PART D
CHAPTER IX

FOUR NEW SPECIES OF MYXOSPORIDIA FROM THE INDIAN FRESHWATER FISH, OPHICHEPHAUS PUNCTATUS, BLOCH.

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Four New Species of Myxosporidia from the Indian Fresh Water Fish

*Ophicephalus punctatus* Bloch

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**SYNOPSIS.** Four new species of Myxosporidia have been described from *Ophicephalus punctatus*, a freshwater fish of North India: *Myxobolus aligarhensis* n. sp., *M. ophicephali* n. sp., *Unicauda basilii* n. sp., and *Henneguya zahoori* n. sp. Observations have been made on some stages of their life cycle other than the spores.

**MATERIALS AND METHODS**

Fish specimens, obtained regularly from the market, were examined for incidence of infection. Minute cysts from the pharyngeal region of the host were removed carefully by fine needles and forceps and the contents of the cysts were fixed immediately in Carnoy’s fixative, Harris’ Delafild’s and iron haematoxylin stains were used. Best staining results were obtained with Harris’ haematoxylin. Lugol iodine was used to study the structure of the iodinophilous vacuole and Giemsa stain to determine the nature of the caudal appendage of the spore. Measurements of 100 organisms were made by a microscrew ocularometer using oil-immersion objectives.

**OBSERVATIONS.**

(1) *Myxobolus aligarhensis* n. sp.

*Cysts.* In an infected fish, the accessory respiratory membrane was found to be packed with cysts up to the base of the gills. The cysts were oval in shape and opaque white when seen under the microscope in fresh preparations. There was always a well-developed cyst membrane. The cysts were 1.5 to 2.0 mm long and 0.43 to 0.55 mm wide.

*Vegetative forms.* Besides spores, cysts also contained a few trophozoites and sporonts. Trophozoites measured 5.4 to 7.1 μm and sporonts 6.2 to 8.7 μm in diameter.

*Spores.* (Fig. 1.) The spores are pyriform with a pointed anterior end and broader and rounded posterior end. The shell valves are smooth and symmetrical. Each spore contains two pyriform polar capsules equal in size. The polar filament, distinctly visible in all preparations, is in the form of a long blue-black coiled streak in each of the polar capsules. The sporoplasm covers most of the extra-capsular region of the spore. The iodinophilous vacuole varies from round to oval in shape and is situated near the posterior end of the spore. Usually there are two nuclei in the sporoplasm of a mature spore lying anterior to the iodinophilous vacuole.

**Dimensions of the spores:**

- Length: 11.4-15.0 μm; width: 6.0-7.92 μm.
- Polar capsules: 7.6-9.2 μm in length and 1.2-2.2 μm in width.
- Thickness of the shell valve: 0.72-1.2 μm.

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(2) *Myxobolus ophicephali* n. sp.

**Cysts.** The location, size and shape of the cysts are the same as for *M. aligarhensis* n. sp.

**Spores.** (Fig. 2.) These are pyriform in shape with a pair of very distinct unequal polar capsules. Due to the unequal polar capsules the shape of the sporoplasm is different from that of *M. aligarhensis* n. sp. The polar filaments are very distinct in all preparations in the form of a spiral streak in the center of each of the polar capsules. The sporoplasm covers most of the extracapsular region of the spore. The iodophilous vacuole stains deeply with iodine and is situated at the anterior central portion of sporoplasm. Usually there are two nuclei lying at the posterior central portion of the sporoplasm.

**Dimensions of the spores:**

- Length: 11.6-13.3 μ; width: 4.0-6.3 μ.
- Polar capsules: 6.7-7.3 μ in length and 1.2-2.0 μ in width.
- Length of polar capsule: 6.9-8.5 μ in length and 1.2-2.0 μ in width.

**Discussion.** Based on diagnostic characters described by Kudo (1920), the above described two species can be classified under the genus *Myxobolus*. Twelve species belonging to the genus *Myxobolus* have been described from Indian fishes by different workers (1-3, 7-9). Establishment of new species has been based by these authors on differences (a) in host, (b) in size of spores and (c) polar capsules. The two organisms described in this paper constitute the first report of myxosporidian parasites from *Ophicephalus punctatus*. Depending upon the size of the spores, shape and size of the polar capsules, these two organisms differ from the rest of the species of *Myxobolus* described so far. These two new species, *M. aligarhensis* and *M. ophicephali*, themselves differ markedly in size and shape of spore and polar capsules. *M. aligarhensis* has equal sized polar capsules whereas those of *M. ophicephali* are unequal.

(3) *Unicauda basiri* n. sp.

**Cysts.** In an infected fish, the cysts were found scattered throughout the mouth cavity attached to the pharyngeal epithelium layer. The cysts are oval in shape, opaque white in color and very minute (0.5 to 1 mm long and 0.1 to 0.3 mm wide). There is always a well-developed cyst membrane. Besides spores, cysts also contained a few trophozoites and sporonts.

**Spores.** (Fig. 3.) The spores are ovoid in shape with two polar capsules at the anterior end. The posterior end of the shell valve of the spore is prolonged into a more or less extended process to form a single caudal appendage. Both the main body of the spore and the caudal appendage stain blue with Giemsa stain. The body of the spore is biconvex and compressed parallel to the sutural plane. The shell valves are smooth and symmetrical. The polar capsules are pyriform in shape and equal in size. The polar filament, in the form of a long dark streak, is distinctly visible in the center of each polar capsule. When fresh spores with unextruded polar filaments are kept 15-30 minutes in Gram stain or Lugol's iodine, the extrusion of the polar filaments occurs. There may be one or two nuclei in the sporoplasm. The position of the nuclei in the sporoplasm varies from spore to spore but usually they are above the iodophilous vacuole. An iodophilous vacuole can easily be distinguished at the posterior base of the sporoplasm in noine stained preparations. 100 spores were measured:

<table>
<thead>
<tr>
<th>Range</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length of the spore (including caudal prolongation)</td>
<td>25.6-39.2 μ</td>
</tr>
<tr>
<td>Length of caudal prolongation</td>
<td>10.0-20.3 μ</td>
</tr>
<tr>
<td>Length of polar capsule</td>
<td>5.0-7.4 μ</td>
</tr>
<tr>
<td>Max. width of polar capsule</td>
<td>1.4-2.0 μ</td>
</tr>
<tr>
<td>Max. width of spore</td>
<td>3.2-4.4 μ</td>
</tr>
</tbody>
</table>

The length of the caudal appendage as compared to the main body of the spore varies in different spores. In 35% of the spores examined the caudal appendage is smaller than the main body. In 22% it is equal, and in 10%, it is longer. In all these spores the iodophilous vacuole is clearly seen, confirming that all these represent mature stages of the spore.

**Discussion.** It has been evident for some time that the genus *Henneguya*, family Myxobolidae, comprises a heterogeneous group of species which differ fundamentally in the structure of the spore and also in the character of the vegetative stage. Davis (4) divided the genus *Henneguya* into three genera, based primarily on fundamental differences in the structure of the spores. "The majority of the species remain in *Henneguya* which is characterized by the spore having a divided posterior process, while the spore body is flattened parallel to the sutural plane. A new genus *Unicauda* is proposed for those species in which the spore has a single, undivided posterior process. A third genus, *Myxobilatus* is erected for those species in which the spore is flattened at right angles to the sutural plane. Based on these criteria the organism described in this paper can be assigned to the genus *Unicauda*. Its characters and size measurements do not show any approximate similarity with any of the 14 existing species of *Unicauda* (4,9). There is only one species *U. ophicephali* which has been reported so far from a fresh water fish of India (9). *U. ophicephali* was described in the name of *Henneguya ophicephali* by Chakravarty, 1939, but later in 1952 Tripathi placed it under the genus *Unicauda* due to its strong affinity with the latter group.
**NEW SPECIES OF MYXOSPORIDIA**

*U. basiri* n. sp. differs from *U. ophiicephali* in many respects: 1) in habitat—whereas the cysts of *U. ophiicephali* have been reported only from muscle, the cysts of *U. basiri* n. sp. have been observed attached superficially to the pharyngeal epithelium only; 2) size of spore: the length and width of the (a) whole body, (b) caudal appendage, and (c) polar capsule of *U. basiri* n. sp. are smaller than in *U. ophiicephali*. Tripathi(9) has categorized the existing 14 species of *Uniцида* into three groups, depending on the size relationship of the caudal appendage of a spore to its main body. In our present study we have found that within the single cysts there may occur spores which are similar in every other respect except the length of the caudal appendage. In our opinion the difference in size relationship between the caudal appendage and the main body of the spore is not a sufficiently diagnostic criterion on which to designate new species or form any type of taxonomical groupings. The structure, shape and size of the main body of the spore, polar capsules and sporoplasm should be taken as main features for diagnostic purposes.

(4) *Heneguya zahoori* n. sp.

**Cysts.** Cysts are found embedded in the tissues of the gill filaments. They are very minute measuring 0.5 mm in diameter. They are whitish opaque and pyriform and are found covered over by the pharyngeal membrane of the host. Besides mature spores, the cysts contain trophozoites and young spores at various stages of development.

**Spores.** The body of the spore is biconvex in shape, compressed parallel to sutural plane, with an attenuated anterior end and extended posteriorly into a bifurcated caudal appendage. With Giemsa stain it can be seen that the spore has a distinct vacuole which is made distinctly visible by treating with Lugol's solution. Dimensions of the spores are as follows:

<table>
<thead>
<tr>
<th>Range</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length of the body</td>
<td>20.0-30.6</td>
</tr>
<tr>
<td>Length of main body (excluding caudal appendage)</td>
<td>8.0-12.0</td>
</tr>
<tr>
<td>Length of the caudal appendage</td>
<td>12.0-18.6</td>
</tr>
<tr>
<td>Width of the main body</td>
<td>2.1-3.0</td>
</tr>
<tr>
<td>Length of the polar capsule</td>
<td>4.9-6.7</td>
</tr>
<tr>
<td>Width of the polar capsule</td>
<td>0.7-1.1</td>
</tr>
</tbody>
</table>

**Discussion.** Since Kudo's(6) classical monograph on Myxosporidia with the description of the genus *Heneguya*, many species have been added to this genus which differ from each other in some fundamental structural difference of the spores. Davis(4) made a systematic and comprehensive study of this assemblage of heterogeneous group of species and divided the genus *Heneguya* into three genera, based primarily on fundamental differences in the structure of the spores. The majority of the species remained in *Heneguya* which is characterized by the spore having a divided posterior process, while the spore body is flattened parallel to the sutural plane. Based on Davis' diagnosis the organism described above can be classified under the genus *Heneguya*. Out of 29 species of *Heneguya* reported so far from fresh water fishes, only 2 have been reported from India. The present species resembles *H. zschokkei* and *H. sahnonis* only in length of the spore(9) but it differs from them in all the other characters. It shows only an approximate similarity of length of main body (excluding caudal appendage) with the Indian species *H. otolithi* and *H. lateris*(5,9); but it differs from them in the width of the spore body, size of polar capsules and caudal appendage. It is also much smaller than *H. ophiicephali* (4) in contrast with the latter, its polar capsules are equal in size.

As the description gives the details by which the species can be distinguished from other species, the authors establish it on an independent basis.

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**REFERENCES**