

Title	Studies on Myxosporidia of Japan
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Citation	Journal of the College of Agriculture, Hokkaido Imperial University, Sapporo, Japan, 10(7), 191-248
Issue Date	1923-03-30
Doc URL	http://hdl.handle.net/2115/12562
Туре	bulletin (article)
File Information	10(7)_p191-248.pdf



# Studies on Myxosporidia of Japan.

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#### With Tables 14 and 2 Plates.

# I. PARASITES OF FLAT FISHES IN HOKKAIDO.

It has been shown that in the North Sea of Europe seven out of twenty three species of the flat fishes are invaded by myxosporidian parasites. This is a low infection rate when compared with that of other fishes in the same sea. Hence, it will be highly interesting to ascertain if the same fact can be shown in the flat fishes in Hokkaido. In Hokkaido we have nearly twenty species of this fish which are easily available for their studies. We have great facility for such purpose, our marine laboratory at Oshoro being near to the principal fishing ground of these fishes. The material for this research was mostly collected from the said locality, but collections also were made from various other districts along the coasts of Hokkaido.

The *Myxospordia* invade, as is well known, all tissues and organs of the fish, but, particularly, they take their abode in the gall bladder. Indeed, some of them are restricted to this organ in their occurrence. Hence, the gall bladder will give the best clue in solving the problem of this infectious disease of the flat fish. With this idea in mind I have restricted my present research solely to the said organ.

The gall bladder in the normal state presents a characteristic bright greenish hue. However, with the presence of the Myxosporidia

[Jour. of the College of Agric. Hokkaido Imp. Univ. Vol. X. Part VII, Feb. 1923.]

this color turns usually to light pink. Thus, one can easily distinguish the healthy bladder from the diseased one. The contents of the affected bladder were studied in the fresh condition when the specimens were brought from Oshoro, while others were preserved mostly in formaline. The fresh ones, too, were sometimes hardened to facilitate precise investigation, using such reagents as Pereny's fluid, picrosulphuric acid or sublimate acetic-alcohol. For staining, haematoxyline (Delafield's or Heidenhein's), picrocarmin, thionine or Giemsa's The extrusion of the filament of the polar capsule solution, was used. was induced by applying solution of potash, ammonia, nitric acid, Ether, which is often mentioned as a glycerine or acetic glycerine. good medium for producing this effect, proved in my case a failure. I have frequently found that acetic glycerine, which seems to have not yet been extensively tried by authors, proved to be far superior to other chemicals for the extrusion.

The number of flat fish examined by me during three years-1913 to 1915—amounts to about 500 in number. It comprises 51 lots, collected from 10 localities. Of these lots, 31 came from what is commonly called the east, but more properly the Pacific coast of Hokkaido, i. e. Kameda, Muroran, Samani, Urakawa, Kushiro and Nemuro, and the remaining 20 lots from the west or the Japan Sea coast, i.e. Yoichi, Oshoro, Ishikari and Soya. The fishes procured from these localities were the following sixteen species :- Paralichthys olivaceus (Temminck & Schlegel), Verasper moseri Jordan & Gilbert, Xystrias gregorjewi (Herzenstein), Hippoglossoides hamiltoni Jordan & Gilbert, Protopsetta herzensteini (Schmidt), Atheresthes evermanni Jordan & Starks, Hippoglossus vulgaris Flemming, Lepidopsetta mochigarei Snyder, Limanda yokohamae (Günther), L. iridorum Jordan & Starks, L. aspera (Pallas), *Platichthys stellatus* (Pallas), *Kareius bicoloratus* (Basilewsky), Clidoderma asperrimum (Temminck & Schlegel), Microstomus stelleri Schmidt, M. kitaharae Jordan & Starks.

To my surprise almost all the species of the flat fish thus examined proved to lodge one or more kinds of the myxosporidian parasites. This notable fact reveals to us, that Hokkaido presents a totally different aspect from the North Sea so far as these parasites are concerned. I have already communicated some of the important observations on the subject to the Zoological Magazine (Tokyo) Vol. 27, No. 232, 1915, with special regard to the percentage of their infection as is illustrated in the following table.

Host	Locality	Number of individuals examined	Number of individuals infected	Percentage of infection	Parasite
Paralichthys olivaceus	{Oshoro Yoichi Ishikari	10 10 8	$\begin{cases} 7' \\ 7 \\ 6 \\ 4 \end{cases}$	70 70 { 75 { 50	Ceratomyxa Ceratomyxa Ceratomyxa Myxidium
Verasper moseri	{Kushiro Nemuro	5 2	I	20 50	Leptotheca Ceratomyxa
Xystrias g <b>r</b> igorjewi	Kushiro	7	{ 3 1	{ 43 14	Ceratomy <b>x</b> a Myxidium
Hippoglossoides hamiltoni	{ Oshoro   Muroran	20 4	{ 8 8 1	{ 40 40 25	Ceratomyxa Myxidium Ceratomyxa
Protopsetta herzensteini	Oshoro Soya Muroran Samani Urakawa Nemuro	20 4 5 4 5 2	17 0 2 5 0	85 0 50 100 0	Ceratomyxa  Ceratomyxa Ceratomyxa
Atheresthes evermanni	{Kameda Urakawa	10 10	4 4	40 40	Ceratomy <b>x</b> a Myxidiu <b>m</b>
Hippoglossus vulgaris	{Muroran Kushiro	5 5	0 0	0 0	
Lepidopsetta mochigarei	Oshoro Voichi Muroran Samani Soya Urakawa Nemuro	45 10 2 6 4 3 2	$\begin{cases} 42 \\ 30 \\ 6 \\ 1 \\ 4 \\ 6 \\ 2 \\ 3 \\ 2 \end{cases}$	<pre>     93     67     60     50     {     67     100     50     100     100 </pre>	Ceratomyxa Myxidium Leptotheca Ceratomyxa Myxidium Ceratomyxa Ceratomyxa Ceratomyxa
Limanda yokohamae	{Oshoro Muroran Nemuro	30 4 2	27 3 0	90 75 0	Lepto!heca Leptotheca —
L. iridorum	{Oshoro Urakawa Nemuro	I2 2 2	9 0 0	75 0 0	Leptotheca —
L. aspera	(Oshoro Yoichi Soya Muroran Urakawa Nemuro	$     \begin{cases}             2 \\             36 \\             5 \\             4 \\           $	$ \begin{cases} 2 \\ 12 \\ 3 \\ 2 \\ 3 \\ 0 \\ 0 \end{cases} $	$     \begin{cases}             IOO \\             33 \\             60 \\             50 \\             40 \\             60 \\             0 \\           $	Leptotheca Ceratomyxa Myxidium Ceratomyxa Leptotheca Ceratomyxa

# TABLE I. List of flat fishes in Hokkaido and their parasites together with percentage of infection.

Host	Locality	Number of individuals examined	Number of individuals infected	Percentage of infection	Parasite
Platichthys stellatus	Oshoro Ishikari Nemuro	20 6 2	$ \left\{\begin{array}{c} 4 \\ 3 \\ 2 \\ 0 \end{array}\right. $	20 {50 33 0	Leptotheca Leptotheca Myxidium
Kareius bicoloratus	{Oshoro Nemuro	17 6	12 4	71 67	Ceratomyxa Ceratomyxa
Clidoderma asperrimum	Kameda Samani Nemuro	48 10 2	$ \left\{\begin{array}{c} 6\\ 1\\ 2\\ 6\\ 0 \end{array}\right. $	{75 13 {20 60 0	Leptotheca Ceratomyxa Leptotheca Myxidium –
Microstomus stelleri	Oshoro Muroran Samani	20 5 4	$ \begin{cases} 20 \\ 4 \\ 1 \\ 4 \end{cases} $	100 { 80 20 100	Ceratomy <b>x</b> a Ceratomyxa Myxidium Ceratomyxa
M. kilaharae	{Oshoro Samani	20 12	19 12	95 100	Ceratomyxa Ceratomyxa

Thus the parasites I have detected in the flat fishes of Hokkaido are represented by three genera, viz., Leptotheca, Ceratomyxa, Myxidium. There are three species of Leptotheca, and four species of both Ceratomyxa and Myxidium. All of them have characteristics, as are described later on, by which they are distinguished from any other allied forms already known to us, and are deemed worthy of being treated as new species.

#### LEPTOTHECA.

# Leptotheca inaequalis nov. sp. Pl. III. Figs. 1-7.

Myxosporidium. Its young form is spherical, measuring 10 to 18  $\mu$  in diameter (Fig. r). The ectoplasm is represented by a thin hyaline layer indistinctly marked off from the endoplasm which is densely packed with coarse granular bodies. At an older stage the myxosporidium emits a broad pseudopodium of about 10  $\mu$  in length (Fig. 2). Still later when one or two spores are formed therein this pseudopodium is extended 60 to 100  $\mu$  in length, and then the ectoplasm is brought into view, particularly in its distal portion (Fig. 3).

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Spore. In lateral view the spore generally arches somewhat with an inconspicuous suture line (Fig. 4). It consists of thin inequivalves, one of which measures 21 to 22  $\mu$  and the other 17 to 18  $\mu$ . The longer valve is slightly attenuated in transverse diameter. This difference in the outline of the valves is more obvious in the anterior view of the spore (Fig. 5). There is a certain variation in such arching as is clearly shown in Figs. 6 and 7. The spore is 13 to 16  $\mu$  in length\*), 12 to 15  $\mu$  in breadth, and 35 to 37  $\mu$  in thickness. The polar capsules in lateral view are oval in shape, measuring 2 to 4  $\mu$  in their longest diameter. The filaments are easily extruded by acetic glycerine. They reach 4 to 5 times as the length of the spore. The sporoplasm does not always completely fill up the valve, but usually leaves at the outer end some vacant space which may amount to one third part of the valve. The variation in the extension of the sporoplasm clearly indicates that the vacant space at the end of a valve is nothing comparable to the so called appendage peculiar to Two nuclei exist in the posterior portion of this some Ceratomyxa. sporoplasm and in proximity of the suture line.

Habitat. This parasite is the most prevalent form of Leptotheca in the flat fish of Hokkaido. I have found it in the following Pleuronectids, viz., Verasper moseri from Kushiro, Lepidopsetta mochigarei from Yoichi, Limanda yokohamae from Oshoro, Muroran and Urakawa, and Clidoderma asperrimum from Kameda and Samani.

The present species no doubt belongs to the second group of *Leptotheca* by Labbè, having spores not oval in shape. So too the majority of the species studied are known to be included in this same group. However, all of their spores have equilateral valves with the thickness relatively less than that in the present form. We consider this parasite, on account of its considerable difference in the general configuration of the shell, as a new species.

# Leptotheca platichthytis nov. sp. Pl. III. Figs. 8-13.

Myxosporidium. In the young stage this has a very irregular

<sup>\*)</sup> The dimensions of the spore are measured in the following way: length is the anterio-posterior diameter of the spore; breadth is the transverse diameter of the spore; thickness is the linear distance between furthest lateral extremities of the spore.

outline, slowly producing some short and broad pseudopodia (Fig. 8). Later it often elongates just as L. elongata does, but its anterior end is generally more broadly expanded than that in the species just mentioned. Moreover, it is truncated in front with numerous minute processes on the edge. Posteriorly it rather rapidly narrows behind to form a slender and elongated process (Fig. 9). The myxosporidia in this stage vary in length, the largest ones often attaining a length of more than 100  $\mu$ . The ectoplasm is thin and inconspicuous, while the endoplasm is filled with fine and somewhat translucent granules. Generally two spores are found therein, but sometimes one-spored and three-spored forms are recognizable (Figs. 10 and 11).

Spore. In anterior view it strongly arches like an inverted V (Fig. 13). Laterally, however, it shows a transversely oblong form with equally blunt ends. The shell is formed of thin and almost asymmetrical valves. The spore is  $13 \mu$  in length, and  $25 \mu$  in breadth and thickness. The polar capsules are globular in lateral view and lie close together with their outer ends converged. Their length is about one third that of the spore. The filaments are  $40 \mu$  in full length and are easily discharged with potash solution. The sporoplasm occupies almost all the space of the shell and is quite homogeneous in nature. It comprises two rather small nuclei which are separated from each other in a moderate distance.

Habitat. This form is at present known to infest Platichthys stellatus from Oshoro and Ishikari. Moreover, even in these districts and in the host mentioned it is infrequently met with.

In its general aspect this parasite may be ranked among the oval group of *Leptotheca*. Nearly all species in this group have their spores less in thickness than that of the present form. Further, they manifest some obvious difference either in the structure of their myxospordia or in the configuration of their spores, so that I feel warranted in considering this parasite as a distinct form.

# Leptotheca limandae nov. sp. Pl. III. Figs. 14–17.

Myxosporidium. At the commencement of the sporulation this body has a very irregular outline with rather short and somewhat branching pseudopodia (Fig. 14). The ectoplasm is thin and not well

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defined except this process, just as in the other species, while the endoplasm is very finely granulated and translucent. In such a stage the myxosporidium reaches a size of about 36  $\mu$  by 16  $\mu$ , and generally comprises two spores (*Fig. 15*).

Spore. In lateral view it exhibits a somewhat transversely coni-Anteriorly, however, it shows an arched outcal form (Fig. 17). line, as in L. inaequalis, and likewise the valves are dissimilar in size and shape (Fig. 16). The larger one, which is 22 to 24  $\mu$  in thickness, arches much more than the complementary one, and tapers to an acuminate extremity. On the other hand the smaller valve of 18 to 20  $\mu$  in thickness makes a slight curvature, and ends more obtusely. The valve is thin, and the suture line is rather faint. The size of the spore is 14 to 16  $\mu$  in length and breadth, and 24 to 26  $\mu$  in thickness. The polar capsules have a globular form, and lie rather near each other. They are about 6 to 8  $\mu$  in long diameter; their filaments are very difficult to extract. The sporoplasm in the majority of cases does not fill up the space of the shell. At least a part of one valve, generally the larger one, is found left vacant. Two nuclei are distinctly seen in the central portion of the sporoplasm.

Habitat. This species seems to predominate on the west coast of Hokkaido, and was detected in two species of Limanda, viz., L. iridorum and L. aspera. These hosts were found among the specimens which came from Oshoro. Later some of the species from Muroran on the east coast were also found to be affected. In either case the rate of infection was comparatively small, although the triad form of this spore is more frequently met with than in the case of other species.

When I made the first inspection of this spore, I thought that it was a varied form of *L. inaequalis.* After making close examinations of specimens from the same host from different districts, and the other hosts from the same district, it becomes ultimately evident that the spore of this parasite in normal condition always manifests certain characteristics in size and general contour, which could never harmonize with those of *L. inaequalis.* Of course it shows many features distinct and different from all allied forms on record.

## CERATOMYXA.

# Ceratomyxa tenuis nov. sp. Pl. III. Figs. 18—21.

Myxosporidium. This is usually round, often producing a short process, and just before sporulation it becomes about 15  $\mu$  in size (Fig. 18). The ectoplasm is distinct, and the endoplasm is occupied by densely packed fine granules, sparingly mingled with some coarse ones of oily nature. Usually this is two-spored, but sometimes the one-spored form is also recognizable (Fig. 19).

Spore. It is large, low and slightly curved in the lateral view (Fig. 20). The shell is formed of thin inequivalves, which strikingly attenuate towards the extremities. One of them is 60 to 69  $\mu$ , and the other 53 to 65  $\mu$  in thickness. The suture is straight and distinct, but not prominent. The sporoplasm usually occupies less than one half of a valve, and in the lateral view this sporoplasmic portion is broader than, and partly constricted from, the outer one. The spore is 10 to 15  $\mu$  in length and breadth, and 108 to 112  $\mu$  in thickness. The polar capsules are subequal and ovoid in lateral view. Their size is 4 to 5  $\mu$  by 3 to 4  $\mu$ , and their filaments are 40 to 50  $\mu$  in length. The sporoplasm is translucent, comprising fine granules and a few coarse spherules. Its two nuclei are rather small and spherical, and lie close together near the suture line.

Habitat. This species was found in Hippoglossoides hamiltoni from Oshoro, Limanda aspera from Oshoro and Soya, and Microstomus kitaharae from Oshoro. In each case the number of the parasites in a single host was comparatively small.

In *Ceratomyxa* there are seven species in which the thickness of the spore exceeds 100  $\mu$ . However, most of them differ from the present form in length and thickness of their spores. In the species under consideration the relative proportion in these measurement is 1:8 to 1:11. *C. sphaerulosa* and *C. attenuata* nearly coincide in this respect with the present one, but their valves are more sharply pointed at the extremities. Further, in the former species the polar capsules are larger, with a highly conspicuous form of the outlets. Such notable difference of the spore suffices to distinguish this parasite from all other similar forms.

# Ceratomyxa robusta nov. sp. Pl. III. Figs. 22-24.

Myxosporidium. In outline it is spherical, but usually it protrudes a nodose pseudopodium which moves sluggishly. Then its size is 13  $\mu$  in diameter (Fig. 22). The ectoplasm is very thin and obscure, and the endoplasm is densely loaded with coarse spherules which often extend to the tip of the process. When older the pseudopodium sometimes elongates twice this size or more, but it still contains spherules now sparingly disposed. In a far advanced stage, when sporulation commences, the ectoplasm becomes more and more distinct (Fig. 23). Now the pseudopodium seems to be formed almost wholly of hyaline ectoplasm. In this stage the myxosporidium attains about 80  $\mu$  in great diameter.

Spore. In general contour this shows a great similarity to the preceding species. However, in lateral view it is higher and has at the same time more blunt extremities than in the latter. The valves are thick and the suture line is very conspicuous. They are quite symmetrical, each of them being about 60  $\mu$  in thickness. The spore is 18 to 20  $\mu$  long, and 115 to 120  $\mu$  thick. The polar capsules are large, and globular to oblong with the outlets wide apart. In lateral view they are 5 to 6  $\mu$  by 4 to 5  $\mu$  in size. The filaments, which are easily extruded by the action of potash solution, reach in some Lepidopsetta as much as 160  $\mu$  in length. The sporoplasm is always disposed asymmetrically in the shell occupying more space in one valve than in the other. It comprises coarse granules and spherules of some oily nature. In its center and near to the suture line there are two well defined but small and oblong nuclei. On the other hand, the nuclei of the polar capsules are small and inconspicuously situated at their posterior portion.

Habitat. The present species was recognized in Atheresthes evermanni from Kameda, Lepidopsetta mochigarei from Oshoro, Soya, and Muroran, and Microstomus kitaharae from Samani.

This is the largest type known to occur in *Ceratomyxa*, except *C. tylosuri*. From this latter it, however, differs completely in general aspect. At a glance the present one is apt to be confounded with *C. tenuis*, but its configuration arising from the relative length and thickness of the shell shows a marked dissimilarity from the latter. In this respect *C. tenuis* has their proportion as I:II, whereas in

this form it is merely I:6. Such being the case the present spore manifests a strong and robust aspect, certainly deserving the new specific name *C. robusta*.

## Ceratomyxa protopsettae nov. sp.

# Pl. III. Figs. 25-34.

Myxosporidium. This is irregular in form usually producing a short and robust pseudopodium (Fig. 25). The ectoplasm is not highly differentiated from the endoplasm which is densely filled with fine granules. With the growth of the spores, which are usually two in number, this process gradually takes a club shape (Figs. 26 and 27). Now when the myxosporidium gets to about 40  $\mu$  it stretches more than 50  $\mu$  (Fig. 28). At the end of sporulation or sometimes in a still earlier stage, as is seen in Paralichthys, it ultimately produces an exceedingly slender and often branched process, which reaches to about 500  $\mu$  in length.

Spore. In anterior view this slightly curves with an indistinct suture line. The valves are thin, like C. tenuis, and are quite inequal. Its short one is 26 to 35  $\mu$  in thickness and has a broad and truncated extremity, while the other is long, being 32 to 40  $\mu$  and consequently has a more slender form. Such notable difference of the extremities, however, is not well pronounced in lateral view (Fig. 31). At all events this spore shows some variations in the curvature of the shell even in the specimens taken from the same host. Figs. 32 and 33 are intended to show some of these variations in a single drop of the gall from Kareius. Further, its abnormal triad form is more frequently recognizable than in other species of Ceratomyxa. The spore is 10 to 12  $\mu$  long, 12 to 13  $\mu$  broad, and 50 to 65  $\mu$ thick. The polar capsules are large and ovate in form, being 6  $\mu$  by 4  $\mu$  in size. They are situated near each other and sometimes very close to the suture line. The sporoplasm in such inequivalves is distributed also asymmetrically. Usually it is gathered more in an elongated valve, extending at least to its middle part. It contains granules, but not so many as in the case of the foregoing species. Besides, its two nuclei resemble those of C. tenuis in their character and disposition. The filaments are expelled with comparative ease by potash solution. They are very long and attain 102  $\mu$  or more in length.

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Habitat. This is the most universally distributed form of Ceratomyxa in flat fishes. Among them the following ten species from quite different and far distant districts are enumerated as the hosts: Paralichthys olivaceus from Yoichi, Oshoro, and Ishikari; Verasper moseri from Nemuro; Xystrias grigorjewi from Kushiro; Hippoglossoides hamiltoni from Muroran; Protopsetta hersensteini from Oshoro, Samani, and Urakawa; Lepidopsetta mochigarei from Samani, Urakawa, and Nemuro; Limanda aspera from Muroran; Kareius bicoloratus from Oshoro, and Nemuro; Clidoderma asperrimum from Kameda and Microstomus stelleri from Oshoro, Muroran, and Samani. Besides, the gall bladder of Amblygaster melanostictum (Temminck & Schlegel) from Mori was also found to be affected with this same parasite.

The remarkable feature of the present form exists in the configuration of its spore, especially in the peculiar aspect of its extremities. It, however, shows a certain affinity to *Ceratomyxa reticularis* and *C. spari*, so far as the size is concerned. As to the configuration the present spore has more or less elongated form. This is evidently proved by the measurement of spore. In the present form it is I:4to 6, while in *C. reticularis* and *C. spari* I:3 to 4 and I:3 to 5 respectively. Moreover, a single glance will reveal to us the marked difference in the general outline of these spores. This is particularly so in *C. reticularis* which has very asymmetrical valves. Such being the fact I have good reason to consider this parasite as a new species. It was named after the flat fish most seriously affected by this form on both sides of Hokkaido.

# Ceratomyxa furcata nov. sp. Pl. III. Figs. 35-36.

Myxosporidium. In its very youngest stage it is quite spherical, being 10  $\mu$  in diameter (Fig. 35), and very seldom producing an irregular process. Two layers of its plasm are hard to discern, and the endoplasm is entirely opaque, being densely packed with granules. Later it becomes gradually clear, and one or two spores are easily perceptible. Then it attains a size of about 25  $\mu$ , but even in this stage it remains as a globular form not producing any obvious process. One and two spored forms are found together promiscuously.

Spore. In lateral view (Fig. 36) it arches so intensely as to take a furcate form. The valves are very thin, but their suture

line is quite distinct. They are inequal, being 50 to 52  $\mu$  and 53 to 57  $\mu$  in thickness, and taper in a marked degree towards the extremities. Further in anterior view they" are compressed, especially so in the outer portion, so that they are seen merely as a thick line. The spore is 15  $\mu$  in length, and 80  $\mu$  or more in thickness according to diversification of the valves. The sporoplasm extends to the middle portion of the valve, and here it often terminates abruptly with a septum-like constriction. Hence, this outer portion might be called an appendage of the spore as often cited in other species. The polar capsules are moderate and round, being 4 to 5  $\mu$  in size.

Habitat. This species was detected in a few Limanda aspera from Oshoro.

In the present form the configuration of the spore is its prominent characteristic, any similar case hitherto known being never yet found in other species of *Ceratomyxa*. Accordingly, I consider this parasite as a new type of the genus.

Quite recently this same parasite was detected in a species of *Osmerus* from the east coast of Hokkaido. In this occasion many matured spores were more easily available than in the present case. Hence their more detailed description will be given in Part III of this work.

#### MYXIDIUM.

# Myxidium crassum. nov. sp. Pl. III. Figs. 37-39.

Myxosporidium. Unfortunately this has not yet been detected, and even the spore was scarcely brought into notice.

Spore. In lateral view it is fusiform often much swollen in the middle portion. Its extremities are abruptly crooked and sharply pointed (*Figs. 37* and *39*). In front view, however, it is narrowly elliptic with rather blunt extremities (*Fig. 38*). The shell is formed of smooth valves with the thickness of about 1  $\mu$ , but its suture line is very difficult to trace out. The measurement<sup>\*</sup>) of the spore is: length, 19 to 21  $\mu$ ; breadth 9  $\mu$ ; and thickness 10 to 12  $\mu$ . The

<sup>\*)</sup> This spore measurement is: length is the diameter between two capsular extremities of the spore; breadth is the widest transverse diameter of the spore; thickness is the diameter between furthest lateral extremities of the spore.

polar capsules are sometimes globular with an elongated neck, and sometimes ovopyriform in shape. They are moderate in size, being 5 to 7  $\mu$  by 4 to 5  $\mu$ . The filaments are easily extruded by acetic glycerine as much as twice the length of the spore.

Habitat. This species was only perceived in Xystrias grigorjewi from Kushiro.

Undoubtedly it belongs to *Myxidium*, of which the species having s-formed spore like the present one invariably affects marine fishes. Of such we have at present five species, i. e. *M. incurvatum*, *M. sphaericum*, *M. bergense*, *M. inflatum*, and *M. depressum*. These spores, except *M. inflatum*, are much smaller in size than those of the present form. On the other hand, the latter species, as its name explains, has the shell considerably inflated at the middle, so that one could not in any way mistake it for the one under discussion.

#### Myxidium microcapsulare nov. sp.

# Pl. III. Figs. 40-42.

*Myxosporidium.* In its somewhat advanced stage containing two well defined spores, it is globular in form, protruding a pseudopodium (*Fig. 40*). It is 43  $\mu$  in length, including this process, and 21  $\mu$  in breadth. The ectoplasm is rather obvious and particularly so towards the distal end of the process. The endoplasm is densely filled with fine granules.

Spore. Laterally it is rather rhombic in shape with short and blunt extremities, and swollen at the middle (Fig. 41). However, in the other front view it is somewhat oblong in outline (Fig. 42). The valves are very thin and present a more or less triangular shape. The spore is 16 to 19  $\mu$  in length, 8 to 11  $\mu$  in breadth, and 9 to 12  $\mu$  in thickness. The polar capsules are small and pyriform, being 4  $\mu$  by 3  $\mu$  in size. They do not stand in one and the same median line of the spore, so that their long axes if prolonged will run parallel to each other. The filaments are very hard to expel, and hence their exact length could not be ascertained. The sporoplasm almost completely occupies the shell, and in its center small and spherical nuclei lic near together.

Habitat. This form was found only in Atheresthes evermanni from Urakawa.

As a *Myxidium* the present spore manifests in its lateral view a very characteristic configuration with a notably small polar capsule which could not be confounded with other similar species already announced.

Myxidium clidodermatis nov. sp.

#### Pl. III. Figs. 43-45.

*Myxosporidium.* In its younger form it presents a spherical outline and is densely loaded with coarse granules so as to obscure its interior view. However, at the commencement of sporulation it gradually turns translucent, and a short and blunt pseudopodium, like in the preceding species, makes its appearance (*Fig. 43*). Then its size is 30  $\mu$  by 20  $\mu$ .

Spore. It is rather oblong in both lateral and front views and more or less swells in its middle portion (*Figs.* 44 and 45). Its extremities accuminate more than those of the former species, and at the same time take s-shape. The valves are thin and smooth, but their suture line is quite distinct. The spore is 16 to 19  $\mu$  long, 7 to 10  $\mu$  broad, and 6 to 8  $\mu$  thick. The polar capsules are rather small and have an elongated pyriform. Their size is 4.5 to 5  $\mu$  by 3.2 to 4  $\mu$ . The polar filaments being very hard to discharge their true nature is totally unknown. Two nuclei of the sporoplasm are small and are situated near together in its center.

Habitat. This species is known at present only in *Clidoderma* asperrimum from Samani, and even in this host it does not occur so profusely as other kindred forms.

Among all known forms of *Myxidium* only two species, *M. sphaeri*cum and *M. bergense*, have spores similar in length to the present one. Nevertheless, the enormous thickness of their valves does not allow us to incorporate this species in either of them. Rather a certain likeness exists between this and another one which I am going to describe. Yet from considerations mentioned in the following chapter I assume this form to be a new species.

# Myxidium oshoroense nov. sp. Pl. III. Figs. 46-50.

*Myxosporidium.* When the sporulation commences it takes a globular form without any obvious process. The ectoplasm is obscure,

and the endoplasm is translucent with many coarse granules. Usually the myxosporidium is two-spored, and the one-spored form is seldom found. The two-spored one in full maturity has a size of about 31  $\mu$  in its widest diameter (*Fig. 46*).

Spore. In lateral view this shows a great resemblance to the previous species (Fig. 47). However, it is more slender, and its extremities are, as a rule, more attenuated and crooked, although there are some individual differences in this respect. In front view (Fig. 48) it is nearly oblong, and its extremities are seen to lie on different levels, more than in the preceding form. The valves are smooth and tolerably thin with a very faint suture line. The spore is 16 to 21  $\mu$  in length, 8 to 11  $\mu$  in breadth, and 7 to 10  $\mu$  in thickness. The polar capsules are rather elongated-conical in outline. In front view they lie straight, but laterally their tips arch in quite opposite directions, so that the axes passing through their long diameters do not run parallel. They are 5 to 8  $\mu$  by 3 to 5  $\mu$  in size. The filaments are very easily extruded either by glycerine or potash solution. They are very slender and attain to about 95  $\mu$  in length. The sporoplasm bears two rather large nuclei, which are situated close to each other near its center.

Habitat. This Myxidium is widely disseminated among flat fishes. Among them Paralichthys olivaceus from Ishikari, Hippoglossoides hamiltoni from Oshoro; Lepidopsetta mochigarei from Oshoro and Samari; Limanda aspera from Yoichi; Platichthys stellatus from Ishikari; and Microstomus stelleri from Muroran are enumerated.

At first sight, this form presents a great similarity to M. clidodermatis, but in its normal spore we can easily recognize some notable differences. In outline it is slender with more curved extremities, and its polar capsules are larger and more dilated at the base. The myxosporidium in this species generally takes a globular form throughout its developemental stages without emitting any process like that of the preceding form Taking all these points together it is valid to consider this as a distinct form from M. clinodermatis.

Having thus been able to determine the nature of the myxosporidian parasites in flat fishes of Hokkaido, the next question to be solved is their distribution. The studies on the distribution of *Myxosporidia* in general were long ago strongly recommended by Thélohan, and their importance has been recognized by some other authors. Unfortunately, on account of the number of specimens treated by the authors, which was not adequate enough to meet this question, such important investigation has hitherto been left neglected. Under the circumstances I have ventured to discuss the matter from the data already thus far described. At first, it will be highly instructive to get a good notion about the actual state of the disease in flat fishes. For this purpose the following table will be of service.

Table II.	Geographical	distribution	of	parasites in flat fishes	
	on east and	west coasts	of	Hokkaido.	

Parasite and Locality	l Leptotheca		Cerate	omyxa	Myxidium	
Host	East	West	East	West	East	West
Paralichthys olivaceus	{ =		·	Yoichi Oshoro Ishikari		Ishikari —
Verasper variegatus	${Kushiro - $	_	Kushiro Nemnro			=
Xystrias grigorjewi	·			_	Kushiro	-
Hippoglossoides hamiltoni	_		Muroran	Oshoro		Oshoro
Protopsetta herzensteini	{ =		Samani Urakawa	Oshoro —	_	
Atheresthes evermanni		·	Kameda	·	Urakawa	
Lepidopsetta mochigarei		Yoichi — —	Muroran Samani Urakawa Nemuro	Oshoro Soya —	Samani — —	Oshoro — —
Limanda yokohamae	{Muroran Urakawa	Öshoro —	_			_
L. iridorum		Oshoro				· -
L. aspera	Muroran	Oshoro	Muroran	Oshoro Soya		Yoichi
Platichthys stellatus	{ =	Oshoro Ishikari	-			Ishikari —
Kareius bicoloratus		_	Nemuro	Oshoro		

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Parasite and Locality	Leptotheca		Cerat	omyxa	Myxidium	
Host	East	West	East	West	East	West
Clidoderma asperrimum	{Kameda Samani		Kameda		Samani —	
Microstomus stelleri	{ =	_	Muroran Samani	Oshoro —	Muroran	
M. kitaharae	<del>.</del>		Samani	Oshoro	·	

Of the three genera considered, Ceratomyxa taken as a whole is disseminated most extensively among the flat fishes, affecting 12 out of 16 species from 10 localities, while Leptotheca attacks 7 species from 8 localities, and Myxidium 9 species from 7 localities. On further inspection it seems to me that Ceratomyxa preponderates on the east coast of Hokkaido. Thus, on that coast 11 out of 14 species of the host are its victims, while on the other coast 8 out of 11 species. In this genus, C. protopsettae infects the host to an alarming extent on both the coasts of Hokkaido. On the other hand Leptotheca frequents the west coast more than the other. In this respect Myxidium is more uniformly distributed around the island. Nevertheless, the east and west coasts have their special representatives. On the east they are represented by the species of Myxidium such as M. crassum, M. microcapsulare, and M. clidodermatis, and on the west by Ceratomyxa In this connection it is noteworthy that a single host often carries at a time more than one species of the parasities from quite different genera. Their most prevalent combination is Myxidium with either Leptotheca or Ceratomyxa, but the last mentioned genera are seldom met with in the same host, as far as my investigation extends. With regard to this fact Limanda aspera ranks first, harbouring five species of parasites from all three genera mentioned, seconded by Lepidopsetta, which bears four species from the same genera. These facts preeminently concern the actual state of parasite infection on flat fishes. If we further want to know the extent of the ravage by them, we ought to investigate the degree of frequency\*) and intensity of this disease pervading among the fish. For this purpose I have summarized the foregoing data into the following tabulated form.

<sup>\*)</sup> Frequency means numbers of species of the host infected, while intensity means numbers of individual of a species infected.

		East	coast		West coast				
Host	No. of fish exam.	Lepto- theca %	Cerato myxa %	Myxi- dium %	No. of fish exam.	Lepto- theca %	Cerato- myxa %	Myxi- dium %	
Paralichthys olivaceus		_			28		71	50	
Verasper moseri	7	20	50			—		_	
Xystrias grigorjewi	7	—	43	14		—		—	
Hippoglossoides hamiltoni	4	_	25		20		40	40	
Protopsetta herzensteini	16	_	75	_	24		85		
Atheresthes evermanni	20	-	40	40	-	·	_	-	
Hippoglossus vulgaris	10	o	о	o				-	
Lepidopsetta mochigarei	13		77	100	59	60	72	67	
Limanda yokohamae	7	86		_	30	90	_	,	
L. iridorum	4	o			12	75	_		
L. aspera	13	40	23	0	47	100	35	60	
Platichthys stellatus	2	0		0	26	. 27	_	33	
Kareius bicoloratus	6	-	67	-	17		71		
Clidoderma asperrimum	20	44	13	60					
Microstomus stelleri	9	-	89	20	20	-	100		
M. kitaharae	I 2	-	100	-	20	-	95	-	

Table	III.	·Number	of fishes	examined and	percentage
		of their	parasites	in Hokkaido.	

To facilitate a rough survey of the intensity of these myxosporidian infections I have arranged its ratio under three headings according to different percentages of the parasites.

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Parasite	100 %		90-1	50 %	50—10 %	
	East	West	East	West	East	West
Leptotheca	0	I	I	3	3	I
Ceratomyxa	r	I	5	5	5	2
Myxidium	I	ο	I	3	3	2

TABLE IV. Rate of intensity of infection of parasites on east and west coasts of Hokkaido.

Referring to tables 3 and 4 we notice that the parasites under consideration prevail more on the west coast of Hokkaido than on the other coast. In this locality their average rate of infection is 93% in frequency and 74% in intensity. On the other hand, on the east coast the frequency is 80% and the intensity 57%. The hosts mostly affected by the parasites are in every case species of Limanda and Microstomus. As to the parasites, Ceratomyxa stands in the highest grade of infection, being more than 90% in frequency, next followed by Myxidium and Leptotheca. These facts are easily discernible even from the specimens caught from a single locality. For instance, in Oshoro, we have II species of the flat fish in which the parasites were met with in the following ratio:-Leptotheca 4/11, Ceratomyxa 8/11, and Myxidium 3/11. Such remarkable state in frequency of Ceratomyxa was also noticed by Auerbach in Norway. There, the flat fishes studied were of five species-three species of Pleuronectes, and one species of Hippoglossus and of Hippoglossoides-of which only one species of Pleuronectes was wholly free from any parasite. The remainder, though not plentiful in number, were found to be attacked by Ceratomyxa in such a high degree as 57 to 100 per cent.

In the literature relating to the subject, we have only two instances in which the rate of infection of such parasites is given in a reliable manner. One of these investigations was Auerbach's work on Norwegian fishes, and the other Awerinzew's on the occasion of his trip to Africa. In 1908, and 1911, Auerbach collected specimens from the Bergen fish market as well as from the sea coast of Norway, thus examining the northern fishes, including *Gadidæ*, quite extensively. Two years later Awerinzew made thorough studies of the myxo-

sporidian parasites in the fishes from the east and west coasts of Africa. They are the tropical fish with *Selachii* as their representative. Now it will be highly interesting to make a comparative study of the infection by the parasites in these localities with ours, as cited before.

Locality	Number	Number examined		infected	Percentage infected		
	Species	Fish	Species	Fish	Species	Fish	
Hokkaido	16	453	15	310	94	68	
Norway	36	823	21	391	58	48	
Africa	30	119	9	29	30	24	

TABLE V. Number of fishes examined and infected in three different districts.

As is illustrated, obviously the flat fish of Hokkaido, when taken as a whole, lodge a higher percentage of the parasites, both in frequency and in intensity, than other fishes from Europe and Africa. Nevertheless, the highest grade in infection is seen to be far superceded by the tropical form.

Locality	100—91%		90—	51%	505%		
	Species	Fish	Species	Fish	Species	Fish	
Hokkaido	2	61	9	267	4	115	
Norway	4	7	9	350	· 8	34	
Africa	8	28	o	о	I	ſ	

TABLE VI. Number of fishes infected in three different districts.

Considering this rate of infection it crossed my mind that there may be a certain close relation between the number of parasites and the situation of the fishing ground, such as its distance from the coast, depth etc. I have collected much valuable information concerning these data from Oshoro, as the following table shows.

		Fishing ground			
Host	Fishing season	Distance from coast	Depth		
Paralichthys olivaceus	VI —VII	2—3 miles	20 fathoms		
Hippoglossoides hamiltoni	$\Pi = \Pi$	27	30—40		
Protopsetta kerzensteini	111 — 111	2—5	20—30		
Lepidopsetta mochigarei	XII — II	716	40—80		
Limanda yokohamae	I III	I-3	8-15		
L. iridorum	II III	o5	030		
L. aspera	I — III	I—5	8-15		
Platichthys stellatus	п — ш	1-5	.8—15		
Kareius bicoloratus	I — III	I-3	815		
Microtomus stelleri	XII — 11	7—16	40—80		
M. kitaharae	I — III	15	815		

TABLE VII. Fishing season and fishing ground of flat fishes at Oshoro.

It is easily seen that the fishing season of these different fishes is almost entirely restricted to the winter time, with a single exception of *Paralichthys*. As to the distance from shore or the depth of the fishing ground there remains something more to be considered. Arranging the fishes according to the depth of their abode we get the following divisions:

TABLE VIII. List of flat flshes at Oshoro arranged according to depth of their fishing ground.

0—15 fathoms	15—30 fathoms	30—80 fathoms
Limanda yokohamae	Paralichthys olivaceus	Hippoglossoides hamiltoni
L. iridorum	Protopsetta kerzensteini	Lepidopsetta mochigarei
L. aspera	Limanda iridorum	Microstomus stelleri
Platichthys stellatus	Platichthys stellatus	
Kareius bicoloratus		
Microstmus kitaharae		

If we refer to this table and examine the parasites as regards the bathymetrical distribution of their hosts, the following assumption can be fairly made. Of three genera of *Myxosporidia* considered, *Leptotheca* seems mostly to abound in shallow water, as it affects in a great measure the species of *Limanda*, which are hauled up from water not deeper than 30 fathoms. As a deep water form we might take *Ceratomyxa*. On the contrary, *Myxidinm* invades the fish irrespective of distance from coast or depth of their abode.

Then turning our attention to the geographical distribution of the parasites in Hokkaido, we will soon notice that the fish in the different localities mentioned above are not affected with this disease to the same degree. Indeed, other things being equal, their infecting ratio seems as a rule to increase, the lower in latitude the locality is. This is fairly well illustrated on the east coast. Here I have collected the specimens from the following six localities, which, enumerated from north to south, are Nemuro, Kushiro, Samani, Urakawa, Muroran and Kameda. These specimens including several species were perceived to lodge the parasites in different ratio according to their abode.

Locality	Frequency . in percentage	Intensity in percentage
Nemuro	38	27
Kushiro	67	21
Samani	100	71
Urakawa	67	57
Muroran	71	44
Kameda	100	43

Table IX.	Frequency and intensity of parasites of	n
	east coast of Hokkaido.	

The same state of things is not well pronounced on the west coast. Nevertheless in Soya, the farthest north on that side of the island, we have 67% in frequency, and 50% in intensity. This is far less than that is mentioned in Oshoro, where the frequency is 100% and the intensity is 76%.

In this connection it is worth of mentioning that these results are

directly opposite to what Auerbach said about the fishes of Norway. The cod fish, particularly Gadus virens, was his main object of research from which the following assertion is derived :---" die Intensität der norwegischen Küste nicht überall die gleiche ist, dass vielmehr eine Zunahme nach Norden hin sich feststellen lässt, und zwar bei jungen und alten Fischen (in Bergen sind von mittelgrossen G. virens etwa 50% gesund, im Norden nur etwa 6-7%)". After making the second trip to the same country he further emphasized his view, saying, "so geht doch aus der Gesamtheit hervor, dass nach Norden zu unstreichbar der Intensität der Infektion ganz bedeutend zunimmt, und zwar bei Alterstufen." It is to be regretted that this assertion only concerns Gadidae, for with reference to the flat fish the number of specimens available to him were too few to draw any such decisive conclusion. These two sorts of fish are equally northern types, and our studies of them, as regard to the distribution of the parasites; have thus finally reached to conflicting results. Before attempting to settle this difficult question it should be borne in mind, that Auerbach made his announcement after studying the same host, that is, G. virens, all along the coast of Norway and by observing the same parasite, that is, Myxidium bergense. Unfortunately, we have no such favorable condition so that table 9 was compiled by taking all flat fishes in a certain locality together, and considering their different parasites as a whole. This different method of procedure in estimating the distribution of parasite may be a reason of the diverse results between us. Besides, they may arise from the nature of the host itself, or from some special conditions involved in the localities, where the specimens are procured. Hence, at the present juncture we must content ourselves with the inference that the cod fish and the flat fish behave differently towards the myxosporidian parasites, at least as regard their distribution.

#### SUMMARY.

1. The flat fish of Hokkaido is more highly susceptible to the infection of the myxosporidian parasites than the allied forms in the North Sea. In Hokkaido the infecting ratio of the parasites is 94% in 16 species of the hosts, and 68% in 453 fishes examined.

2. This fact was affirmed by examining the gall bladder of the fish, which has hitherto been known as the most favorable site of the parasites.

3. The *Myxosporidia* found in the fish are represented by three genera and eleven species: *viz.*, three species of *Letotheca*, and four species of *Ceratomyxa* and of *Myxidium*. All of them are new species. Usually only one of them was perceived in a species of the host. However, more than two species of the parasites from totally different genera were often recognizable in one and the same species of the fish and even in a single individual. In this respect *Myxidium* was seen to coexist more freely with either *Ceratomyxa* or *Leptotheca*, while these two latter rarely associated together.

4. Among others, *Ceratomyxa* ranks first as the serious intruder of the fish, and is followed by *Leptotheca* and *Myxidium* in order. This parasite occurs almost indiscriminately along the coasts of Hokkaido, but it predominates on the east coast. On the contrary, two other genera mostly pervade the west coast.

5. The occurrence of the parasites seems to have a certain relation to the geographical position of the locality from where the fish was available. It increases more and more in frequency and in intensity, the farther south the fish are. In Hokkaido this is fairly well illustrated on the east coast. Nemuro is the region farthest north examined, where less than one half of the fish are affected, while in the south for instance Samani or Kameda, all the hosts were invaded in a large degree.

6. Considering the bathymetrical distribution of the parasites, we have at present but few data to go on for a satisfactory elucidation. Nevertheless, judging from the fish collected from Oshoro, on the west coast of Hokkaido, we must come to the conclusion that some parasites seem to prefer a certain depth as their proper abode. For instance, the majority of *Leptotheca* attack the fish in a shallow sea, while *Ceratomyxa* show a quite different habit abounding mostly in greater depths.

# II.—PARASITES OF THE FLAT FISHES. IN NORTHERN HONSHU.

Having finished the study of the myxosporidian parasities in flat fishes in Hokkaido, I undertook to extend this investigation to the same hosts from totally different districts. For this purpose Honshu, particularly its northern section, which is separated from Hokkaido by

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the Tsugaru Strait, was first taken into consideration. By treating the said parasites in this district, their relation, if any, to the kinkred forms in Hokkaido would be easily shown. With this intention the fishes were collected from the east and west coasts of the district just mentioned through the courtesy of the local Fisheries Experimental Station, and the School of Fisheries, to which I here express my deepest thanks. Thus I had access to five species of the flat fish from Aomori and Miyagi on the east side, and fifteen species from Yamagata, Niigata, and Fukui on the west side. Among them five species, i. e. Pseudorhombus misakius Jordan & Starks, Pleuronectes cornutus (Temminck & Schlegel), Limanda angustirostris Kitahara, Microstomus higeguro Tanaka and Rhinoplagusia japonica (Temminck & Schlegel), which were mostly procured from Japan Sea, while they are not yet found in Hokkaido. Hence their treatment for the present investigation will be a new addition to the studies on the myxosporidian parasites of the flat fishes. It is to be regretted that some of all these specimens, when they arrived, were not carefully preserved in formaline as to allow a close inspection, and at the same time, were not numerous as in the case of Hokkaido. However, in such locality as Fukui, the infection of the parasites is highly pronounced even in a small number of the specimens. The gall bladders of the hosts were treated just as in the former investigation, and here eight species of Ceratomyxa, and one species of Myxidium were found to be their main parasites. The names of the hosts together with the rate of infection will be studied in the next table.

Host	Locality	Numl fi	ber of sh	entage cted	Parasite	
		examin- ed	infect- ed	Perce infe		
Pseudorhombus misakius	Niigata	I	о	0	—	
Verasper variegatus	Fukui	I	I	100	Ceratomy xa	
Hippoglossoides hamiltoni	{Yamagata Niigata	2 3	0 2	0 67	Ceratomyxa	
Xystrias grigorjewi	Aomori Niigata Fukui	4 1 5	1 0 5	25 0 100	Ceratomyxa Ceratomyxa	

TABLE X. Number of flat fishes examined and found infected from northern Honshu.

Tsunenobu Fujita.

Host	Locality	Numt fis examin-	per of sh infect-	Percentage infected	Parasite	
Protopsetta herzensteini	Aomori Miyagi Yamagata Niigata Fukui	5 2 3 6 3	I O O I I	20 0 17 33	Ceratomyxa  Ceratomyxa Ceratomyxa	
Pleuronectes cornutus	{Niigata Fukui	I I	o I	0 100	Sp. incert.	
Lepidopsetta mochigarei	{Aomori Yamagata	4 2	I O	25 0	Ceratomyxa, sp. incert.	
Limanda yokohamae	Yamagata Niigata Fukui	3 2 2	0 0 2	0 0 100	 Ceratomyxa	
L. angustirostris	Aomori Miyagi Yamagata Niigata Fukui	5 2 2 2 2 2	I 0 2 0	20 0 0 100 0	Ceratomyxa  Ceratomyxa 	
Platichthys stellatus	Fukui	I	0	o		
Clidoderma asperrimum	Fukui	I	I	100	Ceratomy xa, sp. incert.	
Microtomus higeguro	Niigata	4	I	25	Ceratomyxa	
	Aomori	5	I	<b>2</b> 0	Ceratomyxa Myxidium	
M. stelleri	Miyagi	5	4	80	Ceratomy xa Myxidium	
	Yamagata Fukui	3 2	0 2	0 100	Ceratomyxa	
M. kitaharae	{Yamagata Niigata	2 4	O I	0 25	Ceratomyxa	
Rhinoplagusia japonica	Yamagata	2	I	50	Ceratomyxa	

Now it is noticeable that these hosts are affected by the parasites in quite different degrees, according to their abode, whether it is on the east or west side of Honshu. This fact is summarized in the annexed table.

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C' 1-	T	Number	of species	s of host	Number of individuals			
Side	Locality	examined	amined infected P		examined	infected	Percentage infected	
Fact	( <sup>Aomori</sup>	5	5	100	23	5	22	
East	(Miyagi	3	I	33	9	4	44	
	(Yamagata	8	I	13	19	I	5	
Mont	Niigata	9	5	56	24	7	3	
west	(Fukui	9	7	78.	18	13	72	

TABLE XI. Infection rate of parasites on east and west coasts of Honshu.

#### CERATOMYXA

# Ceratomyxa japonica nov. sp. Pl. IV. Figs. 51-57.

*Myxosporidium.* It is irregular in outline and produces no obvious process. The ectoplasm is hardly discernible, and the endoplasm is densely loaded with fine granules. When the polar capsule of the spore becomes distinct, the myxosporidium is about 13  $\mu$  in diameter (*Fig. 51*). Usually it is two-spored, and often the one-spored form is also perceived (*Fig. 52*).

Spore. In lateral view this is nearly straight (Fig. 53), or slightly arched (Figs. 55 and 56). As regards its contour there are several different forms, but they are generally represented by two types. In one of them the shell in lateral view tapers gradually but slightly towards the extremities, which thereby ends somewhat acutely (Fig. 53). In the other it more or less elongates with its extremities rounded (Fig. 56). The latter form is generally met with in *Microstomus stelleri* from Fukui. The shell is in every case thin, and the suture line is distinct but not prominent. The valves are inequal, one of them being 17 to 24  $\mu$  and the other 21 to 27  $\mu$  in thickness. Often they are almost asymmetrical, the anterior margin of a valve being more or less depressed (Fig. 55). The size of the spore is 11 to 13  $\mu$  long and broad, and 37 to 49  $\mu$  thick. The polar capsules vary from ovate to long ovate in shape and are in the former case 5  $\mu$  in long

diameter. They converge together so that their exit lies very close to the suture line. The filaments are expelled by nitric acid to almost 40  $\mu$  in length. The sporoplasm is usually gathered in a small space in the mid-anterior portion of the shell. In some forms there is frequently found many small granules loosely and sparingly scattered in the shell (*Figs. 56* and 57). They are well stained by thionine. Two small nuclei in the sporoplasm are found to lie in the mid-central portion of the shell in close proximity to the suture line.

Habitat. This is most generally found in Limanda yokohamae and Microstomus stelleri from Fukui, and Limanda angustirostris, Hippoglossoides hamiltoni, Microstomus higeguro and Protopsetta herzensteini from Niigata. Xystrias grigorjewi from Aomori Bay is also noticed to harbour this same parasite.

This species differs in every respect from all others already recorded, and also from those I have described in the first part of the present investigation. Hence, it is named as *C. japonica* being most extensively distributed in Japan Sea.

## Ceratomyxa toitae nov. sp.

#### Pl. IV. Figs. 58-60.

Myxosporidium. This is quite unknown, the spore being rather scarce.

Spore. It is nearly straight in lateral view with subequal values of 19 to 21  $\mu$  and 21 to 24  $\mu$  in thickness (Fig. 58). In general configuration it somewhat resembles the previous species from which, however, it differs by having more rounded extremities. In anterior view, on the other hand, it arches notably, and the values are asymmetrically disposed, one of them turning more acutely towards the suture line than the other (Fig. 59). Thus they meet together at an angle of about 100 degree. The spore is 13  $\mu$  in length and breadth and 30 to 42  $\mu$  in thickness. Two small polar capsules are 2  $\mu$  in diameter and strongly converge in the vicinity of the anterior end of the suture line. The polar filament was difficult to extract, but is about 20  $\mu$  in length.

Habitat. This is sparingly found in Verasper variegatus from Fukui.

The spore of the present form shows a certain similarity to the

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normal spore of *C. japonica.* Yet this is true only in its lateral view, and when seen from the other side much striking difference is clearly brought out in its general outline. Further, it has much smaller polar capsules. As the consequence we could not in any way amalgmamate these two forms into one and the same species. Though rather rare in occurrence, yet this is a distinct type and is called after Mr. M. Toita, Principal of the School of Fisheries at Obama, Fukui Prefecture, who kindly furnished me the specimens for the present investigation.

# Ceratomyxa microstomi nov. sp. Pl. IV. Figs. 61-62.

Myxosporidium. This is round to oval in shape, and its ectoplasm is more or less distinct only in some advanced stage when a broad process is extruded (Fig.  $\delta r$ ). Then it manifests an anlage of the spore, and is about 20  $\mu$  in long diameter. The endoplasm is finely granulated and translucent. Usually it embraces two spores.

Spore. It slightly arches both in anterior and lateral views (Fig. 62). The shell is thin with a less marked suture line. The valves are symmetrical, but subequal being 39 to 43  $\mu$  and 40 to 46  $\mu$ . They are equally attenuated towards the extremities and their midanterior margin is more or less flattened. The size of the spore is 11 to 13  $\mu$  in length, and 51 to 84  $\mu$  in thickness. The spherical polar capsules are situated rather near together and are 3  $\mu$  in size. The filaments are very difficult to discharge, but seem to be at least 25  $\mu$  in length. The sporoplasm is generally aggregated at the center of the spore, and provides some coarse spherules of oily nature.

*Habitat.* This infects *Microstomus stelleri* from Miyagi, but is remarkably less in number than other *Ceratomyxa* which attack this same host. Nevertheless, it is often found associated with a *Myxidium* which I will describe later on.

The characteristics of the present spore lie in the general outline and the large polar capsule. They are not at all recognizable in any known species and deserve to be called as new form C. microstomi after the generic name of the host.

Ceratomyxa microcapsularis nov. sp.

Pl. IV. Figs. 63-65.

Myxosporidium. Its young form is unknown, and when two spores

are distinctly formed it takes a globular shape with some irregular outline. However, any process could not be detected in this and still later stages. The ectoplasm is generally more or less distinct, and the endoplasm is homogenous and translucent. In an advanced stage the myxosporidium is 27  $\mu$  in diameter (*Fig. 63*).

Spore. Its general contour presents a slight curvature in anterior view (Fig. 65), whereas in lateral view it is nearly straight (Fig. 64). The shell is very thin and the suture line is rather faintly represented. The valves are attenuated with acute extremities, particularly so when seen laterally. They are subequal being 32 to 37  $\mu$  and 34 to 40  $\mu$  in thickness. They are also asymmetrical, and in lateral view the anterior margin of larger valve is usually depressed in its outer one third portion. The spore is 11 to 13  $\mu$  long, 13 to 14  $\mu$  broad, and 53 to 72  $\mu$  thick. The polar capsules are almost perfectly spherical and 2  $\mu$  in diameter. They are set apart and make their exit by means of straight narrow canals. The filaments are loosely discharged to 14  $\mu$  in length. The sporoplasm is collected in a small mass in the center of the shell. Its nuclei are small and round, situated moderately apart.

Habitat. Microstomus stelleri, but totally different individuals from that which lodges C. microstomi. Of specimens examined one comprised simultaneously a species of Myxidium.

Although this parasite invades the same host as *C. microstomi*, yet its distinctive feature is easily discernible. Amongst others the polar capsule of the spore particularly attracts our attention by its small size and spherical form. These characters are unique in the spores of *Ceratomyxa*, so that this species is deemed worthy to be designated as *C. microcapsularis*.

# Ceratomyxa fukuiensis nov. sp.

## Pl. IV. Figs. 66-68.

Myxosporidium. Its young form is rarely found. More advanced and sporulated ones are of general occurrence. Then it is usually rather elliptical in form with a slight wavy contour. The ectoplasm is quite indistinct, and the endoplasm is highly homogenous and translucent. The myxosporidium contains generally two spores, and when about to liberate them it reaches 40 by 30  $\mu$  in size (Fig. 66).

Spore. It is large and arches but slightly in any view (Figs. 67 and 68). The shell is very thin with a distinct suture line and with more or less flattened anterior margin. The valves are almost cylindrical in form with obtusely rounded extremities but subequal, being 32 to 40  $\mu$  and 35 to 40  $\mu$  in thickness. The size of the spore is 11 to 13  $\mu$  long and broad, and 65 to 75  $\mu$  thick. The polar capsules are large ovate, not lying in close proximity. Moreover they do not converge and consequently their exits are separated from each other. Their size is 5  $\mu$  by 3  $\mu$ . The filaments are easily extruded by potash solution to almost 50  $\mu$  in length. The sporoplasm sometimes exists only in the central portion, and sometimes extends nearly to the end of the shell. Its nuclei are small and are situated in the mid-central portion of the shell.

Habitat. Protopsetta herzensteini from Fukui at the depth of 100 to 150 fathoms.

The spore of this parasite is likely to be confounded with that of *C. microstomi*. Nevertheless, this latter has a larger and more attenuated shell with a small polar capsule. Such character suffices to distinguish these two forms, and this species is nominated after the locality whence the host was procured.

# Ceratomyxa limandae nov. sp. Pl. IV. Figs. 69-74.

Myxosporidium. The youngest stage observed is one having an anlage of the spore. Generally it is spherical in contour and is 13  $\mu$  in size (Fig. 69). Its ectoplasm is faintly differentiated and presents quite irregular outline, while the endoplasm is densely granulated. The characteristic of this form is that even in immature stage the nuclei in the sporoplasm, as well as in the polar capsule, are clearly brought out by staining with Giemsa's solution (Fig. 70).

Spore. Its thin shell arches and is more or less elevated at the mid-anterior margin of the suture line (Figs. 71 and 73). On the other hand it is almost perfertly straight in its anterior view (Figs. 72 and 74). The values are asymmetrical and subequal. One value is a little shorter and has a more rounded extremity than the other. It is 19 to 23  $\mu$  in thickness in contradistinction to the complementary one of 21 to 25  $\mu$ . Such difference of values is also seen tolerably well even in the lateral view. The size of the spore is 11 to 13  $\mu$  in

length, 9 to 10  $\mu$  in breadth, and 43 to 45  $\mu$  in thickness. The polar capsules are large, though generally asymmetrical in a minor degree. The smaller valve carries, as a rule, also a smaller capsule. Nevertheless, they are equally pyriform and converge together with their exit in close proximity to the suture line. Their larger one is 7  $\mu$  by 5  $\mu$  in size, thus occupying one-third length of the spore. The filaments are discharged by nitric acid to 60  $\mu$  in full extent. The nuclei of the sporoplasm are tolerably large and are 2  $\mu$  in size. They are set apart in the posterior portion of the shell. The nuclei of the polar capsule on the other hand are indiscernible, although in the young stage of the spore they are as well manifested as those of the sporoplasm.

Habitat. This is most profusely found in Limanda angustirostris from Aomori Bay, at the depth of about 30 fathoms. The other flat fishes from the same site such as Protopsetta herzensteini, Microstomus stelleri, Xystrias grigorjewi, and Lepidopsetta mochigarei seem in all probability to harbour this same parasite. Unfortunately almost all of them were destitute of its matured spore, so that I could not ascertain the true nature of the parasite.

The spore of this species presents many prominent features, of which the large polar capsule and also the large nucleus in the sporoplasm are highly attractive. Consequently, even at a glance, this is easily distinguished from all other forms.

# Ceratomyxa majimae nov. sp. Pl. IV. Figs. 75–79.

Myxosporidium. In a stage just before leaving the shell it is perfectly spherical and is  $12 \ \mu$  in diameter (Fig. 75). At the beginning of sporulation there arises a thick and strong pseudopodium (Fig. 76). Here two layers of the plasm are distinct only at the tip of this process. The endoplasm is seen as a fine and homogenous granule. In the later stage when two spores are already distinct the myxosporidium is about  $42 \ \mu$  in size.

Spore. In extreme cases it presents dimorphic characteristics. In the normal form the shell is, in lateral view, enormously curved, so that two valves meet together at an angle of about 80 degrees (*Fig.* 78). On the contrary, the other form has the shell moderately arched, but much more elevated, and hence it manifests quite a robust appear-

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ance (Fig. 79). The shell is not thin in either case but has a distinct suture line. In normal form the valves are elongated, and are subequal being 37  $\mu$  and 36  $\mu$  in thickness. They are at the same time asymmetrical, and one of them turns more strongly toward the suture line than the other at its first one-third portion. The spore is 16 to 17  $\mu$  long and 46 to 50  $\mu$  thick. The polar capsules are moderately large and perfectly spherical being 5  $\mu$  in diameter. They are connected with the outlet by a narrow canal which either converges or runs straight upward. The filaments are easily expelled by acetic glycerine to 84  $\mu$  in length. The sporoplasm generally occupies the center of the shell, which often at the same time comprises fine and coarse granules easily stained with thionine. The nuclei are small in size, and are usually kept at a wide distance.

Habitat. Of several species of flat fishes procured from Yamagata, Rhinoplagusia japonica alone was found to infect this parasite.

The present species is highly remarkable by having large and spherical polar capsules in the spore. Further, its general contour will no doubt attract our attention, so as to realize it is a new species. Hence, I propose to designate it as *C. majimae* as a token of my best regards to my colleague Mr. T. Majima who has special interest in the study of Japanese flat fishes, and who has kindly helped me in the collection of the specimens for the present investigation.

# Ceratomyxa robusta Fujita

Habitat. Microstomus kitaharae from Niigata.

The description of this species is given in the first part of the present work.

#### MYXIDIUM.

#### Myxidium microstomi nov. sp.

#### Pl. II. Figs. 80-85.

Myxosporidium. The young stage accessible presents already a sporulated form. It is more or less spherical in outline without emitting any process. Here the ectoplasm is hardly distinguishable as such, and the endoplasm is rather opaque being loaded with fine granules. The myxosporidium contains two spores and is about 13  $\mu$  in size (Fig. 80).

Spore. It is somewhat oblong in lateral view with sharpened and

abruptly crooked extremities (Fig.  $\delta I$ ), while in front view it is elliptical with a broad end. The shell is thick and the suture line is more or less distinct. The valves are equal and symmetrical. They are inflated at the portion just posterior to the polar capsule, so that in anterior view their optical cross section presents a spherical form (Fig.  $\delta J$ ). On the valve there are some five or six faint striations which run parallel with the long axis of the spore. The spore has a length of 14 to 19  $\mu$ , and breadth and thickness of 8–9  $\mu$ . The polar capsules are ovopyriform to pyriform, and are 4  $\mu$  by 3  $\mu$  in size. The filaments are 40 to 60  $\mu$  in length. The full matured spore has the sporoplasm much concentrated in the shell. In such a stage only one nucleus is recognizable in its center.

Habitat. In the majority of cases the present species was detected to associate with either *Ceretomyxa microstomi* or *C. microcapsularis* in *Microstomus stelleri* from Miyagi.

The spores of Myxidium that have the striation on their shells and therefore a certain resemblance to the present form are comparatively few in number. Among them *M. macroscapsulare* shows some similarity to the present one in general outline. Nevertheless, as its name implies the polar capsules are larger and moreover the striations run oblique instead of straight as in this species.

#### SUMMARY.

I. The flat fish in Honshu were collected from its northern section in order to study their myxosporidian parasites with special reference to the same disease in Hokkaido. The localities selected for such collection were Aomori and Miyagi on the east, and Yama-gata, Niigata, and Fukui on the west coast of Honshu. The specimens from these localities were found to harbour, on the whole, eight species of *Ceratomyxa*, and one species of *Myxidium*.

2. These parasites occur equally on these two coasts with high frequency and comparatively low intensity of the infection. Thus, on the east coast, the frequency rate was 75%, while that of the intensity was as low as 28%. So, too, on the west coast these rates were found to be 50% and 34% respectively.

3. Every locality from where the specimens were procured seems to have a certain special form of the parasites. This is most noticeable in Aomori, where all the flat fishes were recognized to be affected with one and the same *Myxosporidia*, which was totally unknown

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in other localities, even on the same east coast.

4. Further as to the rate of infection of the parasites there is some notable variations among the localities on the same coast and not far distant apart. Such example was furnished by the fishes from Yamagata and Fukui. In the former the fishes were perceived almost entirely immune. While in the latter, they were suffered so greatly as to make 100% in intensity. Fukui lies farther south than Yamagata, but the specimens at hand being not numerous enough I must hesitate at the present juncture to make an assertion that the myxosporidian parasites in Honshu also tend to increase towards the south just as in the case of Hokkaido.

5. The myxosporidian parasites in Honshu so far studied are almost totally different in nature from those which infect the same or identical hosts in Hokkaido. Thus, their existence being locally confined in these two districts free communication of the host seems to be barred by Tsugaru Strait.

# III. ON MYXOSPORIDIA IN SALMONID FISHES OF HOKKAIDO.

The salmonid fishes in this investigation were collected from lakes and rivers on the east and west sides of Hokkaido. They comprise the following species: viz. Oncorhynchus nerka (Walbaum); O. keta (Walbaum); O. gorbuscha (Walbaum); O. masou Brevoort; Salvelinus kundscha (Pallas); Plecoglossus altivelis Temminck & Schlegel; Osmerus dentex Steindachner; Os. lanceolatus Majima, and Hypomesus japonicus (Brevoort).

For this purpose the gall bladder only mostly preserved in formaline, were thoroughly studied. It was soon found that among the specimens only three genera, e. i. Oncorhynchus, Salvelinus, and Osmerus, were infected with the Myxosporidia in various degrees of intensity. The parasties I have thus detected were six species, represented by three genera, which are one Ceratomyxa, one Myxidium and four Chloromyxum. Among them, the species of Myxidium and Chloromyxum are quite new forms, and the Ceratomyxa is one which I have already found in certain flat fish in Hokkaido.

The rate of infection of these parasites together with their distribution in Hokkaido will be studied in the following table:

Host	Locality	Number	of fish	Percentage of fish	Parasite	
		examined	infected	infected		
Oncorhynchus nerka	Kuttaraushi Lake	ю	o	о	<del></del>	
0. nerka	Shikotsu Lake	10	о	о		
0. nerka	Doya Lake	5	о	о		
0. keta	Ishikari River	50	o	o	_	
0. keta	Chitosé River	20	8	40	Chloromyxum	
0. keta	Nishibetsu Rlver	16	6	38	Chloromyxum	
O. gorbuscha	Nishibetsu River	ю	5	50	Chloromyxum	
0. masou	Ishikari River	50	6	12	Myxidium	
0. masou	Mukawa River	2	о	о		
0. masou	Nishibetsu River	10	6	6 <b>0</b>	Chloromyxum	
0. masou	Nishibetsu River	ю	I	10	Myxid <b>iu</b> m	
Salvelinus kundscha	Shikotsu Lake	8	2	25	Chloromyxum	
Plecoglossus altivelis	Kuromatsunai	20	о	о		
Osmerus dentex	Ishikari River	IO	о	O	-	
Os. lanceolatus	Mukawa River	<sup>.</sup> 20	4	20	Ceratomyxa	
Hypomesus japonicus	Ishikari River	20	· 0	0		

# TABLE XII. Number of fishes infected with parasites in different localities of Hokkaido.

#### CERATOMYXA

#### Ceratomyxa furcata Fujita.

# Pl. II. Figs. 84-86.

*Myxosporidium*. Generally it is irregularly rounded, and no process could be seen through its developmental stage. The endoplasm containing dense granules becomes translucent only in a later stage, and then the spore formation is recognizable. When the spore attains full maturity the size of this form is about 30  $\mu$  (*Fig. 84*).

Spore. It is laterally attenuated and usually strongly arched posteriorly so as to present a furciform. The values are subequal and thin, and are either straight (Fig. 85) or somewhat inflected anteriorly.

In every case the suture line is distinct, The larger value is 50 to 60  $\mu$  and the smaller one 45 to 55  $\mu$  in thickness, of which about 1/3 to 1/2 is occupied by the sporoplasm. The length of the spore is 11 to 13  $\mu$ . The polar capsules are spherical, and are 3  $\mu$  in diameter with the filaments about 60  $\mu$  in length. The nuclei in the sporoplasm are round, and set wide apart in its posterior portion.

Habitat. In Osmerus lancelolatus from Mukawa River on the east side of Hokkaido,

This is no doubt the same species that infects *Limanda aspera* from Oshoro, as already shown in the first part of this work, although the spores from these two hosts often manifest some difference in their dimensions.

#### MYXIDIUM

# Myxidium oncorhynchi nov. sp. Pl. II. Figs. 87-89.

Myxosporidium. This is most sparingly found in the gall bladder. At the stage of sporulation it generally takes a globular form, and is about 27  $\mu$  in diameter. Frequently, however, a broad lobate process is perceptibly protruded, which chiefly consists of thin and transparent ectoplasm. The endoplasm is very finely granulated, mingled with coarse, translucent, and colorless spherules. Usually it comprises two spores (Fig. 87).

Spore. In front view (Fig. 89) it is elliptical in form, but laterally (Fig. 88) it is spindle-shaped, neither sharply pointed nor curved at extremities. The shell is formed of rather thick valves, which are equilateral and appose each other with more or less elevated suture This suture is so highly conspicuous as to form a prominent margin. ridge. This ridge corresponds to the median long axis of the shell, not inclining in any way as in many other spores of Myxidium. On the valves there are four or five faint striations, which run parallel to each other, and also to the suture line. The size of the spore is II to 12  $\mu$  long, 5 to 8  $\mu$  broad, and 3 to 5  $\mu$  thick. The polar capsules are oval to globular in form, and are rather small being 3 to 4  $\mu$  in long diameter. Long and slender polar filaments are extruded with ease to the length of about 45  $\mu$ . The sporoplasm contains two rather oblong nuclei, which lie side by side in its middle portion.

They are rather large and noticeable, being easily stained with carmine.

Habitat. Oncorhynchus masou from Ishikari River on the west, and from Nishibetsu River on the east, sides of Hokkaido.

The present form is a species of *Myxidium*, which has a straight outline of the spore. In this genus we have at present only two species, viz., *M. giardi* and *M. pfeifferi*, which present some similarity to the present form so far as the general outline and size of their spores are concerned. However, *M. giardi* has the shell more inflated at the middle and more abruptly constricted at the extremities than that of the spore under consideration. Further, it has distinct and more numerous striations on its valves. On the other hand *M. pfeifferi* distinguishes itself by its more attenuated extremities and the larger size of the polar capsules. Considering all these particulars the present species is quite a new form, and is designated after its host.

## CHLOROMYXUM.

# Chloromyxum salvelini nov. sp. Pl. II. Figs. 90–92.

Myxosporidium. Its youngest form is generally not profusely recognizable. In a somewhat advanced stage it takes a globular form, and is 28  $\mu$  in long diameter. Sometimes there is seen a thick and globose pseudopodium stretched out like Myxidium oncorhynchi (Fig. 90). Again two layers of plasms show an identical structure with those of the latter species. The endoplasm generally contains two spores, but rarely the one-spored form is also perceptible.

Spore. It is almost perfectly spherical, and 10 to 13  $\mu$  in diameter (Fig. g1). The shell is formed of equilateral values of moderate thickness, which, however, become thin only at their suture margin. The surface of the values are provided with very remarkable sculpture in the form of ridges. These ridges are numerous, narrow in breadth, and lower in height than the suture margin. They are rather irregularly disposed, but in the majority of cases, however, some middle ones, circumscribing the shell, run parallel to each other as well as to the suture line (Fig. 92). All others arranged in outside of them stand in a more or less radiate direction. At all events the extremities of the ridges, which can be seen frontally as projections streching

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out of the shell margin, are computed to be about 35 in number. The polar capsules are four in number, and are all equally pyriform. Three of them, which lie on the same level, are large being 5  $\mu$  in long diameter. The other one, that stands more posteriorly, is small and less significant. The polar filaments are comparatively short being 20  $\mu$  in length. The nuclei in the sporoplasm is small and lie apart in its central portion.

Habitat. In Salvelinus kundscha from Shikotsu Lake. In this lake, situated about 300 m above sea level and totally landlocked, we have still another species of salmon, viz., Oncorhynchus nerka. This latter form was many years ago transplanted from Akan Lake. Since then its artificial propagation has been annually carried out with good success, and now it has become quite indigenous. However, it is note-worthy that this Oncorhyuchus though living together with Salvelinus is, so far as I am aware, quite free from the parasite under consideration.

The present species belongs to *Chloromyxum*, but differs considerably from *C. truttae*, which is the only known form of this genus parasitic to European trout. The sculpture on the valve of the spore of that parasite will show by mere inspection some notable differences from that of the present form. There are still two other species, which likewise have similar sculpture on their spores, e. i. *C. cristatum* and *C. koi*. Nevertheless, the present one differs considerably from them in form, number, and thickness of the ridge on the valve.

# Chloromyxum chitosense nov. sp.

# Pl. II. Figs. 93–96.

Myxosporiduum. This is irregular in outline having no definite form, but usually it is spherical, and thrusts forth a thin and short pseudopodium (Fig. 93). The ectoplasm is distinct only in this process, but else it is confluent with the endoplasm. This later is more or less opaque, comprising granules of various size. The spores contained in it vary from one to seven in number. In the stage of complete sporulation this form is 30  $\mu$  or more in long diameter.

Spore. In anterior view (Fig. 96) it manifests a nearly spherical form, while laterally (Fig. 94) its outline is very slightly angurated at the anterior and two lateral edges. The valves are equilateral, thin, and perfectly smooth. Its suture margin is not prominent. The size

of the spore is 8  $\mu$  in length and breadth, and 9  $\mu$  in thickness. Of four polar capsules three are equally large and ovopyriform, and 4  $\mu$ in long diameter. The smaller one, on the other hand, is hardly discernible being laid more posteriorly. The polar filaments are easily thrown out to the length of about 15  $\mu$ . The sporoplasm is provided with two rather oblong nuclei, which lie near each other at the median posterior portion of the spore (*Fig. 95*).

Habitat. In Oncorhychus keta from Chitosé River on the west side of Hokkaido. During many years, numbers of this fish from Ishikari River were subjected to scrutinous examination for myxosporidian parasite, but the effect was then everytime negative. A few years ago I was lucky enough to have an occasion to collect the same fish from Chitosé River, a tributary of Ishikari River. Then the present form of *Chloromyxum* attracted my attention, and, subsequently, the infection of the *Myxosporidia* to the salmon on the west side of Hokkaido was fully ascertained.

Of many species of *Chloromyxum C. magnum* manifests a certain conformity with the present one, having similar size and smooth valves of the spore. Nevertheless, its general configuration shows such marked difference to that of the spore now in question, that they can not be treated as one and the same species.

# Chloromyxum giganteum nov. sp. Pl. II. Figs. 97-101.

Myxosporidium. At the commencement of sporulation this form shows an irregular outline and rarely pushes out the pseudopodium (Fig. 97). It is completely burdened with very fine and deep greenish granules mixed with some coarse ones of the same color, which totally obscure the presence of the spores. Hence, there is hardly any distinction between its ecto- and endoplasms. Then its size is about  $30 \ \mu$  in long diameter. In a still further advanced stage, when it reaches to  $40 \ \mu$ , the endoplasm becomes translucent, and although it is still loaded with greenish granules, four or five spores contained therein are now clearly brougnt out into view (Fig. 98).

Spore. It is perfectly spherical in form when seen anteriorly (*Fig. 101*). However it is slightly elongated antero- posteriorly in other views. This is more particularly pronounced in its lateral aspect, where it takes somewhat an oval form (*Fig. 100*). The shell is

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formed of rather thin valves, and its suture margin is not so highly illustrated. On its surface there is a sculpture something like that of C. salvelini. Here its ridges have almost the same thickness as the valve. They are arranged very irregularly, but some of them, that lie near the center of the spore, run generally parallel to the suture margin. Their projections seen out of the shell margin are 30 to 40 in number. The size of the spore is 14 to 16  $\mu$  in length, and 13 to 14  $\mu$  in breadth and thickness. The polar capsules are elongated pyriform and convergent in lateral view, and are all identical in size being 4  $\mu$  by 3  $\mu$ . However, as in other forms one of them is situated slightly more posteriorly than the complementary ones. The filaments are about 30  $\mu$  in length. The sporoplasm contains two small nuclei, which stand rather near together and occupy almost the central position of the spore (Fig. 99).

Habitat. In Oncorhynchus gorbuscha from Nishibetsu River on the east side of Hokkaido. Its gall bladder was greatly distented with a gelatinous mass consisting mainly of tremendous numbers of this parasite in different stages of growth. Sometimes, however, there was found in the same host still another small but conspicuous form of *Chloromyxum*, which will be described later on. Such miserable state of the disease was also recognizable in other salmons as *O. keta* and *O. masou* that live in this same river.

Of known species of *Chloromyxum* the spore of this parasite is the largest one, and at the same time presents strikingly different features. From *C. salvelini* it differs not only in the size of the spore, but also in its general outline. Further, these two forms manifest a certain dissimilarity in the disposition and numbers of the ridges on the valve.

# Chloromyxum quadriforme nov. sp. Pl. II. Figs. 102-106.

Myxosporidium. This shows an appearance similar to that of C. giganteum so far as its contour and granular content are concerned (Fig. 102). However, the endoplasm is more obvious in this species than the latter one, especially so in an advanced stage, when a short and blunt pseudopodium is seen to be protruded. In such a stage the myxosporidium is about 24  $\mu$  in long diameter, and generally contains 3 to 12 spores (Fig. 103). Sometimes there is seen only one-spored form, which is 14  $\mu$  in size.

Spore. In lateral view (Fig. 105) it is rather quadriform in outline, being formed of two somewhat triangular valves. Its anterior and posterior extremities are, however, more broadly rounded, while in the other view it presents a spherical aspect (Figs. 104 and 106). The valves are thick, particularly so at their lateral angulated edges. The suture margin has almost the same thickness as the valves, and is The surface of the shell is furnished with rather highly conspicuous. thin and insignificant ridges. They are few in number, and, further, there is no pattern in their arrangement. Their projections seen on the shell margin are usually less than 30 in number. The size of the spore is 10  $\mu$  in length and breadth, and 12  $\mu$  in thickness at the widest angular edge. The polar capsules are pyriform with rather long necks. One that lies more posteriorly than the others is imperceptibly smaller in size. The larger capsules are 5  $\mu$  in long diameter, so that their posterior extremities reach as far as to the center of the spore. The polar filaments are easily expelled to about 25  $\mu$ in length. The sporoplasm embraces two nuclei just as in other cases, but here they are located in its mid-posterior portion.

Habitat. At first this was found in Oncorhynchus keta from Nishibetsu River. Later, it was also detected in O. gorbuscha and O. masou from the same river. In some O. masou the present form was also found simultaneously with Myxidium oncorhynchi.

The characteristic which distinguishes the present form from all other allied ones chiefly lies in the configuration of its spore. Such shape of the spore has never yet been mentioned in *Chloromyxum*. Hence, this alone suffices to make us consider the present species as a new type of this genus.

# IV. ON MYXOSPORIDIA IN SOME DEEP SEA FISHES OF HOKKAIDO.

The deep sea fishes subjected to the studies of the myxosporidian parasite have hithertofore been confined to those of the Gadoid family. Nevertheless, they were so extensively and thoroughly investigated that in Europe seven genera and fourteen species were recognized to be infected with eight genera and seventeen species of *Myxosporidia*. These parasites invade different organs of the host, but in the majority of cases they seem to prefer the gall bladder as their favorable site. Such state of affairs will be easily seen from the following table.

Parasite	Gall bladder	Liver	Kidney	Urinary bladder	Cartilage	Gill	Total
Leptotheca	2	0	o	o	o	0	2
Ceratomyxa	2	о	o	о	o	o	2
Sphaerospora	o	о	I	о	o	о	I
Sphaeromyxa	2	I	Ó,	о	I	o	4
Myxidium	I	о	о	I	о	о	2
Zschokkella	0	o	0	I	o	0	I
Chloromyxum	I	о	<b>o</b>	I	o	0	2
Myxobolus	o	o	o	о	I	2	3
Total	8	I	I	3	2	2	17

Table	XIII.	Specie	s c	of M	[yxospo	rid	<i>ia</i> and	their	infecting
		organs	in	$\operatorname{cod}$	fishes	of	Europ	e.	

In Japan we have far less species of cod fish than in Europe, and in Hokkaido only its three species from three quite different genera; viz., Gadus macrocephalus Tilesius, Theragra chalcogramma (Pallas), and *Eleginus navaga* (Kölreuter) are highly esteemed as valuable commercial fishes. Amongst others Gadus has widest distribution being found almost along the whole coast of Hokkaido, while Theragra is generally procured in its south western coast. On the other hand *Eleginus* is the sole production of the northern coast of the island. In Hokkaido we have still other sorts of the deep fish. The most easily accessible ones among them are the Sebastoid family, of which at present four species from three different genera, i. e. Sebastobolus macrochir (Günther), Sebastodes flammeus Jordan & Starks, S. vulpes (Steindachner & Döderlein), aud Sebastichthys trivittatus (Higendorf) are enumereted. All these Gadoid and Sebastoid fishes were treated mostly in fresh state and after careful research their gall bladders were ultimately found to harbour six species of the myxosporidian parasite. They belong either Leptotheca or Myxidium.

Host	Locality	Numb <b>er</b>	of fishes	Percentage of fish	Parasite	
	2000,009	examined	infected	infected		
Gadus macrocephalus	Ochotsk Sea	5	I	20	Myxidium	
G. macrocephalus	Riishiri	50	о	о		
G. macrocephalus	Hanasaki	II	3	37	Myxidium	
Theragra chalcogramma	Iwanai	40	10	- 25	Myxidium	
Eleginus navaga	Nemuro	20	о	o		
Sebastabolus macrochir	Nemuro	3	о	0		
S. macrochir	Muroran	Io	. 2	20	Leptotheca	
Sebastodes flammeus	Nemuro	3	о	о	·	
S. flammeus	Muroran	Io	2	20	Myxidium	
S. vulpes	Yoichi	5 '	5	100	Leptotheca	
Sebastichthys trivittatus	Yoichi	5	2	40	Leptotheca	

TABLE XIV. Number of fishes infected with parasites in different localities of Hokkaido.

#### LEPTOTHECA.

# Leptotheca constricta nov. sp. Pl. II. Figs. 107-111.

Myxosporidium. It is almost perfectly spherical in contour, and this is so, even in an advanced stage when it contains an anlage of the spore (Fig. 107). However, then it often manifests a somewhat wavy outline and is about 16  $\mu$  in size. The ectoplasm is thin and obscure without emitting any obvious process, while the endoplasm is densely granulated, colorless and opaque. Usually this contains only one spore.

Spore. In its lateral view this shows transversely reniform with obtuse extremities (*Fig. 109*). Anteriorly, however, it is perfectly straight in outline (*Fig. 110*). The shell is thin and inequivalved, with a distinct suture line. Generally, the valves are slightly narrowed at the suture junction as if constricted there. Such characteristic

feature of the spore is recognizable in the lateral as well as in the anterior views (*Fig. 110*). The length and breadth of the spore is 10  $\mu$ , and its thickness is 19  $\mu$ . The polar capsules are rather large and are spherical to ovopyriform, more generally diverging in some distance from the suture line as is shown in *Fig. 111*. The sporoplasm completely fills the extra- capsular portion of the shell, and comprises two small nuclei.

Habitat. In the gall bladder of Sebastobolus macrochir off the coast of Muroran at a depth of about 100 fathoms.

The general configuration of this spore together with its large polar capsule is highly striking as the most characteristic feature of the species, which notably deserves to be treated as a new form.

# Leptotheca yoichiensis nov. sp. Pl. II. Figs. 112—117.

Myxosporidium. In the youngest stage this form is almost spherical, with a mass of dense granules therein and is 5  $\mu$  in size (Fig. 112). When it gets some growth of 12  $\mu$  a trace of the spore is more or less discernible (Fig. 113). When it has reached 17  $\mu$  a blunt process is seen to be protruded (Fig. 114). In an advanced stage with well matured spores the granular content of the endoplasm remains obscure so as to prevent a clear insight of the spore.

Spore. The spore is elongated-oblong and never curves in any view (Figs. 116 and 117). The shell is thin, with slightly asymmetrical valves. One of them is 12  $\mu$ , while the other is 9  $\mu$  in thickness. The extremity of this shorter half is more rounded. The size of the spore is 9  $\mu$  in length and 21  $\mu$  in thickness. The polar capsules are large and globular being 3  $\mu$  in size. The polar filaments are about 13  $\mu$  long and are loosely discharged. The sporoplasm almost fully occupies the shell, and contains two large nuclei in its mid-posterior portion.

Habitat. In Sebastichthys trivittatus, and Sebastodes vulpes from the coast near Yoichi.

This species differs from all known forms of *Leptotheca* in the general feature of the spore. In size alone it shows a certain similarity to *L. inermis*, but the later has a more robust shell, which distinctly curves backward. This is easily recognizable in a glance, and we could not consider these two forms as one and the same species.

#### MYXIDIUM.

# Myxidium ochotense nov. sp. Pl. II. Figs. 118—120.

*Myxosporidium*. This is totally unknown, the spore maturing almost at the same time.

Spore. In the front view (Fig. 119) it is spindle in form with straight and rather acuminated extremities. Even in the lateral view these extremities never crook in any way, although the axes of the polar capsules do not lie in the same straight line (Fig. 118). The shell is thin, smooth, and equivalved with a distinct suture line. Its size is 12 to 14  $\mu$  in length, 7  $\mu$  in breadth, and 6  $\mu$  in thickness. The polar capsules are conical with a length of about one fourth that of the spore. The polar filaments are extruded 40  $\mu$  in full length. The nuclei in the sporoplasm are usually two, and are rather large, situated in its middle portion (Fig. 120).

Habitat. At first this was detected in the gall bladder of Gadus macrocephalus hauled up from Ochotsk Sea, Lat.  $50^{\circ}$  54' N., Long. 156° E., at the depth of 25 fathoms. Many years later the same parasite was also found in this cod fish from Hanasaki near Nemuro, on the east coast of Hokkaido.

Of the straight forms of spores of *Myxidium*, that of *M. procerum* might be taken as one nearly allied to the present species, but it is much larger in size and its extremities are somewhat truncated instead of acuminated as in the spore under consideration. Such striking differences suffice for us to assume this parasite as a new species.

# Myxidium theragrae nov. sp. Pl. II. Figs. 121–124.

Myxosporidium. It is spherical to oblong in shape with an irregular outline rarely finely fringed (Fig. 121). Two layers of plasms are ill-defined, and the endoplasm is loaded with compact and translucent granules. Usually this comprises two spores and attains 19  $\mu$ in size.

Spore. Frontally it is biconcave-lenticular in outline, but is not inflated at the middle (*Fig. 123*). In the lateral view the extremities, though not much sharpened, curve in s- form (*Fig. 124*). The shell

is thin, smooth, and equivalved, with an indistinct suture line. It is 19  $\mu$  long, 8 to 10  $\mu$  broad, and 6  $\mu$  thick. The polar capsules are long oval and equally large, being 4  $\mu$  by 3  $\mu$  in size. The filaments are easily extruded to 29  $\mu$ . The nuclei are large and distinct in the center of the sporoplasm, and also in the posterior portion of the polar capsules.

Habitat. In Theragra chalcogramma at a depth of 200 fathoms off the coast of Iwanai.

The characteristic of this spore lies in its smooth but curved shell with a rather large polar capsule. This remarkable feature distinguishes the present species from all kindred forms at issue.

## Myxidium fusiforme nov. sp.

## Pl. II. Figs. 125-127.

Myxosporidium. This is quite rare in occurrence. In a sporulated stage it presents an irregular spherical contour with a diameter of 20  $\mu$  (Fig. 125). The ectoplasm is thin and inconspicuous without any obvious process. 'The endoplasm is packed with colorless, transparent, and fine granules. It generally contains one to two spores.

Spore. This is fusiform and perfectly straight in any view (Figs. 126 and 127). However, when seen frontally it is more attenuated in the extremities and inflated at the middle portion. The shell is formed of rather thin, smooth, and equilateral valves, which appose each other with a faint suture line. The size of the spore is  $30 \mu$  in length and  $9 \mu$  in thickness. The polar capsules are elongated pyriform in shape, and are  $9 \mu$  by  $3 \mu$ . Thus each of them is one third of the spore in length. Their filaments are about  $50 \mu$  in length, the proximal one third of which is thicker than the other. Two rather large nuclei in the sporeplasm lie closely together in its center.

Habitat. In Sebastodes flammeus from Muroran.

At first sight the spore of this parasite seems to have much resemblance to that of M. ochotense. This is so when the front view of the latter one is compared to the lateral view of the present form. However, if these two spores are brought into the same front or lateral view, we can easily see they do not have the same features in their general configuration. They are of totally different natures, and hence, this parasite is to be regarded as a new species.

# Myxidium tsudae nov. sp. Pl. II. Figs. 128-131.

*Myxosporidium*. It presents no definite outline but, as a rule, it is irregularly elliptical without emitting any process (*Fig. 128*). Its two layers of plasms are totally indiscernible the endoplasm having a very densely aggregated mass of fine granules. It contains one to many spores, and when they are all completed it attains  $25 \mu$  in size.

Spore. In the front view it is broadly spindle shaped with the extremities not attenuated but sharpened (Fig. 129). However, when seen laterally, it is more slender in outline, and the extremities are curved in s-form (Fig. 130). The shell is moderate in thickness with a very obscure suture line. There are four or five faint striations on its surface. The length of the spore is 16 to 19  $\mu$  and its breadth is 8 to 9  $\mu$ . The polar capsules are rather large pyriform, more or less laterally compressed. Their size is 4 by 3  $\mu$  with the filaments 28  $\mu$  in length. In the sporoplasm there is only one large nucleus situated in its central portion. The nuclei of the polar capsules are also large and distinct.

#### Habitat. In Sebastobolus macrochir from Muroran.

Almost all *Myxidium* spores which have striated values are, so far as my knowledge extends, straight in their outline. Perhaps the only exception will be found in *M. macrocapsulare*. The present species is also to be included in this exceptional case. Nevertheless, it is larger in size with a more slender outline in its lateral view, and with more acute extremities in front view, than in the case of the latter species. Hence it is a new form, and is designated after Mr. M. Tsuda of the Fisheries Experimental Station of Hokkaido as a token of my gratitude for his kind service in furnishing me the Sebastoid fishes in this work.

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# **Explanation of Plates.**

Figures are drawn with Abbé's camera, and unless mentioned are magnified × 600.

Plate I. Figures 1-7. Leptotheca inaequalis.

1. Myxosporidium of very young stage, fresh.

2. ditto, more advanced stage with pseudopodium, fresh.

3. ditto, with two matured spores, fresh.

4. Spore of normal form, lateral view, fresh.

5. ditto, slightly anterior view.

6. ditto, stained, lateral view.

7. ditto, an abnormal form, lateral view.

#### Figures 8-13. L. platichthytis.

8. Myxosporidium of very young stage with processes, fresh.

9. ditto, with elongated posterior process, fresh.

10. ditto, with one spore.

11. ditto, with three spores.

12. Spore in lateral view, stained.

13. ditto, anterior view.

#### Figures 14–17. L. limandae.

14. Myxosporidium with two spores, fresh.

15. ditto, with two triad spores.

16. Spore of normal form, anterior view.

17. ditto, lateral view.

#### Figures 18-21. Ceratomyxa tenuis.

18. Myxosporidium of very young stage, fresh.

19. ditto, with one spore.

20. Spore stained, lateral view.

21. ditto, anterior view.

#### Figures 22-24. C. robusta.

22. Myxosporidium of young stage with pseudopodium, fresh.

23. ditto, in a far advanced stage with two spores, fresh.

24. Spore stained, lateral view.

## Figures 25-34. C. protopsettae.

25. Myxosporidium of very young stage, fresh.

26. ditto, commencement of sporulation, fresh.

27. ditto, more advanced stage, with pseudopodium, fresh.

- 28. ditto, before discharging spores, fresh.
- 29. ditto, a more advanced stage with prolonged pseudopodium, fresh.
- 30. Spore of normal form, anterior view.

31. ditto, lateral view.

32. ditto, abnormal form stained.

33. ditto, abnormal and much curved form, lateral view.

34. ditto. abnormal triad form.

#### Figures 35-36. C. furcata.

35. Myxosporidia in different stages of development, fresh.

36. Spore, lateral view.

## Figures 37-39. Myxidium crassum.

37. Spore, lateral view.

38. ditto, front view.

39. ditto, different form in lateral view.

# Figures 40-42. M. microcapsulare.

40. Myxosporidium with two matured spores, fresh.

41. Spore, lateral view.

42. ditto, stained, front view.

#### Figures 43-45. M. clidodermatis.

43. Myxosporidium, with two spores, fresh.

44. Spore, lateral view.

45. ditto, stained, front view.

# Figures 46-50. M. oshoroense.

- 46. Myxosporidium with two spores, fresh.
- 47. Spore, lateral view.
- 48. ditto, stained, front view.
- 49. ditto, a slightly varied form in lateral view.
- 50. ditto, an abnormal form in lateral view.

Plate II. Figures 51-57. Ceratomyxa japonica.

- 51. Very young stage of myxosporidium with anlage of two spores, fresh.
- 52. ditto, with one spore.
- 53. Spore in lateral view, stained.
- 54. ditto, anterior view.
- 55. Abnormal spore in lateral view.
- 56. ditto, from *Microstomus stelleri* from Fukui in lateral view.
- 57. ditto, anterior view.

# Figures 58-60. C. toitae.

58. Spore, lateral view.

- 59. ditto, anterior view.
- 60. Abnormal spore, anterior view.

#### Figures 61-62. C. microstomi.

61. Myxosporidium with two spores, fresh.

62. Spore, lateral view.

#### Figures 63-65. C. microcapsularis.

63. Myxosporidium with advanced stage of spores, fresh.  $\times$  340.

- 64. Spore in lateral view, stained.
- 65. ditto, anterior view.

## Figures 66-68. C. fukuiensis.

66. Myxosporidium with matured spores.

- 67. Spore in lateral view, stained.
- 68. ditto, anterior view.

# Figures 69–74. C. limandae.

69. Myxosporidium of young stage.

70. ditto, with one spore, stained.

71. Spore in lateral view, stained.

72. ditto, anterior view.

73. Abnormal spore, lateral view.

74. ditto, anterior view.

## Figures 75-79. C majimae.

75. Myxosporidium about to leave the shell, fresh.

76. Young stage of myxosporidium, fresh.

77. ditto, with two distinct spores, fresh.

78. Spore stained, lateral view.

79. Abnormal spore, lateral view.

#### Figures 80-82. Myxidium microstomi.

80. Myxosporidium with two spores.  $\times$  860.

81. Spore stained, lateral view.  $\times$  860.

82. ditto, front view.  $\times$  860.

83. Optical cross section of spore. × 860.

Plate 4. Figures 84–86. Ceratomyxa furcata.

84. Myxosporidium of an advanced stage.

85. Spore stained, lateral view.

86. ditto, an abnormal form in lateral view.

Figures 87-89. Myxidium oncorhynchi.

87. Myxosporidium of much advanced stage, fresh.

88. Spore, lateral view. × 990.

89. ditto, front view, stained.  $\times$  990.

#### Figures 90-92. Chloromyxum salvelini.

90. Myxosporidium with one spore.

91. Spore stained, front view.  $\times 1620$ .

92. ditto, anterior view.  $\times 1620$ .

Figures 93-96. C. chitosense.

93. Myxosporidium with many spores in advanced stage.
94. Spore, lateral view. × 1620.
95. ditto, stained, front view. × 1620.
96. ditto, anterior view. × 1620.

Figures 97-101. C. giganteum.

97. Myxosporidium with anlage of spores.
98. ditto, with many matured spores.
99. Spore stained, front view. × 1620.
100. ditto, lateral view. × 1620.
101. ditto, anterior view. × 1620.

Figures 102–106. C. quadriforme.

102. Myxosporidium with anlage of spores.
103. ditto, with many matured spores.
104. Spore, anterior view. × 1620.
105. ditto, lateral view. × 1620.
106. ditto, stained, front view. × 1620.

Figures 107–111. Leptotheca constricta.

107. Myxosporidium with anlage of spore. x 860.

108. ditto, with immature spores.  $\times$  340.

109. Spore, lateral view.  $\times$  860.

110. ditto, anterior view.  $\times$  860.

111. Abnormal spore in lateral view. × 860.

#### Figures 112-117. L. yoichiensis.

112. Myxosporidium of very young stage. × 860.

113. ditto, with anlage of spores.  $\times$  860.

114. ditto, with pseudopodium.  $\times$  860.

115. ditto, with two spores.  $\times$  860.

116. Spore, stained, lateral view.  $\times$  860.

117. ditto, anterior view.  $\times$  860.

# Figures 118-120. Myxidium ochotense.

- 118. Spore, lateral view.  $\times$  1620.
- 119. ditto, front view.  $\times 1620$ .
- 120. ditto, stained, lateral view. In cod fish from Hanasaki.

# Figures 121-124. M. theragrae.

- 121. Myxosporidium with two matured spores.
- 122. ditto, with one spore in same stage.
- 123. Spore, front view. × 860.
- 124. ditto, stained, lateral view.

# Figures 125-127. M. fusiforme.

- 125. Myxosporidium with one immatured spore, fresh.  $\times$  860.
- 126. Spore, front view, stained. × 860.
- 127. ditto, lateral view.  $\times$  860.

## Figures 128-131. M. tsudae.

- 128. Myxosporidium with matured spores, fresh.  $\times$  860.
- 129. Spore stained, front view. × 860.

130. ditto, lateral view.  $\times$  860.

131. Abnormal spore in lateral view.  $\times 860$ .



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