Studies on Myxosporidia from the fishes of Bengal, with a note on the myxosporidian infection in aquaria fishes.

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With plates 8—10.

Contents.

Introduction.

Very little work on the group Myxosporidia has been done in India. Four species, viz. *Myxidium mackiei* Bosanquet (1910), *Myxobolus nodularis* Southwell and Prasad (1918), *Thelohanellus rohita* (Southwell and Prasad) Kudo (1933), *T. seni* (Southwell and Prasad) Kudo (1933) and two undetermined species, viz. *Myxobolus* sp. Southwell (1915) and *Sphaerospora* sp. Southwell and Prasad (1918), have so far been reported from India. Ganapati (1936) has added

a new species of *Henneguya*. From the economic standpoint RAY (1933) published a note on 'Preliminary observations on myxosporidia from India'. The present paper deals with the descriptions of a number of myxosporidians infesting the common food fishes of Bengal. I have also discussed at the end of this paper the causes of fish mortality.

I take this opportunity to record my indebtedness to Dr. H. N. RAY for the materials which he placed at my disposal and for his helpful and valuable suggestions. I wish also to offer my sincere thanks to DR. B. L. BHATIA for his suggestions and for including in his sporozoa volume of the Fauna of British India (in press) the new myxosporidians described below. I am grateful to Mr. D. MUKERJI and Mr. J. L. BHADURI for helping me in various ways. I am also thankful to Prof. H. K. MOOKERJEE for affording me ample facilities of work on this line.

Material and method.

The fishes which form the material for the investigation were either collected from the tank within the college compound or purchased from the local markets. Fish fries, as well as, young fishes of different species were kept in large aquaria under constant observation.

Macroscopic examination of the fishes were undertaken to detect the presence of cysts or tumours. Microscopical examination of the internal organs such as, gall bladder, liver, kidney testis, ovary, gills, etc. were made for the detection of the parasites. The parasites were observed under fresh conditions and their measurements recorded. Hanging drop preparations were made after the improved method of NEMECZEK (1926) for the study of the fresh spores. Serial sections of tissues infected with the parasites and their cysts were taken. The following method was adopted. 1 to 10 % of KOH was chiefly used for the extrusion of filaments. I find however the following method more satisfactory. Smears were drawn along the length of the slide with the help of a pippete and were fixed by adding several drops of Methyl Alcohol. They were then washed in water and stained with giemsa. The filaments were fully extruded and stained red. LUGOL'S solution was also used for the detection of iodinophilous vacuoles. For section cutting and smear preparations, the infected organs and tissues were fixed in SCHAUDINN'S fluid, as well as in BOUIN-DUBOSCQ, the latter fluid was found more suitable for section cutting. DELAFIELD'S and Iron-alum haematoxylin were chiefly used for staining both smears and sections.

Description of species.

Ceratomyxa gobioidesi n. sp.

Host: *Odantoamplyopus (Gobioides) rubicundus* (Ham.).

Habitat: Gall bladder.

Locality: Calcutta.

Vegetative form. A few trophozoites (Pl. 8 Fig. 1) circular or disc-shaped in form were found in the smear preparations of the gall bladder. No sharp line of demarcation is visible between the ectoplasm and the endoplasm of the parasites; small nuclei were seen scattered throughout its cytoplasm. Size of the trophozoites vary from 500 to 650 μ in diameter. The trophozoites are disporous. Spore — Large number of crescent shaped spores (Pl. 8 Fig. 2—4) were found to float in the bile. The valves of the spores are symmetrical and their terminal end is blunt. The sutural plane is distinct. The polar capsules are spherical and equal; they are placed one on each side of the sutural plane and are provided with distinct coiled filaments. The extra capsular cavity is filled with finely granular sporoplasm. The sporoplasm contains two nuclei, one on each side of the sutural plane. Nuclei of the polar capsules are distinct in stained spores. Dimensions: breadth of the spore 14—15 μ, sutural diameter of the spore 4—5 μ, polar capsules 2.5—3 μ in diameter, polar filament 15 μ long.

Remarks: The myxosporidian mentioned by Ray (1933) infesting the liver, gall bladder, kidney and ovary of the same species of the fish, agrees with the descriptions of *Ceratomyxa gobioidesi* given here. The spores of *C. gobioidesi* under report resemble to some extent those of *C. monospora* Davis, but differ from the latter in possessing equal and symmetrical valves and symmetrically placed sporoplasm. More over, the vegetative forms of these two species widely differ.

Ceratomyxa hilsae n. sp.

Host: *Hilsa ilisha* (Ham.).

Habitat: Gall bladder.

Locality: Calcutta.

Vegetative form. Large number of spherical trophozoites were found in the smear preparations of the gall bladder. Two spores were often found lying side by side and this suggests that the trophozoites are disporous.

Spore. The spores appear elliptical in front view (Pl. 8 Fig. 6) but when seen laterally their anterior side is arched while the

posterior side is almost straight (Pl. 8 Fig. 5). The valves are tapering and end in rounded extremities or blunt points. The sutural plane is prominent and divides the spore into two equal parts, each of which is provided with a polar capsule. The polar capsules are spherical and equal in size and are provided with distinct coiled filaments. The sporoplasm which is placed asymmetrically does not fill the entire extra-capsular cavity. It contains two nuclei placed side by side. Dimensions: breadth of the spore 25—40 μ, sutural diameter of the spore 10 μ, polar capsules 5 μ in diameter, polar filament 35—40 μ long.

Remarks: The spores of *C. hilsae* approach those of *C. limandae* FUJITA generally but slightly differ from the latter in shape, in having valves equal and symmetrical and in the nature of polar capsules.

Myxidium glossogobi n. sp.

Host: *Glossogobius giuris* (HAM.).

Habitat: Gall bladder.

Locality: Calcutta.

Vegetative Form: Not found.

Spore. The shape of the spores are elongately oval with rounded extremities (Pl. 8 Figs. 7, 8). The valves of the shell are nonstriated. The sutural plane is not distinctly observable in fresh spores. The polar capsules are situated, one at each end of the spore. They are pyriform but become slightly ovoidal after the extrusion of the filaments. The openings through which the filaments are thrown out are marked by elevated areas of the shell situated just in front of the capsules. The sporoplasm is uniformly granular and occupies, the middle portion of the spore. The sporoplasm contains two nuclei at its centre and several black granules are noticeable in fresh spores. Dimensions: length of the spore 12—15 μ, breadth of the spore 8.5—10 μ, polar capsules 3.1—4.1 μ long, filament 40—50 μ long.

Remarks: The spores of *M. glossogobi* resemble those of *M. glutinosum* DAVIS, but they differ in size and shape. Further, the shape of *M. glossogobi* occupies an intermediate position between *M. glutinosum* DAVIS and *M. oviformi* PARISI.

Myxobolus calbasui n. sp.

Host: *Labeo calbasu* (HAM.), *L. rohita* (HAM.) and *Cirrhina mrigala* (HAM.).

Habitat: Gall bladder. One of the specimens of *Cirrhina mrigala* measuring about 9 inches long was found highly infected and almost

all the organs viz. liver, heart, intestine, muscles etc. contained mature cysts.

Locality: Calcutta.

Vegetative form. Young trophozoites, measuring 30 μ to 25 μ in diameter, were abundantly found in the smear preparations of the gall bladder. They are spherical or slightly oval and contain large number of nuclei scattered irregularly in the cytoplasm. The cysts are almost spherical measuring 300—350 μ in diameter and are surrounded by a membrane formed by the tissues of the host (Pl. 8 Fig. 9). In serial sections, these cysts contain in the centre a large number of mature spores and in the periphery generative and vegetative nuclei. They are polsyporous.

Spore. The spores are roughly oval in front view (Pl. 8 Figs. 10, 11, 13, 14) with anterior extremity pointed and the posterior rounded and lenticular in lateral view (Pl. 8 Fig. 12). The shell is moderately thick and the valves are symmetrical. The sutural ridge is distinct but the sutural line is not well marked. Two pyriform polar capsules are present and one of the capsules is smaller than the other. Coiled filements of the polar capsules are well marked in fresh spores and when extended they were found to be unequal in size. The sporoplasm is granular and occupies the posterior region of the spore. It contains two nuclei and a spherical iodinophilous vacuole. The nuclei of the polar capsules are also distinct even in fresh spores. Dimensions: length of the spore 12.4—15 μ, breadth of the spore 8.2—10 μ, thickness of the spore 6.18 μ, thickness of the shell valves 1.03 μ, polar capsules 6.18 $\times$ 4.12 μ and 4.12 μ $\times$ 3.09 μ, maximum lengths of the longer and shorter polar filaments 125 μ and 60 μ, iodinophilous vacuole 4.1 μ in diameter.

Remarks. RAY (1933) mentioned in his short report this myxosporidian from the liver of *Cirrhina mrigala*. The spores of *M. calbasui* resemble in shape those of *M. permagnus* WEGNER, but differ from the latter in size, in having unequal polar capsules and in the absence of folds on the valves.

Myxobolus mrigalae n. sp.

Host: *Cirrhina mrigala* (HAM.).

Habitat: Scales.

Locality: Calcutta.

Vegetative form. Large number of cysts were found on the scales (Pl. 9 Figs. 20, 21). They are oval, opaque white in colour and contained a large number of mature spores. The fish scales

seemed perforated after taking out the cysts. The cysts were distributed in all parts of the body except the head and the fins. They are bounded by a fibrous inner layer which was surrounded by an outer layer of cells derived from the epidermis of the host (Pl. 8 Fig. 15). The dimensions of the cysts vary from .75—1.5 mm. in length and .75—1 mm. in breadth. The cysts are polysporous.

Spore. The spores are spherical or slightly oval in front view (Pl. 8 Figs. 16, 17 and Pl. 9 Fig. 18) and lenticular in lateral aspect (Pl. 9 Fig. 19). The shell is moderately thick and symmetrical. The shell valves exibit several triangular markings which are well marked in front view. The sutural ridge is prominent but the line is indistinct. Two unequal polar capsules are pyriform, and show distinct coiled filaments of unequal length. The sporoplasm occupies the posterior region of the spore and contains two distinct nuclei and an iodinophilous vacuole. Nuclei of the polar capsules are distinct and are situated at the base of the capsules. Dimensions: length and width of the spore 7.21—8.24 μ, thickness of the spore 6.18 μ, thickness of the shell 1.03 μ, polar capsules 5.15 by 3.09 μ and 3.09 by 2.06 μ, iodinophilous vacuole 3.1 μ in diameter.

Remarks. The spores of *M. mrigalae* are more or less similar in shape and size to those of *M. squamosus* KUDO, but the former differ from the latter in having unequal polar capsules and in the absence of an intercapsular appendix.

Henneguya ophicephali n. sp.

Host: *Ophicephalus punctatus* BLOCH.

Habitat: Branchiae and muscles.

Locality: Calcutta.

Vegetative form. A fair number of cysts occur attached to the gill filaments or muscles of the cephalic region. The cysts (Pl. 9 Figs. 22, 23 and Pl. 10 Fig. 27) are spherical or oval and bounded by a fibrous layer derived from the tissues in which they are embedded. They are about 2 mm. in diameter and polysporous.

Spore formation. The vegetative and generative nuclei can easily be distinguished in the smear preparations of the early stages of the plasmodial forms, as well as, in the periphery of the cysts. The vegetative nuclei (Pl. 10 Fig. 31) are large, generally circular in shape measuring 4.12 to 6.18 μ in diameter. They contain chromatin granules scattered at the periphery just below the nuclear membrane and one or two nucleolus in the centre. By further division of these vegetative nuclei, the generative nuclei (Pl. 10 Fig. 32)

are formed. The latter can easily be distinguished from the former by their small size, the dense chromatin network, a comparatively large karyosome and an island of protoplasm, surrounding the nuclei. The generative nuclei increase in size and each is transformed into a pansporoblast.

The spore formation in myxosporidians have been studied by a number of workers [1]) whose descriptions by no means agree with one another. In *Henneguya ophicephali*, the nucleus of the early pansporoblast first divides unequally into a large and a small nucleus. The former divides again into two, from which the two sporoblasts (Pl. 10 Fig. 33—35) are developed. The small nucleus placed in between the two developing sporoblasts underwent a single division. The small nuclei formed are without any definite chromatin granules and nucleolus. Little rounded or oval bodies deeply stained were found in each sporoblast and they are similar in nature to those described by KUDO (1926) in *Myxosoma catostomi.*

Each of the sporoblasts by further division of its nucleus gives rise to a single spore (Pl. 10 Fig. 36).

Spore. The shape of the spores are more or less ovoidal or oblongate. The anterior end is broader and rounded while the posterior end is tapering and is prolonged as the tail. In the spores obtained from the gills the tail was found bifurcated and devaricated (Pl. 9 Fig. 25 and Pl. 10 Fig. 26) while in those from the muscle the bifurcations are approximated (Pl. 10 Figs. 29—30). The length of the tail is shorter in the spores obtained from the muscles than those obtained from the gills. The valves of the shell are symmetrical and uniformly thick. The sutural ridge is prominent. The polar capsules are pyriform and one is slightly smaller than the other. They are provided with distinct coiled polar filaments. The sporoplasm is oblong and contains two nuclei and at the posterior region a spherical iodinophilous vacuole. Dimensions: Total length of the spore including the tail 41.5 to 52.5 μ, breadth of the spore 6.18 to 7.21 μ, thickness of the spore 4.12 μ, polar capsules 6.18 to 9.27 μ by 2.06 to 3 μ and 5.15 to 8.24 μ by 2.06 to 3 μ. length of the tail 20—32 μ, polar filaments 26—32 μ in length, iodinophilous vacuole 2.06 μ in diameter.

Remarks. RAY (1933) reported this myxosporidian from the gills of the same host, but did not give and description of the species referred to here. The spores of *H. ophicephali* show close affinities to those of *H. gurleyi* KUDO. But they differ in the fact that

[1]) For a detailed description, the reader is referred to KUDO (1926).

the anterior end of the spore of *H. ophicephali* is broader and more rounded than those of *H. gurleyi* KUDO and have unequal polar capsules.

Myxosporidian infection in aquaria fishes. It has already been discussed by a number of workers[1]) that myxosporidian infections in fishes are of considerable practical importance, in as much as, they cause fatal diseases and destroy thousands of fishes. It is well known that *Myxobolus pleifferi* causes a fatal disease in barbel while *M. cyprini* is responsible for the carp pox, and *Unicapsula muscularis* is the cause of the pathological condition known as'wormy' halibut.

KUDO (1934), however, is of opinion that death is primarily not due to these protozoan parasites but occurs as a result of secondary bacterial or fungous infections of the tissues affected. RAY (1933) however, is of opinion that infection of *Ceratomyxa* in *Gobioides rubicundus* proved fatal under laboratory conditions. In the fishery laboratory attached to this department I have observed mortality among fishes infected with the myxosporidian. I find the fishes which showed severe myxosporidian infection were greatly emaciated and reduced in weight. Death occurred when infection was heavy. Further investigations in these lines are being carried on by me in this laboratory.

The accompanying table will indicate the number of fishes parasitised out of the total number of fishes examined, with their seat of infection and locality.

Name of fish	No. of fishes examined	No. of fishes infected	Seat of infection	Parasite	Locality
1. *Odanto amplyopus rubicundus*	10	4	Gall bladder	*Ceratomyxa gobioidesi*	Calcutta
2. *Hilsa ilisha*	10	6	„	*Ceratomyxa hilsae*	„
3. *Glossogobius guiris*	31	5	„	*Myxidium glossogobi*	„
4. *Labeo calbasu**	15	15	„	*Myxobolus calbasui*	„
5. *Labeo rohita**	73	70	„	„	„
6. *Cirrhina mrigala**	50	48	Gall bladder liver, heart intestine, muscles etc.	„	„
7. „	8	7	Scale	*Myxobolus mrigalae*	„
8. *Ophicephalus punctatus*	30	5	Gills	*Henneguya ophicephali*	„

* These fishes were kept in aquaria.

[1]) HOFER (1904), KEYSSELITZ (1908), DAVIS (1924), DUNKERLY (1925).

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To save space all the papers are not mentioned here. For a detailed list please refer to KUDO 1920 and 1933.

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Explanation of plates.

Plates 8—10.

Figures were drawn under Camera lucida, unless otherwise stated.

Plate 8.

Ceratomyxa gobioidesi n. sp.

Fig. 1. Vegetative form from a statined smear × 103.

Fig. 2 and 3. Two fresh spores × 1666.

Fig. 4. A stained spore × 1666.

Ceratomyxa hilsae n. sp.

Fig. 5. Lateral view of a fresh spore × 1666.

Fig. 6. Front view of a fresh spore × 1666.

Myxidium glossogobi n. sp.

Fig. 7. A fresh spore × 1666.

Fig. 8. A stained spore × 1666.

Myxobolus calbasui n. sp.

Fig. 9. Photomicrograph of a cyst in section from heart muscles. × 140.

Figs. 10 and 11. Front view of two spores × 1666.

Fig. 12. Lateral view of a fresh spore × 1666.

Figs. 13 and 14. Two stained spores showing nuclei and iodinophilous vacuole × 1666.

Myxobolus mrigalae n. sp.

Fig. 15. Photomicrograph of a cyst in section × 120.

Figs. 16 and 17. Front view of two fresh spores × 1666.

Plate 9.

Myxobolus mrigalae n. sp.

Fig. 18. A stained spore × 2700.

Fig. 19. A fresh spore in side view × 1666.

Fig. 20. A portion of Cirrhina mrigala showing cysts on the scale. Natural size.

Fig. 21. A single scale enlarged to show the position of the cyst × 6.

Henneguya ophicephali n. sp.

Fig. 22. Head of Ophicephalus punctatus showing the cysts attached to the gill filaments. Natural size.

Fig. 23. Photomicrograph of a section of a cyst from the gills.

Fig. 24. Lateral view of a fresh spore from gills × 1666.

Fig. 25. Front view of a fresh spore from gills × 1666.

Plate 10.

Henneguya ophicephali.

Fig. 26. Front view of a fresh spore from gills × 1666.

Fig. 27. Photomicrograph of the section of muscles of ophicephalus punctatus showing a large number of cysts × 38.

Figs. 28 and 29. Front view of two fresh spores from muscles × 1666.

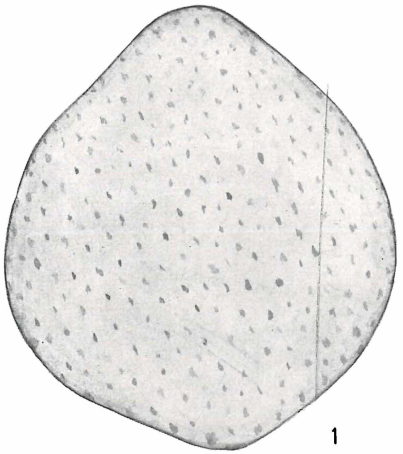
Fig. 30. Lateral view of a fresh spore from muscles × 1666.

Fig. 31. Vegetative nucleus × 1666.

Fig. 32. Generative nucleus × 1666.

Figs. 33—35. Pansporoblasts in different stages × 1666.

Fig. 36. Sporoblast showing a developing spore × 1666.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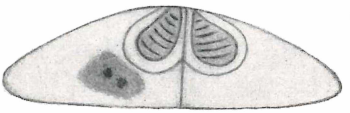
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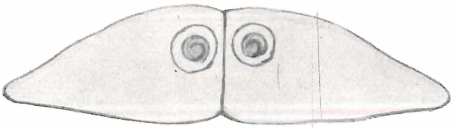
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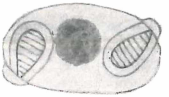
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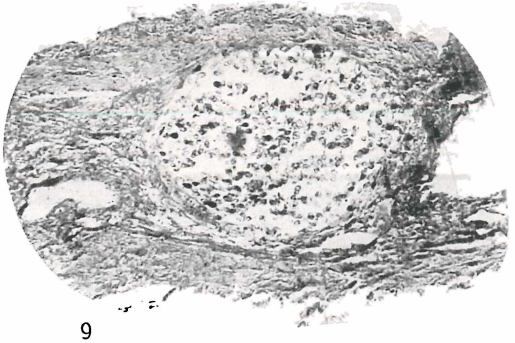
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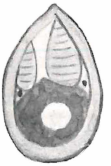
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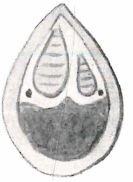
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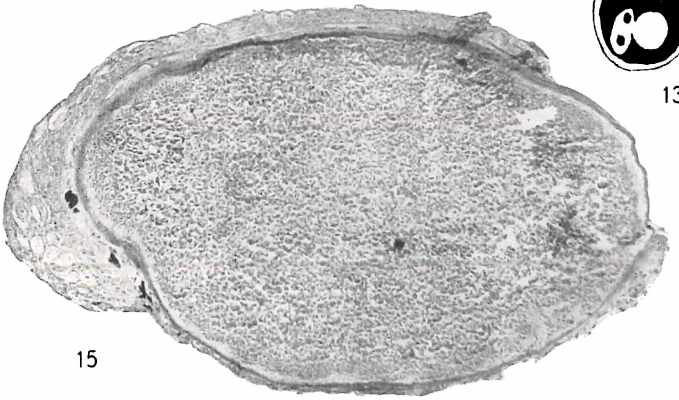
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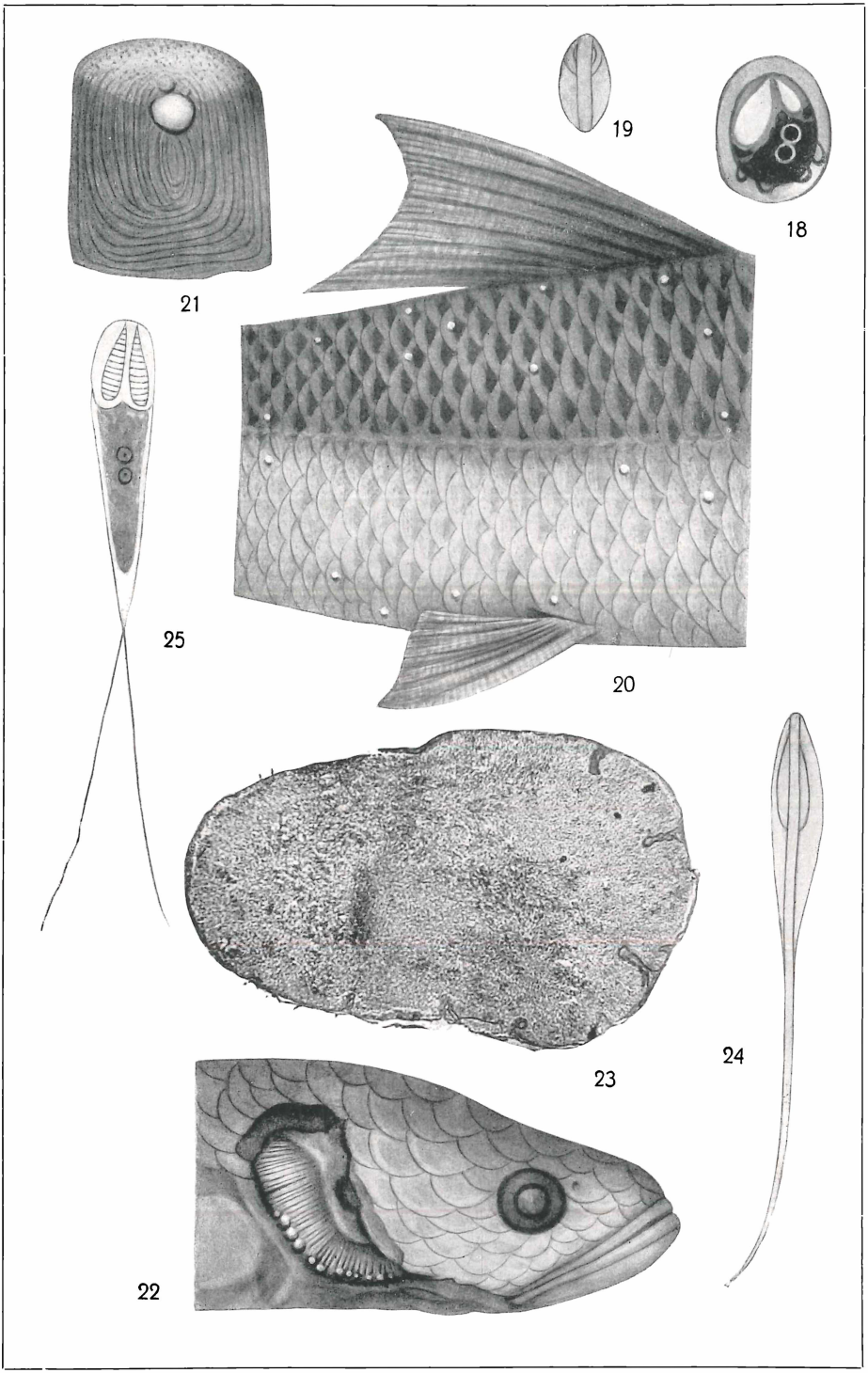
VERLAG VON GUSTAV FISCHER IN JENA



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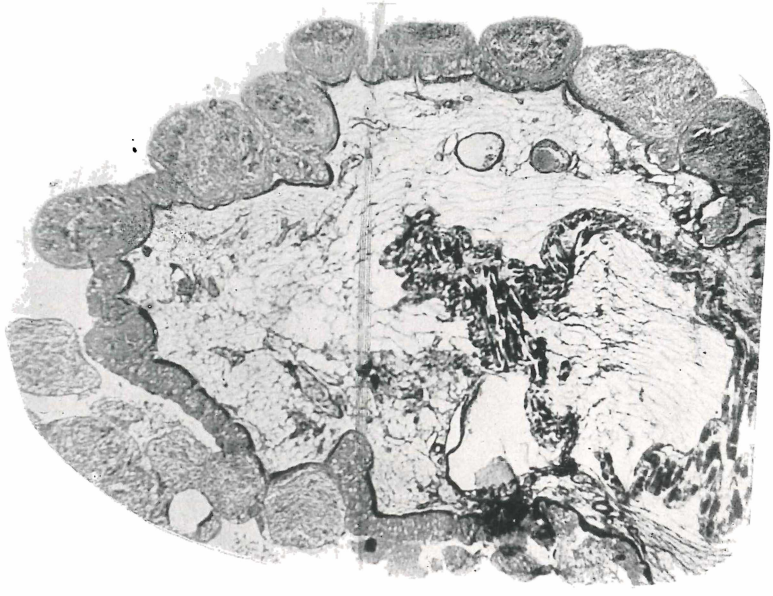

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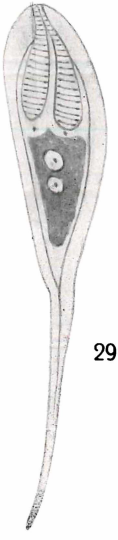

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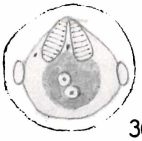

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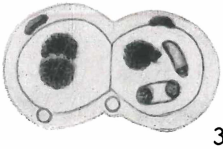

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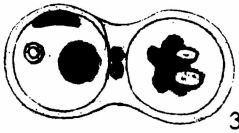

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VERLAG VON GUSTAV FISCHER IN JENA