MORPHOLOGY AND LIFE HISTORY OF MITRASPORA CYPRINI
FUJITA, PARASITIC IN THE KIDNEY OF GOLDFISH

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SUMMARY: Mitraspora cyprini was found in the diseased kidney or ureter
or both of goldfish, most of which were collected seasonally from representative
goldfish farms in Edogawa Ward, Tokyo. Histological observations of both healthy
and diseased kidneys were made. Observations were also made of smeared and
stained preparations and living specimens of M. cyprini.

The spore is oblong with slightly attenuated anterior end. Each of the two
shell valves bears eight striae. It has eight caudal filaments, two polar capsules
and a binucleate amoebula.

The trophozoites multiply in the renal tubules by the processes of plasmotomy
and gemmation and infect the epithelial cells. The trophozoites are polysporous
and the spore is monosporont.

Though this Mitraspora differs slightly in taxonomic features from the previous
authors', the author identified it as M. cyprini.

Infection takes place through the mouth. The liberated binucleate amoebulae,
penetrating the wall of the small intestine, reach the tubules through glomerules
by blood. Intracellular and intraluminal life follow multiplication and sporogony
in the tubules. The whole life cycle takes a year to complete.

INTRODUCTION

During the course of the study on the kidney enlargement disease (KED) of
goldfish in Japan, the author found this Myxosporidian from 100% of the diseased
kidneys and later proved that this Mitraspora cyprini was the causative agent of
the disease (unpublished data). Fujita (1912) first reported this myxosporidian from
the renal tubule of goldfish and carp, and coined the name Mitraspora cyprini.
Kudo (1920) reported this parasite from the renal tubules of a carp and he adopted
the name Mitraspora. Hoshina (1968) found this Myxosporidian from the diseased
kidney of goldfish, collected from a farm in Edogawa, Tokyo.

The previous authors gave, however, no detailed descriptions of the morphology
and life history of M. cyprini. Above all, considering from the view point of the
importance of this species as the causative agent of KED of goldfish (unpublished
data), the author felt much interest in this, made investigations during the period
from July, 1969 to December, 1971, and established the life cycle of M. cyprini.
KED is characterized by marked swelling of the kidney, or the ureter, or both (Fig. 2, 1). The swelling of these organs makes the abdomen externally distended to such an extent as to deform the whole body.

MATERIALS AND METHODS

Diseased and healthy fish were collected seasonally from ponds of four goldfish farms in Edogawa Ward, Tokyo.

Collected fish were fixed on the spot or transported to the laboratory of Tokyo University and kept in Laboratory aquaria for a short time until observations were made.

In each sampling the kidneys were fixed in Bouin’s or Zenker’s solution. Ten percent formalin was used for fixation of the whole fish body in the field when necessary. Paraffin sections of 3-8\(\mu\) in thickness were made in ordinary ways and stained with Mayer’s haemalum and eosin or safranin, Mallory’s triple stain or May-Giemsa’s stain. Sections were made in similar ways from ureters, intestines and other organs.

Smear preparations were made from the kidneys of diseased as well as normal fish. They were stained with May-Giemsa’s stain and sometimes with Mayer’s haemalum. Spores were stained with Lugol’s solution to test for iodonophilous vacuole of sporoplasm.

For observing living trophozoites, polytrophozoites, spores, etc., of M. cyprini a small piece of tissue was taken from the diseased kidney and crushed with a slide. The fluid was spread on the slide, diluted with physiological saline when necessary, and observed immediately under a microscope. For observing polar filaments of spore, an ordinary method using a 1-5% sodium chloride solution, a 2.5% potassium hydroxide solution, a dilute pepsin hydrochloric acid solution, or sometimes mechanical pressure by a cover slip was employed. Among them a 5% NaCl solution gave the most satisfactory result.

RESULTS

The spore: (Fig. 2, 2 to 5)

The external view of a mature spore resembles a monk’s hood, slightly attenuated at the anterior end. In fixed preparations (in smears) the spore is oblong with the pointed anterior and truncated posterior extremities. In profile it is spindle-shaped, while from the above it is rather ovoid.

The spore is covered with a shell composed of two valves usually symmetrical in shape and size, which combine with each other in the sutural plane. The shell is very thin and has uniform thickness, except two points of the truncated posterior end where it presents a knot-like appearance. The sutural line is straight, more or less thick, forming the sutural ridge. Each shell valve has generally eight distinct striae running longitudinally in almost parallel to the suture of the shell. The striae appear as fine spinous projections around the entire margin of the spore in its optical section. The most characteristic feature of this species is the presence of generally eight moderately long filiform caudal filaments of 5.8\(\mu\) long on the average, attaching themselves in a single row to the posterior margin of the spore. The point of attachment appears to meet with the end of the striae.
TABLE I

Dimensions of spores and polysporoblasts of Mitraspora cyprini

<table>
<thead>
<tr>
<th>No. of spores measured</th>
<th>Length (μ)</th>
<th>Width (μ)</th>
<th>Thickness (μ)</th>
<th>Length of polar capsules (μ)</th>
<th>Diameter of polar capsules (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Mean</td>
<td>12.2</td>
<td>6.3</td>
<td>6.1</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>11.2-14.0</td>
<td>5.6-7.0</td>
<td>4.3-7.0</td>
<td>2.4-2.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of spore measured</th>
<th>Length of polar filaments (μ)</th>
<th>No. of striation</th>
<th>No. of caudal filaments</th>
<th>Length of caudal filaments (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Mean</td>
<td>53.8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>35-77</td>
<td>7-10</td>
<td>7-10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of polysporoblasts studied</th>
<th>Length (μ)</th>
<th>Width* (μ)</th>
<th>No. of spores per polysporoblast***</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Mean</td>
<td>27.5</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>16.0-64.6 (129)**</td>
<td>13.6-61.2</td>
<td>3-21</td>
</tr>
</tbody>
</table>

* Measurement was made on polysporoblasts in sections.
** One exceptionally large one.
*** Number of spores were counted mostly with polysporoblasts in smears. Measurement of spore was done with smeared specimens.

The measurements of the spore are summarized in Table I and compared with the data reported by Fujita (1912), Kudo (1920) and Hoshina (1968) in Table II. The dimensions of fixed spores are 12.2 (11.2-14.0) μ in length, 6.3 (5.5-7.0) μ in width and 6.1 (4.3-7.0) μ in thickness.

The anterior part of the spore is occupied by two symmetrical pyriform polar capsules. They are generally 4.0 μ in length and 2.9 μ in diameter, divergent, and connected with the foramina in the spore membrane of the anterior tip. The young spores are found to carry the rudiments of the capsulogenic nuclei. The polar filament is 42-65 μ in length when extruded. The filaments are hollow tubes.

The sporoplasm occupies the extracapsular cavity in the posterior region of the spore, and of granular structure. It is binucleate and visible in both fresh and stained preparations. A round iodinophil vacuole is found in sporoplasm in some spores.

Schizogony

The shell valves of fresh mature spores under mechanical pressure or in contact with chemicals become separated along the sutural line. The amoebula (Fig. 2, 4 and 6) makes its way out through the opening of the spore valves. The movement of the amoebula is rather slow and the pseudopodia are lobate. The natural way of spore germination was observed in the intestinal lumen of goldfish in June. Two nuclei of the amoebula are found independent from each other, laying side by side (Fig. 2, 6). Each nucleus is round and about 2 μ in diameter. The diameter of the
TABLE II
Comparison of biometrical data of M. cyprini among different authors

<table>
<thead>
<tr>
<th>Biometrical key</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The author</td>
</tr>
<tr>
<td>Host</td>
<td>Carassius auratus &amp; C. carassius</td>
</tr>
<tr>
<td>Habitat</td>
<td>Intracellular &amp; intraluminal</td>
</tr>
<tr>
<td>Diameter of polysporoblast (μ)</td>
<td>16-64.6(129)** (length) (27.5*)</td>
</tr>
<tr>
<td></td>
<td>13.6-61.2 (width) (24.6)</td>
</tr>
<tr>
<td>Number of spore per polysporoblast</td>
<td>3-21(6) (3-9)</td>
</tr>
<tr>
<td>Length of spore (μ)</td>
<td>11.2-14.0(12.2)</td>
</tr>
<tr>
<td>Width of spore (μ)</td>
<td>5.6-7.0(6.3)</td>
</tr>
<tr>
<td>Thickness of spore (μ)</td>
<td>4.3-7.0(6.1)</td>
</tr>
<tr>
<td>Length of polar capsules (μ)</td>
<td>2.4-4.2(4)</td>
</tr>
<tr>
<td>Diameter of polar capsules (μ)</td>
<td>2.6-2.9(2.9)</td>
</tr>
<tr>
<td>Length of polar filaments (μ)</td>
<td>35-77(53.8)</td>
</tr>
<tr>
<td>Number of striations per valve</td>
<td>7-10(8)</td>
</tr>
<tr>
<td>Number of caudal filaments</td>
<td>7-10(8)</td>
</tr>
<tr>
<td>Length of caudal filaments (μ)</td>
<td>4.2-7(5.8)</td>
</tr>
<tr>
<td>Sporoplasm vacuole</td>
<td>Occasionally present</td>
</tr>
<tr>
<td>Sporoplasm nuclei</td>
<td>Present (2)</td>
</tr>
</tbody>
</table>

* Average
** Maximum diameter

Fresh amoebula is about 5 μ. The cytoplasm is slightly granular in the living state. The amoebula can be differentiated from the binucleate trophozoites fundamentally by their smaller size and small nucleic closely attached to each other.

The zygote is formed by the union of the amoebula nuclei after germination (Fig. 2, 7). It is round and has reticulate cytoplasm and a large nucleus with an eccentric nuclei. The diameter of a zygote (in smear) is about 6 μ. The zygote nucleus divides into two and a young trophozoite of about 7 μ in diameter, with a large vegetative nucleus with scattered chromatin and a smaller deeply stained compact generative nucleus is formed (Fig. 2, 8). The vegetative nucleus stains light red or pink, while the generative nucleus stains blue or purple in dried smears stained with May-Giemsa’s stain. The vegetative or generative nucleus divides into two and a trinucleate body is formed (Fig. 2, 9). Then the remaining vegetative or generative...
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nucleus divides into two and a tetranucleate body is formed (Fig. 2, 10). The nucleus divides by amitosis. The tetranucleate body divides into two binucleate bodies each containing a vegetative and a generative nuclei. The division is ascertained to be plasmotomy. Thus multiplication takes place and the binucleate trophozoites infect the epithelial cells (Fig. 2, 12). In most smear preparations made during the months of June to August, observed were bi, tri and tetranucleate bodies attached together (Fig. 2, 11), forming body looking rather like the external budding reported by some authors (Cohn, 1896; Doflein, 1898; Noble, 1941).

During the months of September, October and even in early November when the trophozoites are found in the distal region of the epithelium (Fig. 2, 13), the generative nucleus of the trophozoite increases in size. The centric nucleolus becomes distinct, being surrounded by a clear area, and the chromatin granules are situated in the margin of the nucleus. The nucleus is surrounded by a specially differentiated area of coarsely granular cytoplasm. The vegetative nucleus does not show such changes and is slightly smaller. The chromatin reticulum is evenly distributed and no specially differentiated area of cytoplasm is observed.

The trophozoites gradually move toward the middle and bottom layers of the epithelium and intracellular growth takes place through the nuclear division (Fig. 2, 14). Most trophozoites become nucleate with four to six nuclei and start leaving the epithelium for the lumen. In this stage, degenerative trophozoites appear, their nuclei being shifted to one or opposite sides of the body, which forms the so-called ring-type cell of rather uniform in size, though a little smaller than nondegenerative trophozoites.

Sporogony

Completing their intracellular life, the trophozoites (Fig. 2, 15) become free in the lumen and develope into polysporoblast. The young polytrophozoites with gradual nuclear division and cytoplasmic growth develop into large polytrophozoites (Fig. 2, 16). During December, the polytrophozoites are found to contain one or two (usually one) round bodies composed of hyaline ectoplasm surrounding a granular endoplasm. These bodies are gemmules or internal buds (Kudo, 1922) (Fig. 2, 16). They are usually trinucleate with one vegetative and two generative nuclei. They should not be confused with the vacuoles which are empty and smaller in size. Fully developed gemmules make their way to the periphery of the mother body and thereafter pince off to the exterior through the ectoplasmic layer. Most of the gemmae become free in the lumen and grow to polytrophozoites, while some of them degenerate. This gemmation seems to be one of the reasons for the phenomenon that trophozoites of various sizes are found at the same time in the lumen. Occasionally the gemmules may be retained in the body of mother trophozoite for a long time. In such a case, they increase in size and the nucleus increases in number to a certain extent. They usually degenerate later and form the so-called ring-type cell (Fig. 2, 17).

The cytoplasm of young trophozoites is poorly differentiated (Fig. 2, 15), while that of polytrophozoites clearly differentiated (Fig. 2, 16) into hyaline ectoplasm producing pseudopodia and slightly granular endoplasm containing refractile globules. Often a large vacuole is observed. The body is colorless. They move slowly with pseudopodia. Young trophozoites produce lobate pseudopodia from one localized part, while polytrophozoites produce short and broad lobes from localized parts or from the
entire surface of the body.

Both polytrophozoites and polysporoblasts are seen either attached to the epithelial lining or free in the lumen (Fig. 2, 17). In the attached form they are simply attached to the epithelial cells by root or comb-like ectoplasmic processes. No sign of pathological alterations were observed in epithelial cells, though Davis (1917, 1944) reported destruction of the epithelial cells attached by large trophozoites of Sphaerospora polymorpha and Myxobilatus asymmetricus.

In the later stages, polytrophozoites carry large number of nuclei. The vegetative nuclei are rather larger than the generative nuclei and usually scattered in the peripheral region of body. They are circular or oval in shape with a large nucleolus. The generative nuclei or the reproductive nuclei, although varying considerably in size, are rather smaller in size and occur singly or in groups. Usually in February and March many spore cells are found in the polytrophozoites (Fig. 2, 18). The spore cell is originally believed to be formed from a small body or a pansporoblast produced by the union of two generative or propagative cells. The pansporoblast nucleus divides and ultimately a six nucleate spore cell which develops into a single spore (Monosporont) is formed. During the multiplication stage the generative nuclei separate soon after division, but in the pansporoblast the daughter nuclei remain closely associated.

The spore cells gradually develop into spores inside the polysporoblasts. Figure 1 represents diagramatically the life cycle of M. cyprini and the formation of mature spore from a spore cell. Of the six nuclei, two take part in the formation of the valves of the spore with striae, suture, and caudal filaments, two the formation of capsulogenous cells in which the polar capsules develop, and the other two the formation of the sporoplasm (d-f).

Sporogony continues and the polysporoblasts carrying several spores are observed (Fig. 2, 19). The vegetative nuclei which remain unchanged during the spore formation are disintegrated or discharged after the rupture of the sporoblast.

The size of polysporoblasts varies from 16 to 64.6 µ in length and from 13.6 to 61.2 µ in width (Table I), though occasionally large polysporoblasts of over 100 µ in diameter were found. The maximum observed was 129 µ in diameter. Usually fully developed polysporoblasts of M. cyprini contain three to nine spores. The maximum number of spores observed in fresh and smeared materials was as large as 21.

On the rupture of the polysporoblast, the spores make their way out and become free in the lumen of the kidney tubules. They are usually excreted or discharged out of the host into pond water.

**IDENTIFICATION**

As stated earlier, Fujita (1912) coined the name Mitraspora cyprini for this myxosporidian. Kudo (1920) and Hoshina (1968) also adopted this name. Bykohovskaya-Pavlovskaya et al. (1964) used the name Mitraspora cyprini as the synonym of Sphaerospora cyprini under the family Sphaerospora.

At first sight the spore of M. cyprini presents some resemblance to that of S. cyprini, as both carry caudal filaments, arranged almost in the same way, and pyriform polar capsules. According to Bykohovskaya-Pavlovskaya et al. (1964), S. cyprini bears two spores in each sporoblast. The present species, however, generally forms
three to nine spores in each sporoblast. The spore of *S. cyprini* has five to six caudal filaments of 3-3.5 μ in length instead of eight long caudal filaments of 4.2-7 μ in *M. cyprini*. The spore shell is rather thick in *S. cyprini*. These differences in shape and contour of spore do not admit that *M. cyprini* should be taken as the synonym
Fig. 2-1.
Fig. 2-2.
Abbreviations

S., Section preparation; Sm., smear preparation; F., fresh preparation; HE., Hematoxylin (Mayer)-Eosin staining; HS. Hematoxylin (Mayer)-Safranin staining; Az., Azan staining; MG., May-Giemsa's staining; Lu., Lugol's staining; v.n., vegetative nucleus; g.n., generative nucleus; gem., gemmule; vac., vacuole. Time of sample collection in parentheses.

Fig. 2.

1. Internal feature of a diseased (KED) fish showing highly enlarged kidney, pushing other internal organs and the swimming bladder is rather displaced. (December, '72)

2. Photomicrograph of the front view of a stained spore showing typical structure of a M. cyprini spore. The polar filaments which are shot out under the action of NaCl solution (5%) possessing unstained narrow canals. Sm. MG. ×2,900. (March, '70).

3. A stained spore shell showing striations (Striae) and caudal filaments. Sm. MG. ×2,900. (May, '70).

4. A fresh spore with amoebula and remnants of the capsulogenic nuclei. F. ×1,800. (June, '71).

5. A stained spore with iodinophilous sporoplasm vacuole. Lu. ×1,800. (April, '72).

6. Two living binucleate amoebulae. F. ×1,800. (June, '71).

7. A stained zygote. Sm. MG. ×1,800. (July, '70).

8. A binucleate young trophozoite with its vegetative (lightly stained) and generative (deeply stained) nuclei. Sm. MG. ×1,800. (July, '70).

9. A trinucleate young trophozoite with two vegetative and a generative nuclei. Sm. MG. ×1,800. (July, '70).

10. A stained tetranucleate young trophozoite with two vegetative (v. n.) and two generative (g.n.) nuclei. Sm. MG. ×1,800. (July, '70).

11. Young trophozoites produced by plasmotomy. Sm. MG. ×1,800. (July, '70).

12. Multiplying young trophozoites are attached to and invading the epithelial cells of the kidney tubules. S. HE. ×494. (July, '70).

13. Trophozoites with distinct generative nuclei and mitosis in the distal part of the epithelium. S. HE. ×607. (October, '71).

14. Trophozoites are in the middle and bottom layers of the pseudostratified epithelium. S. HE. ×220. (November, '69).

15. A fresh pentanucleate trophozoite, just freed into the lumen, after completing the intracellular life. F. ×1,200. (December, '71).

16. A stained polytrophozoite with a trinucleate gemma (gem.) and a vacuole (vac.). Lobate pseudopodiae are produced all around the body. Sm. MG. ×494. (December, '71).

17. Growing polytrophozoites free in the lumen as well as attached to the epithelial layer by comb-like ectoplasmic process. A gemmule (gem) is retained in the mother trophozoite. Ring-type cells (arrow) are also visible. S. HE. ×314. (February, '71).

18. Large polytrophozoites showing the formation of spore cells. S. Az. ×494. (February, '72).

19. A part of the highly enlarged tubule containing polysporoblasts with spores in the lumen. S. HS. ×494. (March, '71).
of S. cyprini. It may be mentioned here that the genus Sphaerospora, whose type species in Sphaerospora divergens (Thélohan, 1895; Auerbach, 1912), is monosporous, disporous and polysporous, and the spore is spherical or subspherical. The ectoplasm of the vegetative body is without real pseudopodia; whereas, genus Mitraspora whose type species is Mitraspora cyprini Fujita, is disporous (Kudo, 1920) and polysporous (Fujita, 1912; Hoshina, 1968). The spore is oblong and the vegetative forms move with lobate pseudopodia.

From all the above facts, the present myxosporidia should not be classified as Sphaerospora. The genus Mitraspora should be maintained as a justified name.

Of the four known species of the genus Mitraspora, M. cyprini Fujita, M. caudata (Parisi) Kudo, M. elongata Kudo and M. caspialosae Dogiel, M. caudata and M. caspialosae are quite different in spore morphology from the present species.

The intracellular life in the early stages of development and gemmation in the later stage and polysporous nature of the present species resemble those of Kudo’s Mitraspora elongata, parasitic in the kidney of Leopomis cyanellus. This myxosporidia, however, aside from the difference in host, also differs from the present species in the biometry and morphology. The spore of M. elongata is rather longer (15–17 μ) than that of the present species and carries 14–16 longitudinal striation instead of 7–10. Caudal filament is absent in M. elongata.

Neither Fujita (1912) nor Kudo (1920) made any mention of the presence of iodinophilous vacuole and the phenomenon of kidney enlargement in carp and goldfish infected with M. cyprini. On the assumption that M. cyprini described by Fujita does not cause KED in goldfish, there may be a possibility that the present species is quite a new one.

The above mentioned differences, however, seem not to be necessarily enough to justify the establishment of a new species for the present Mitraspora. The author, therefore, identified it as Mitraspora cyprini.

Kudo (1954) placed the genus Mitraspora in the family Wardiidae under the suborder Eurysporea. Recently Hoffman (1967) put the genus Mitraspora under a new family, Elongidae. He writes “This genus was formerly in the suborder Eurysporea, but the position of the sutural plane fits the suborder Platysporea: a new family, Elongidae is proposed to contain it and Myxobilatus.” The author follows Hoffman.

**DISCUSSION**

Of the 231 parasitic Myxosporidia of Pisces, complete morphology and life cycle of only about 30 species are known. The remarkable differences between Mitraspora cyprini and the other 30 species are the causation of abnormal kidney enlargement (KED), monosporont nature of formation of spores in the polysporoblast and the peculiar phenomenon of reinfection in M. cyprini.

The mode of Infection: Regarding the mode of infection of the kidney there are some hypotheses most of which are mere conjecture. According to Ohlmacher (1893), in a frog infection occurred by way of the cloaca to the bladder, eventually lodging in the kidney. Kudo (1922) stated, “It may seem probable that the liberated amoebulae pass through the alimentary canal into the cloaca where the ureters open, as was thought by Davis (1916) in S. dimorpha, and make their way up the ureters,
and further into the uriniferous tubule of the kidney”. According to Joseph (1907), in the case of a toad the liberated amoebulae penetrate through the wall of the digestive tract, appear in the coelomic fluid, and finally reach the nephrostome through which they further make their way into the lumen of the uriniferous tubule of the host kidney.

The most accepted route of infection, which the author also believes, is that on reaching the intestine of the host spores germinate under the influence of digestive fluids (Davis, 1917; Thélohan, 1895; Auerbach, 1910), allowing the sporoplasm to creep out as a minute amoeba which penetrates into the blood vessels and finally gains access to the kidney and ureter.

The author examined the water of a KED prevailing pond on June 14, 1971, and the result showed that the water contained 17 spores per litre. Naturally a large number of spores accumulate at the bottom of a pond. It is likely that during the months of May to August spores are abundant in the pond. In the histological study free and germinating spores were often found in the intestinal lumen during those months. Germinating spores in the digestive tracts were also observed in the fish to which homogenized diseased kidney containing spores had been orally administered.

Amoebula was once observed in a blood smear. So it seems probable that amoebulae penetrate into the blood vessels in the intestine. The initial stage of infection of the renal tubules with *M. cyprini* is the appearance of amoebulae, zygotes or young trophozoites in the lumen. They are free or attached to cilia of epithelial cells. Then they begin to invade the epithelial cells. In this and successive stages they were never found entering into the proximal region of epithelial cells through the basement membrane of the tubules. From this observation it is certain that they invade into the lumen via glomerules, though the author could not detect them in the glomerules.

Multiplication: Multiplication is of special importance for *M. cyprini* as the young trophozoites increase in number, thus causing remarkable pathological changes and cellular proliferation in the tubules before spore formation. Regarding the mode of multiplication, plasmotomy is held by many authors such as Doflein (1898), Kudo (1920), Bremer (1922) and Noble (1944). In *M. cyprini* multiplication is accomplished by (1) plasmotomy and (2) gemmation or endogenous budding.

The multiplicative young trophozoites resembled the external budding reported by Cohn (1896) in *Myxidium lieberkuhni*, Doflein (1898) in *Myxoproteus ambiguous*, and Noble (1941) in *Ceratomyxa blennius*, when they were placed on a slide glass. But according to Laveran and Mesnil (1902), the trophozoites formed by plasmotomy may attach themselves to the larger individuals, thus giving rise to the appearance erroneously interpreted as budding. While, according to Davis (1916), both plasmotomy and budding are abnormal processes. The author, however, believes that in *M. cyprini* the usual way of multiplication of trophozoites is by plasmotomy, and that the attachment of the young trophozoites themselves formed the body resembling rather external budding. Though Auerbach (1910) and Erdmann (1911) termed the multiplicative division of trophozoites in *M. cyprini* as binary fission, plasmotomy is accepted as the best term to express this phenomenon.

Regarding gemmation, Davis (1916) reported eight nucleate gemma in *Sphaerospora dimorpha*. Kudo’s (1920) trinucleate gemma in *Leptotheca ohlmacheri* and Noble’s (1941) trinucleate internal buds (gemma) are quite similar to that of *M.
cyprini even in the way of formation, extrusion and nature in relation to mother trophozoite. Erdmann (1911) observed similar bodies in the disintegrating trophozoites of Chloromyxum leydigi. Recently Dogiel et al. (1965) referred gemmation only as endogenous budding and they considered budding, especially exogenous budding, as plasmotomy.

Development of Trophozoites, Sporogony and Reinfection: Intracellular development of trophozoites, which later get free in the lumen and grow to polytrophozoites in M. cyprini, has been detailed for the first time, though Kudo (1920) observed the trophozoites of Mitraspora elongata in both lumen and tissue of the kidney tubules of Lepomis cyanelles. There are a few reports of myxosporidian trophozoite parasitic to the epithelial cells but nothing is known about the relationship with the intraluminal trophozoites, which has been made clear in M. cyprini.

Comparing the later development of trophozoites and sporogony of M. cyprini with other polysporus myxosporidia closely related to M. cyprini, Kudo (1920) reported monosporont in Mitraspora elongata, whereas the polysporoblasts of Myxidium gasterson (Noble, 1943), Sphaerospora dimorpha (Davis, 1916) and Myxidium bergense (Auerbach, 1910, 1912) form disporonts.

The union of two generative cells (Mavor, 1916; Georgévitch, 1936) producing a small body or pansporoblast which ultimately develops into a single spore in M. cyprini was also observed by Kudo (1917, 1920) in Myxobolus toyamai and Mitraspora elongata. Similar observations of the formation of a spore from six cells (a spore cell) have been reported by many authors, such as Davis (1916, 1923), Kudo (1920) and Noble (1944).

After the spores are formed and become free in the lumen, the tubules whose epithelia have not been seriously or even not at all infected by young trophozoites get infected with young trophozoites. Though the presence, way of multiplication and development of the trophozoite are quite similar to those in the freshly infected 0-age fish, germinating spores and empty spore shells are found only in the former case. Therefore, the formerly infected kidneys are believed to be reinfected by the amoebulae, germinated from the spores produced in the same kidney. This phenomenon which has so far been observed only in M. cyprini may be called reinfection.

From all the above facts, the life cycle of M. cyprini which takes a year to complete is summarized as follows:

- Pond water (Spores)
- May Intestine (Germination) through mouth
- July Blood (Amoebula)
- Renal tubules
- August Schizogony (Plasmotomy, intracellular growth and development) →Sporogony (Intraluminal growth and development of polytrophozoites, gemmation, development of spore cells into spores in the polysporoblast)
- March
- May
- Pond water (Spores)
As stated before, complete morphology and life cycle of a few myxosporidians are known. So this study on the morphology and life history of *Mitraspora cyprini* would be of help in the study of other parasitic myxosporidians. The internal life history of *Mitraspora cyprini* in goldfish kidney would also be helpful in the study of such human internal parasites as *Toxoplasma gondii* and *Leishmania donovani*.

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