyst measures $750\ \mu$ long and $500\ \mu$ thick. Within this cyst there are always multitudes of the monosporogenous myxosporidium all in nearly the same stage of development. In free state this myxosporidium is quite spherical, but in the cyst being compressed together as a mass it is generally transformed into a polygonal shape so that the mass itself now will show an appearance of honeycomb (*Fig. 3*).

Myxosporidium. In the specimen at hand this is represented by an advanced stage of the sporoblast with the ripe spore of about $16\ \mu$ in size. The ectoplasm is quite indistinct, and the endoplasm is translucent and homogenous.

Spore. The majority of this spore present in front view somewhat a semilunar form with rounded corner (*Fig. 4*). Nevertheless quite spherical one often occurs mixed with this normal form (*Fig. 5*). In lateral view these forms, however, equally present a spindle shape (*Fig. 6*). The valves are thick, symmetrical and smooth externally. Internally the spore has a wedge-shaped process at the mid-anterior portion of the shell and just between the polar capsules. The suture line is rather faint, but its margin is distinct being as thick as the valve. The size of the normal spore is $9\ \mu$ long, $6\ \mu$ broad, and $12\ \mu$ thick, and the spherical one is $9\ \mu$ both in length and breadth. The polar capsules are long ovoid and sometimes very slightly asymmetrical, and are so strongly convergent that their outlets often lie very close together that they might appear to cross each other at their neck. The size of the larger capsule is $5.5\ \mu$ in long diameter. The polar filaments are discharged very loosely, but have a length of about $15\ \mu$. Comparatively large nuclei of the sporoplasm are situated at the mid-center of the spore.

Habitat. In the skin of *Acheilognathus lanceolatum*. This is rather rare in occurrence, its infection rate being only ten per cent.

The distinctive feature of the present species lies in general configuration of the spore and also in large polar capsules. Of about twenty species of *Leptotheca* to which this form no doubt belongs there is none comparable to the present one in the character of the spore just mentioned above. It is a special type of this genus worthy to be treated as a new species, *L. acheilognathi*.

MITRASPORA

M. plecoglossi nov. sp.

Figs. 8-12.

Myxosporidium. The youngest form of this parasite is generally seen to be embedded in the wall of the uriniferous tubule of the kidney (*Fig. 8*). However, when further developed it finally leaves the wall and is set free in the tubule. Nevertheless there is not yet found any diffused form intercellularly. The myxosporidium most generally met with in the specimen is in young stages with many immature spores. Then it is very irregular in outline with a short blunt process and is $35\ \mu$ in long diameter (*Fig. 9*). The ectoplasm is discernible only at this stretched portion, while the endoplasm comprises many obscure but homogenous granules.

Spore. In front as well as in anterior views the spore shows perfectly spherical forms (*Figs. 10 & 12*). Laterally, however, it presents a somewhat ovoid form truncated behind (*Fig. 11*). The shell is formed of symmetrical and moderately thick valves. The suture is represented merely by a distinct but fine line destitute of any obvious ridge. Parallel to this line there run about ten striations on each valve. They are very fine and their extremities do not extend beyond the outer margin of the shell. Nevertheless at each of their posterior ends a fine and moderately long filamentous process is seen to be appended. These processes are almost all equal in length and seem to be stiff always stretching to their full extent. The spore is $7.5\ \mu$ long and broad, and the posterior process is about one half the length of the spore. The polar capsules are asymmetrical, but are equally pyriform. The larger one is $5\ \mu$ by $2.5\ \mu$ with filament four to five times the length of the long diameter of the spore.

Habitat. In the kidney of *Plecoglossus altivelis*. The infection rate of this parasite is twenty per cent.

Considering from the general aspect of the spore of the present form it is a species of *Mitraspora*. However it differs completely from three other forms in this genus either by general configuration of the shell or by number and length of the posterior process. These distinctive features lead the author to consider it as a new species and to call it *M. plecoglossi*.

MYXIDIUM

M. ischikauiae nov. sp.

Figs. 13-15.

Myxosporidium. Its young form is not yet recognizable and even the sporoplasm of a very advanced stage with spores is not perceived but rarely. The sporoblast is, as a rule, spherical without any distinct process and is $23\ \mu$ in diameter. Its ectoplasm is very thin hyaline, and envelops dark and finely granulated endoplasm. A two-spored form is most prevalent (*Fig. 13*).

Spore. It is oblong frontally but laterally its two extremities are more or less acuminate and at the same time slightly curved in s-form (*Figs. 14 & 15*). The shell is provided with thick symmetrical valves with a distinct but thin and oblique suture line. Running straight and equidistantly along this line there are three or four striations on each valve. This is very distinct in front view (*Fig. 14*). The size of the spore is $9\ \mu$ long and $5\ \mu$ broad. The polar capsules are large oval without any marked neck, and are $3\ \mu$ by $2\ \mu$ in size. The polar filaments are made out only by potash solution to measure at least to five times the long diameter of the spore. Nuclei of the sporoplasm are rather large and are set somewhat wide apart.

Habitat. In the gall bladder of *Ischikauia stenackeri* with infection rate of about thirty per cent.

The present *Myxidium* manifests a close resemblance to *M. macrocapsulare* in the general outline of its spore. Still the striations on the valve in my case are less numerous and run straight instead of oblique as in the species previously cited. With such remarkable difference between them we could not esteem these two as one and the same species. Of course there is nothing in common with any known species of *Myxidium*. The new specific name is derived from the host.

M. uchiyamae nov. sp.

Figs. 16-20.

Myxosporidium. This is an intercellular parasite, the myxosporidium generally being found as a non-capsulated and loose mass among the general structure of the kidney (*Fig. 16*). The sporoblast

is spherical usually comprising one spore and is $20\ \mu$ in size (*Fig. 17*). The ectoplasm is indistinct and the endoplasm is full of rather dark coarse granules.

Spore. The front view of this spore resembles that of the previous form; but this is much larger and not so much rounded at the extremities (*Fig. 18*). On the other hand the lateral view of these two spores reveals a totally different aspect, that is, in this species the shell is perfectly straight but narrower and slightly more pointed at the extremities than the other (*Fig. 19*). The shell is formed of thick, smooth and symmetrical valves. The suture line is faint being hardly recognizable, but its ridge is just as well developed as the valve. The spore measures $13.5\ \mu$ long, $8\ \mu$ broad and $6\ \mu$ wide. Often there is found some aberrant form, the valve of which makes an abnormal growth. In such a case one valve overgrows the other and consequently the suture line conspicuously arches to one side. At a glance such spore is apt to be claimed as a *Zschokkella* species. The polar capsules are large ovoid with short neck, and are $6.5\ \mu$ in length. Consequently their large posterior portion almost touch each other pushing the sporoplasm aside completely. The polar filaments are difficult to make out but they are slender extending to about $56\ \mu$. The sporoplasm occupying a very narrow, midlateral space of the spore comprises two rather large nuclei.

Habitat. In the kidney of *Anguilla japonica*. The parasite affects this host at a rate of twenty per cent.

The polar capsule of the present species is perhaps the largest one among all known *Myxidium* spores. Even that of *M. macrocapsulare* is about one third the length of the spore, but in this case it is hardly less than one half of the same. This single fact substantiates a real difference of the species under consideration from any *Myxidium* thus far treated. The new species name *M. uchiyamae* is dedicated to Mr. K. Uchiyama, formerly an expert in the Fisheries Experimental Station, Shiga prefecture, who helped the present author much in supplying the materials for the present investigation.

M. fusiforme nov. sp.

Figs. 21-23.

Myxosporidium. Just like the preceding form and often associated with it the present species is also found diffused in the general structure

of the kidney. The difference between these two forms of myxosporidium is not so obvious as their spores. Yet the size is much larger in this than in the other being $29\ \mu$ in diameter. The endoplasm of this myxosporidium is packed likewise with coarse dark granules (*Fig. 21*), and usually comprises on only one spore.

Spore. This is spindle shaped in outline; but in front view it is more inflated at the middle and also more acuminate at the extremities (*Fig. 22*). The valves are very thin and perfectly smooth, and the suture line is fine but distinct with a well developed ridge. The size of the spore is $19\ \mu$ long, $5\ \mu$ broad, and $4\ \mu$ wide. The polar capsules are in front view moderately large ovate with obscure neck. Laterally, however, they are thin and less conspicuous. It is $4\ \mu$ in length. The filaments are not protractible by chemicals of ordinary use. The sporoplasm completely fills up the shell, and two large nuclei are often seen distinctly without staining.

Habitat. In the kidney of *Anguilla japonica* with infection rate of twenty per cent. As to the intensity of infection in an individual this species certainly excels the preceding form.

The present species belongs to the small sized *Myxidium* and especially to its straight and smooth shell form. From any of this form it differs by the large polar capsule on one hand and by the well pronounced suture ridge on the other. These two main characteristics suffice to consider the present one as a new species *M. fusiforme* after its spore shape.

ZSCHOKKELLA

Z. parasiluri nov. sp.

Figs. 24-26.

Myxosporidium. Its young stage is unknown; but when two to four spores in more or less advanced stage are recognizable the sporoblast generally manifests an irregular oblong form. Then it is about $20\ \mu$ to $25\ \mu$ in long diameter (*Fig. 24*). Its ectoplasm is not easily discernible, and the endoplasm is filled up with homogeneously coarse granules, which is, however, easily clarified by potash solution.

Spore. In lateral view (*Fig. 26*) it is long elliptical sometimes with one extremity more or less narrowly rounded. In the other view its extremities are equally enlarged and the shell slightly arches to one

side resembling the silk worm cocoon (*Fig. 25*). It is formed of thin smooth equivalves, which apply closely to one another with a very faint suture line. Nevertheless the suture ridge is distinct and has almost the same thickness as the valve. This ridge is seen to traverse the shell obliquely passing through the portion very near to, and almost in the same level with the outlets of the polar capsules. The size of the spore is about $15\ \mu$ long, and $7\ \mu$ in breadth and width. The polar capsules are large ovoid with thick wall and with distinct but short neck. They are equally oval in form but are disposed of in totally different manner from other species of *Zschokkella*, i. e. one of them opens at the narrowly rounded extremity, and the other exclusively on the lateral or often on so called the ventral side of the shell. They are $47\ \mu$ in long diameter. The external outlet of this cavity is rather large with a distinct wall. Six or seven whorls of the polar filaments which are distinctly brought out by acetic glycerine are easily extruded to about $60\ \mu$.

Habitat. In the gall bladder of *Parasilurus asotus* with about twenty five per cent infection rate.

From the outline of the spore this parasite is certainly a species, of *Zschokkella*. This genus is at present represented by five species, and most of their spores have a semicircular form. In this respect the present spore manifests a marked dissimilarity. Further, if we trace its minor details such as the outlet of the polar capsule, the position of suture ridge etc. we will soon become aware that this parasite has a quite different form from all known species of *Zschokkella*. Hence it should be called as a new species, *Z. parasiluri*.

LENTOSPORA

L. gigi nov. sp.

Figs. 27-32.

Cyst. In the kidney of a species of *Fluvidraco* there are found number of minute black dots scattered here and there, which can be perceptible even with naked eye. This dot is the cyst of *Lentospora*, which is generally spherical in form and varies from $80\ \mu$ to $100\ \mu$ in size. It is enclosed within a thin, smooth and fibrous membrane, and comprises multitudes of dark granulated pansporoblast and sporoblast together with jet black coarse spherules (*Fig. 27*).

Myxosporidium. The pansporoblast and the sporoblast are equally irregularly spherical and sometimes protrude a blunt process (*Fig. 28*). Their ectoplasm is hardly distinguishable, and the endoplasm unless cleared by reagents is quite opaque being highly granulated. An advanced stage of the sporoblast is as a rule two-spored and measures $25\ \mu$ in size.

Spore. It is ovate in front view with pointed anterior extremity (*Fig. 29 a*). In lateral view it manifests a somewhat spindle shape (*Fig. 30*). The valves are thick and smooth with wide and conspicuous suture ridge. As a rule its posterior border more or less thickens and at its middle portion there is found a fold in one or other form. The fold is at first merely a slit that runs lengthwise along the middle portion of this border. While in another complex and well established form it is represented by two well defined wedge-shaped processes situated slightly apart at the same border. Between these two modifications there are various grades in transformation, which are clearly shown in (*Fig. 29 a-d*.) The size of the spore is $12\ \mu$ long, $5\ \mu$ broad and $6\ \mu$ wide. The polar capsules are pyriform but slightly asymmetrical and are $7\ \mu$ in length. Usually they stand straight in close application to one another, and thus occupy all the space of the anterior half of the shell. However, towards the anterior end the capsules join together to form a common short neck, which finally opens at the midanterior portion of the spore. The polar filaments are three to four times the long diameter of the spore. There is no idinophilous vacuole.

Habitat. In *Fluvidraco nudiceps* with an infection rate of seventy per cent.

At present there is no case recorded in which the ripe spore of *Lentospora* has any form of fold in its shell border like that of some *Myxobolus* species. This sole character is enough to distinguish the present form from all known ones in that genus. The new name *L. gigi* is derived from the Japanese name of the host.

L. kawabatae nov. sp.

Figs. 33-37.

Cyst. The general feature of this cyst is so similar to the preceding species that these two forms are often confounded as one and the same species. Such perplexity naturally will arise as these two are most likely to occur in the kidney of the same host. However,

this differs from the other by having a larger diameter but rather thinner wall. Further it comprises less myxosporidia in number (*Fig. 34*).

Myxosporidium. The nature of this form shows a certain similarity to that of preceding one with the single exception of its larger size which measures 30 μ .

Spore. Different from the spore of *L. gigi* the present form is larger in size with the anterior extremity obtusely rounded in both front and lateral views (*Figs. 35 & 36*). The valves are smooth and moderately thick without any fold at its posterior border as in the case of the preceding form. The suture ridge is prominent being a little thicker than the valve. The spore is 16 μ long, 8 μ broad and 6 μ wide. The polar capsules are long pyriform, and as a rule quite asymmetrical. One of them is larger provided with a much prolonged and narrow wavy neck. This neck finally comes to meet with that of the other smaller and straight capsule at its anterior end so that in this species too the polar capsules open with one and the same outlet just as in the case of *L. gigi*. Sometimes, however, there is found a more inflated spore with the capsules arranged symmetrically, but such is conceivable as an abnormal one (*Fig. 37*). The larger capsule in normal form is 12 μ , the smaller one 9 μ in length, and 3 μ in breadth in both cases. The polar filaments are three to four times the length of the spore. The nuclei are large and distinct in the sporoblast and also in the polar capsule.

Habitat. In *Fluvidraco nudiceps*. Its infection rate is almost the same as the preceding species.

The spores of some *Lentospora* frequently manifest almost similar configuration with that of the present form. Nevertheless the nature of the polar capsule will soon betray their uniformity. Indeed the asymmetrical form of this capsule is quite unique among many features of the present form and is never yet recorded in any *Lentospora* at issue. The present species, *L. kawabatae*, is named as a token of the author's gratitude to Mr. J. Kawabata, Director of the Fisheries Experimental Station, Shiga Prefecture, who had kindly given every facility in performing the present work.

L. leucogobiana nov. sp.

Figs. 38-40.

Myxosporidium. This is totally unknown.

Spore. Frontally this is short oval to spherical, and laterally it

is biconvex lens in form (Fig. 39). The valves are symmetrical, thick and smooth. The suture ridge is somewhat thicker than the valve. The spore measures $12\ \mu$ long, $10\ \mu$ broad and $7.5\ \mu$ wide. The polar capsules are asymmetrical and more or less convergent. The larger capsule is $7.5\ \mu$ long and $3.5\ \mu$ wide occupying more than one half of the shell. The outlet of the capsules lie rather apart, and not on the same straight line but in obliquely opposite position, that is one of them on the right, and the other on the left, anterior side of the shell. By applying potash solution five or six whorls of the filament were clearly seen to lie slanting towards the center of the spore. Nevertheless the filament is very hard to extrude in any way. In the specimen examined the nucleus in the sporoplasm was one and large situated at its mid-center.

Habitat. In the kidney of many goby fishes with an infection rate of generally about forty per cent; but sometimes it gets as high as ninety per cent in *Pseudogobius esocinus*.

Of *Lentospora* species the present form has a certain resemblance either to *L. elliptica* or *L. spherica* so far as the outline of the spore is concerned; but it differs strictly from either of them by having comparatively thick wall and larger capsules. No other species than these two mentioned show any bit of conformity to the present form in the nature of their spores. Hence the latter is called as *L. leucogobiana* after the host.

CHLOROMYXUM

C. sphericum nov. sp.

Figs. 41-45.

Myxosporidium. This is quite unknown.

Spore. As a rule it is perfectly spherical in any view (Figs. 41 & 43); but sometimes in lateral view it is seen slightly elongated antero-posteriorly (Fig. 42). The shell consists of symmetrical valves of moderate thickness. The suture line is fine and obscure but its ridge is elevated being much thicker than the valve. On each valve nine or ten striations run equidistantly and parallel to these ridges. Though distinct they are fine and delicate so that their extremities never exceed the shell margin. The spore is $8\ \mu$ to $9\ \mu$ in diameter. The polar capsules are long oval to pyriform and are all similar in size usually

being $6\ \mu$ by $3\ \mu$ in size. However, they converge notably so as to occupy the full anterior space of the shell (*Fig. 43*). Consequently their outlets are laid in a certain distance. The filaments are easily extruded to about $32\ \mu$.

Habitat. In the gall bladder of *Parasilurus asotus* and *Zacco platypus*. They were infected at the rate of twenty five per cent. Nevertheless in an individual host they were found to be less numerous than other species of *Chloromyxum*.

This *Chloromyxum* distinguishes itself by the nature of the striation on the valve. In all known species of this genus such striation, if present, is always heavier and at the same time more complex in arrangement than that of the present form. This fact though simple suffices to cause the author to deem it as a new species.

C. parasiluri nov. sp.

Figs. 44-47.

Myxosporidium. In an advanced stage it is generally spherical but sometimes its outline shows some irregularity. The ectoplasm is hyaline and rather distinct, while the endoplasm is very finely granulated and comprises besides a few pansporoblasts or sporoblasts numbers of coarse black spherules which seem finally to dissolve in the further course of the development. The sporoblast in somewhat advanced stage measures $14\ \mu$ to $15\ \mu$ (*Fig. 44*).

Spore. In front view it is perfectly spherical (*Fig. 45*). However, in lateral as well as in anterior view it is soon recognizable to be more or less asymmetrical, that is one valve is laterally more expanded than the other one (*Fig. 46*). The valves are thick but the suture is very simple represented merely by a fine line. The striation on the valves are ten to twelve, of which only four or five just next to the suture line are fairly discernible. Their arrangement is quite similar to that of *C. sphericum* mentioned before. However, in this case they are longer so that their extremities about sixteen in number can be distinctly seen from the shell margin as projections. The spore is small, measuring $7.5\ \mu$ long and broad, and $6\ \mu$ wide. The polar capsules are quite asymmetrical, their form varying from oval to long pyriform. Two of them are more elongated and lie crosswise with the other two short ones. Hence each valve carries these two different sorts of capsules. They are rather closely disposed of so that their outlets stand near

to each other. The large capsule measures $6\ \mu$ by $2\ \mu$ and the small one $5\ \mu$ by $2\ \mu$. The polar filaments could not be extruded with ease.

Habitat. In the gall bladder of *Parasilurus asotus* with infection rate of twenty per cent.

In some respects such as the fine striation on the valve the present form bear a certain resemblance to *C. sphericum*. Nevertheless the general configuration of the spore particularly shown in lateral and anterior views together with the lack of the suture ridge will no doubt utterly disprove the similarity of these two species. At all events there are absolutely no similar form in *Chloromyxum* comparable to the present species. Hence this is legitimately to be enlisted as a new species.

C. cyprini nov. sp.

Figs. 48-52.

Myxosporidium. The sporoblast is irregular in form, usually emitting some obtuse process and enclosing in an advanced stage, many spores in different grades of development (*Fig. 48*). The ectoplasm is hardly discernible as such except at the tip of the process, and the endoplasm is of dark consistency from the presence of a rather coarse granular mass. The sporoblast with ripe spore is $58\ \mu$ in long diameter.

Spore. The youngest form of the spore in the sporoblast at the author's disposal is $8\ \mu$ in size. There is not yet any differentiation either to valve or capsule (*Fig. 49*). Sometime afterwards the suture line is fully established immediately succeeded by the formation of the polar capsule. This capsule has always its anterior neck protruded out of the anterior border of the shell. Such a notable character is seen to persist in the ripe spore as its striking characteristic. Then the next stage of development of the spore is the appearance of numbers of striations on the valves. In this case the middle two run antero-posteriorly as is illustrated in the figure, while all others outside of them will take quite a different course as radial direction to them. The ripe spore is perfectly spherical in anterior view (*Fig. 50*), while in other view it is slightly elongate anteroposteriorly (*Fig. 51*). Its valves are thin and the suture ridge is less prominent being more or less thicker than the valve. On the other hand the striations on the valve are equally thick with this valve and particularly their central four are so

very pronounced that their extremities projecting out from the shell margin will attract our attention far more than the others. At any rate all such projections are computable as eight or ten on each valve. The size of the spore is $10\ \mu$ to $12\ \mu$ in length and $10\ \mu$ in breadth and thickness. The polar capsules are long pyriform slightly convergent but in anterior tip they bend in opposite directions so that their outlets are laid in a moderate distance. This curved portion, as is mentioned before, usually projects out of the shell in the same length as the height of the striation. Such characteristic behaviour of the capsule is distinctly recognizable even in the anterior view of the spore as a minor protuberance (*Fig. 52*). The capsule measures $5\ \mu$ long or a little less than one half the length of the spore, and the filaments are $50\ \mu$ to $60\ \mu$ long. The sporoplasm packs the shell densely and has two nuclei.

Habitat. In gall bladder of *Cyprinus carpio* with twenty per cent infected.

In this *Chloromyxum* the nature of the polar capsule is very characteristic and can be looked upon as the sole feature which distinguishes the present form from all known species of this genus. Being a new species the author has ventured to call this parasite *C. cyprini* after its host.

MYXOBOLUS

M. uniporus nov. sp.

Figs. 53-56.

Cyst. This parasite was found in the general structure of the kidney as a spherical form of about $120\ \mu$ in diameter. It is enclosed with rather thick and finely striated membrane (*Fig. 53*). Within this membrane numbers of the myxosporidia in different stages are densely packed, which, however, are obstructed from view by the presence of clusters of dark coarse spherules.

Myxosporidium. Its outline is quite indefinite and its content is also hardly differentiated into two layers of plasm. Nevertheless in some extended form it is about $40\ \mu$ in long diameter and contains two spores (*Fig. 54*).

Spore. In front view it is somewhat spindle shaped slightly sharpened anteriorly; but laterally it is quite oblong (*Figs. 55 & 56*).

The shell is formed of symmetrical smooth and moderately thick valves. There is absolutely no fold on the shell border as is often recognizable in other forms. The spore is 12 μ long, 3 μ broad and 6 μ wide. Two polar capsules are often asymmetrical but equally long pyriform. They are about 7 μ in length and therefore their posterior ends usually reach far beyond the center of the spore. They meet together with their anterior neck just in inside of the shell, and open in a common mid-anterior orifice of the shell. The filaments are about five times the length of the long diameter of the spore. In sporoplasm there is a comparatively large idoinophilous vacuole which is easily seen without staining.

Habitat. In the kidney of *Parasilurus asotus* with less than twenty per cent infection rate.

From the configuration of the ripe spore with an idinophilous vacuole in its sporoplasm the present form is beyond doubt a species of *Myxobolus*. Of about fifty species in this genus one that might be conceivable in general view as congenital to the species under consideration is either *M. cyprini* or *M. neurobius*. By close inspection it becomes certain, however, that besides the form and size of the spore, which within a certain extent are negligible, *M. cyprini* manifests a marked difference from the present form in the character of the polar capsules, the necks of which stand crosswise anteriorly instead of uniting in the present case. *M. neurobius* on the other hand agrees in this respect with my specimen, but such union occurs in this case before the neck of the capsules are passing through the valve. Besides the cyst in the present form is usually larger and often highly elongated without any notable envelope. Even its spore if seen laterally will show a totally different aspect than the other being shorter but generally much inflated spindle form. If such differences are considered together I must hesitate to call the present species *M. neurobius*. It will be far more rational to announce it as a new species, *M. uniporus* from the character of its polar capsules.

SUMMARY.

1. In Biwa, the largest lake in Japan, we have 46 genera and about 60 species of fishes that will represent almost all fresh water forms of the Empire. Of these 19 genera and 21 species comprising 210 fishes were examined for the present purpose. It was finally

recognized that 38 fishes that belong to 10 genera and 10 species were invaded by the Myxosporidian parasites. Consequently their rate of infection was 53 per cent in genus, 48 per cent in species and 18 per cent in number.

2. The *Myxosporidia* thus found were, with a single exception, almost exclusively endoparasitic in nature lodging either in the gall bladder or in the kidney, and sometimes in both these organs. In this case the kidney was perceived to be more a favorable site for the parasite than the gall bladder. Thus 26 fishes from 6 species were affected in the former organ, while 12 fishes from 4 species in the latter. This is quite contrary to the case of marine fishes in which the gall bladder is most persistently attacked by such form of parasite. The *Myxosporidia* in the kidney were represented by many different forms such as *Mitraspora*, *Myxidium*, *Lentospora* and *Myxobolus*. On the other hand those infecting the gall bladder were few and quite different except *Myxidium*. They were *Zschokkella* and *Chloromyxum*. Besides there was one ectoparasitic form, viz. *Leptotheca* which was found embedded in the skin of some acheilognathoid fish. Of all these genera *Lentospora* was most frequently met with on this occasion so that its infection rate often reached as high as 90 per cent. At all event the parasites in the fishes under consideration were enumerated as 7 genera and 13 species.

3. At first sight it is easily comprehensible that the fishes collected from a comparatively narrow space of the lake, that is near Hikoné, will equally harbour any form of the parasite almost indiscriminately. However, as a matter of fact the fish that is carnivorous in nature or seeks muddy bottom for prey is most liable to be invaded by some special form of the parasite and at the same time at a higher rate of their infection.

In conclusion I wish to acknowledge my deepest obligation to the Tokugawa Three Hundred Anniversary Commemoration Society and also to the Imperial Academy for the funds furnished for the present investigations.

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EXPLANATION OF FIGURES.

Figures unless specially mentioned are magnified X 1620.

1. Figures 1-7. *Leptotheca acheilognathi*.

- Fig. 1. Head of *Acheilognathus lanceolatus* showing the parasite in a form of wart(w).
nat. siz.
Fig. 2. Section of wart with a mass of myxosporidia partly illustrated. X 56.
Fig. 3. Myxosporidia in wart, magnified. X 600.
Fig. 4. Normal spore stained, front view.
Fig. 5. Abnormal spore, front view.
Fig. 6. Spore, lateral view.
Fig. 7. Ditto, anterior view.

2. Figures 8-12. *Mitraspora plecoglossi*.

- Fig. 8. Section of kidney of *Plecoglossus altivelis* with myxosporidia(m).
Fig. 9. Spore, blast with many spores. X 860.
Fig. 10. Spore, front view.
Fig. 11. Ditto, lateral view.
Fig. 12. Ditto, anterior view.

3. Figures 13-15. *Myxidium ischikataiae*,

- Fig. 13. Myxosporidium with two spores. X 860.
Fig. 14. Spore stained, front view.
Fig. 15. Ditto, lateral view.

4. Figures 16-20. *M. uchiyamae*.

- Fig. 16. Section of kidney of *Anguilla japonica* with myxosporidia(m). X 340.
Fig. 17. Sporoblasts and spores from macerated structure of kidney. X 600.
Fig. 18. Spore stained, front view.
Fig. 19. Ditto, lateral view.
Fig. 20. Abnormal spore, lateral view.

5. Figures 21-23. *M. fusiforme*.

- Fig. 21. Sporoblasts in different stages of spore formation. X 340.
Fig. 22. Spore stained, front view.
Fig. 23. Ditto, lateral view.

6. Figures 24-26. *Zschokkella parasiluri*.

- Fig. 24. Sporoblast with advanced stages of spores. X 860.
Fig. 25. Spore, front view.
Fig. 26. Ditto, lateral view.

7. Figures 27-32. *Lentospora gigi*.

- Fig. 27. Cyst, clarified. X 600.

- Fig. 28. Sporoblast, clarified. X 600.
- Fig. 29. Spore with different modifications in posterior margin, front view.
- Fig. 30. Spore, lateral view.
- Fig. 31. Ditto, anterior view.
- Fig. 32. Normal form of polar capsules.

8. Figures 33-37. *L. karwabatae*.

- Fig. 33. Section of kidney of *Fluvidraco nudiceps* showing cyst(c) of the parasite, clarified. X 220.
- Fig. 34. Cyst, clarified. X 600.
- Fig. 35. Spore, front view.
- Fig. 36. Ditto, lateral view.
- Fig. 37. Abnormal spore, front view.

9. Figures 38-40. *L. leucogobiana*.

- Fig. 38. Spore stained, front view.
- Fig. 39. Ditto, lateral view.
- Fig. 40. Ditto, anterior view.

10. Figures 41-43. *Chloromyxum sphaericum*.

- Fig. 41. Spore, front view.
- Fig. 42. Ditto, lateral view.
- Fig. 43. Ditto, anterior view.

11. Figures 44-47. *C. parasituri*.

- Fig. 44. Sporoblast, clarified. X 860.
- Fig. 45. Spore, front view.
- Fig. 46. Ditto, lateral view.
- Fig. 47. Ditto, anterior view.

12. Figures 48-52. *C. cyprini*.

- Fig. 48. Myxosporidium with young spores, clarified. X 600.
- Fig. 49. Young spores(a-c) in different stages of development.
- Fig. 50. Spore stained, front view.
- Fig. 51. Spore, lateral view.
- Fig. 52. Ditto, anterior view.

13. Figures 53-56. *Myxobolus uniporus*.

- Fig. 53. Cyst, partly clarified. X 390.
 - Fig. 54. Sporoblast, clarified. X 860.
 - Fig. 55. Spore, front view.
 - Fig. 56. Ditto, lateral view.
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