

STUDIES ON PARASITES OF INDIAN FISHES.

III. PROTOZOA 2 (MASTIGOPHORA AND CILIOPHORA) ¹

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Only two species, one each of the classes Mastigophora and Ciliophora, viz., *Trypanosoma clariae* Montel (1905), var. *tabrachi* de Mello and Vales 1936 and *Zoothamnium horai* Khajuria and Pillay 1952 from Indian fishes, have been fully described. Lingard (1904) recorded unnamed species of *Trypanosoma* from *Barbus carnaticus*, *Gobius giurir*, *Macrone seenghala*, *Ophicephalus striatus*, and *Trichogaster fasciatus*. Jones and Job (1938) have also recorded *Zoothamnium* sp. from the mouth of *Acentrogobius neilli* from Madras.

During the course of my studies on the parasites of Indian freshwater food fishes, a new species belonging to the class Mastigophora and three new species of the class Ciliophora were found, and are described below.

The parasites were first studied in the living condition after staining with the vital stains, methylene blue and neutral red, and then fixed in osmic vapour or aqueous Bouin's fluid and stained with Ehrlich's hæmatoxylin and eosine.

Class MASTIGOPHORA

Order PROTOMANADINA Blochmann.

Family BODONIDAE Butschli.

Genus *Bodomonas* Davis, 1947.

***Bodomonas rebae*, sp. nov.**

(Text-fig. 1, a-c)

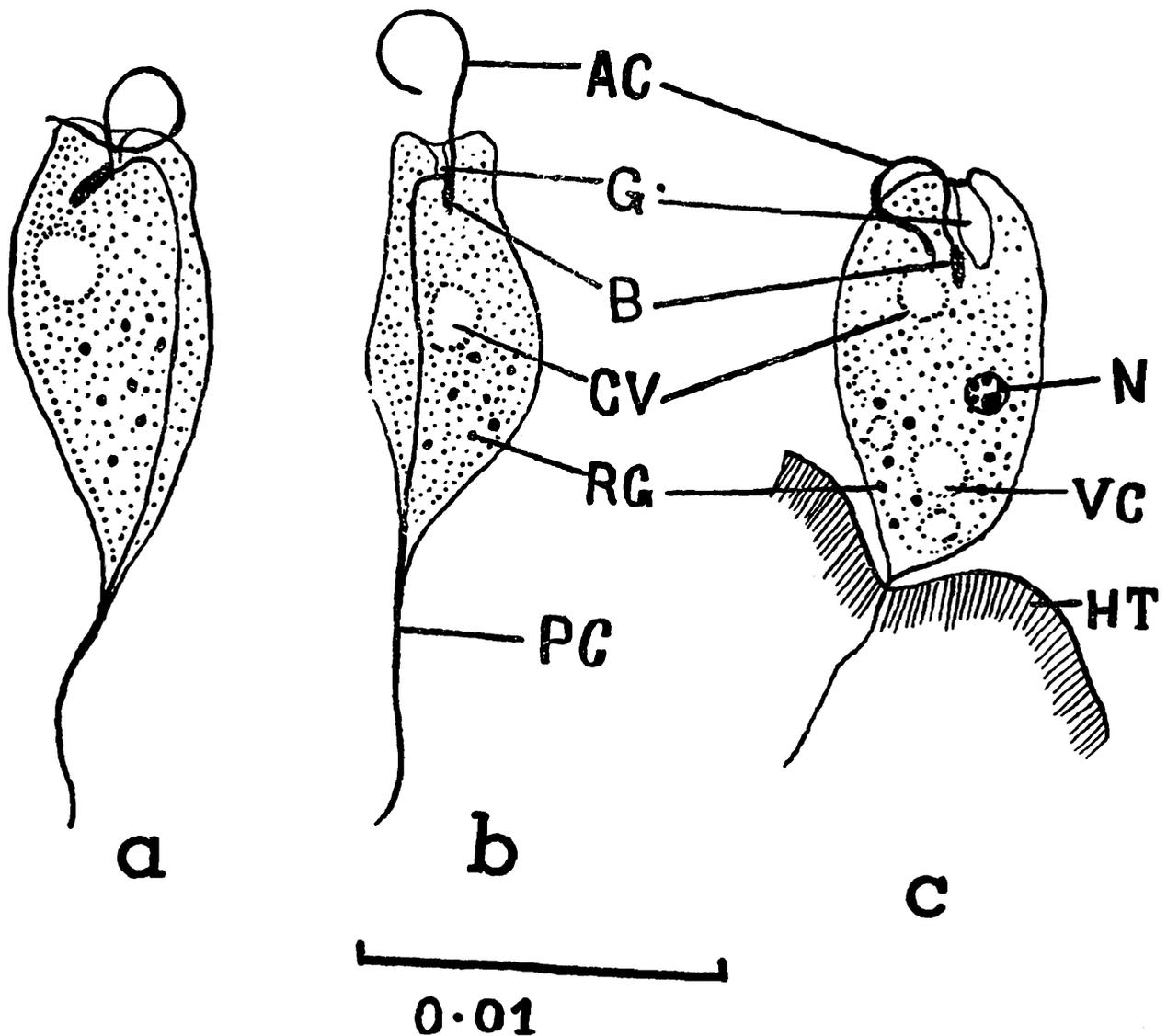
This flagellate parasite was found on the gills of the fry of *Cirrhina reba*, *C. mrigala*, *Labeo rohita* and *Catla catla* (1.8-2.5 cms. long) during August 1953. The parasite is so small that it is apt to be missed unless examined under a high power objective with $\times 20$ or $\times 12$ eye-piece. 59 per cent of the 200 fry examined from two consignments were found infected with this parasite. The following table shows the incidence of infection in individual species.

Species.	No. examined.	No. infected.	Per cent infection.
<i>Labeo rohita</i>	50	38	76
<i>Cirrhina reba</i>	50	45	90
<i>C. mrigala</i>	50	25	50
<i>Catla catla</i>	50	10	20

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The parasite is attached by its posterior flagellum to the end of the gill filaments.

Body long, tapering posteriorly, anterior end broad and flattened; Middle part of the body broadest. Protoplasm granular with refringena granules especially in the posterior half of the body. Contractile vacuole spherical, posterior to the elliptical blepheroplast. Cytopharynx, small depression at the anterior end. Nucleus spherical containing granules of chromatin which stain deeply with methylene blue. Two flagella, the anterior small and curved, the posterior long and trailing behind the body, serving as an anchor.

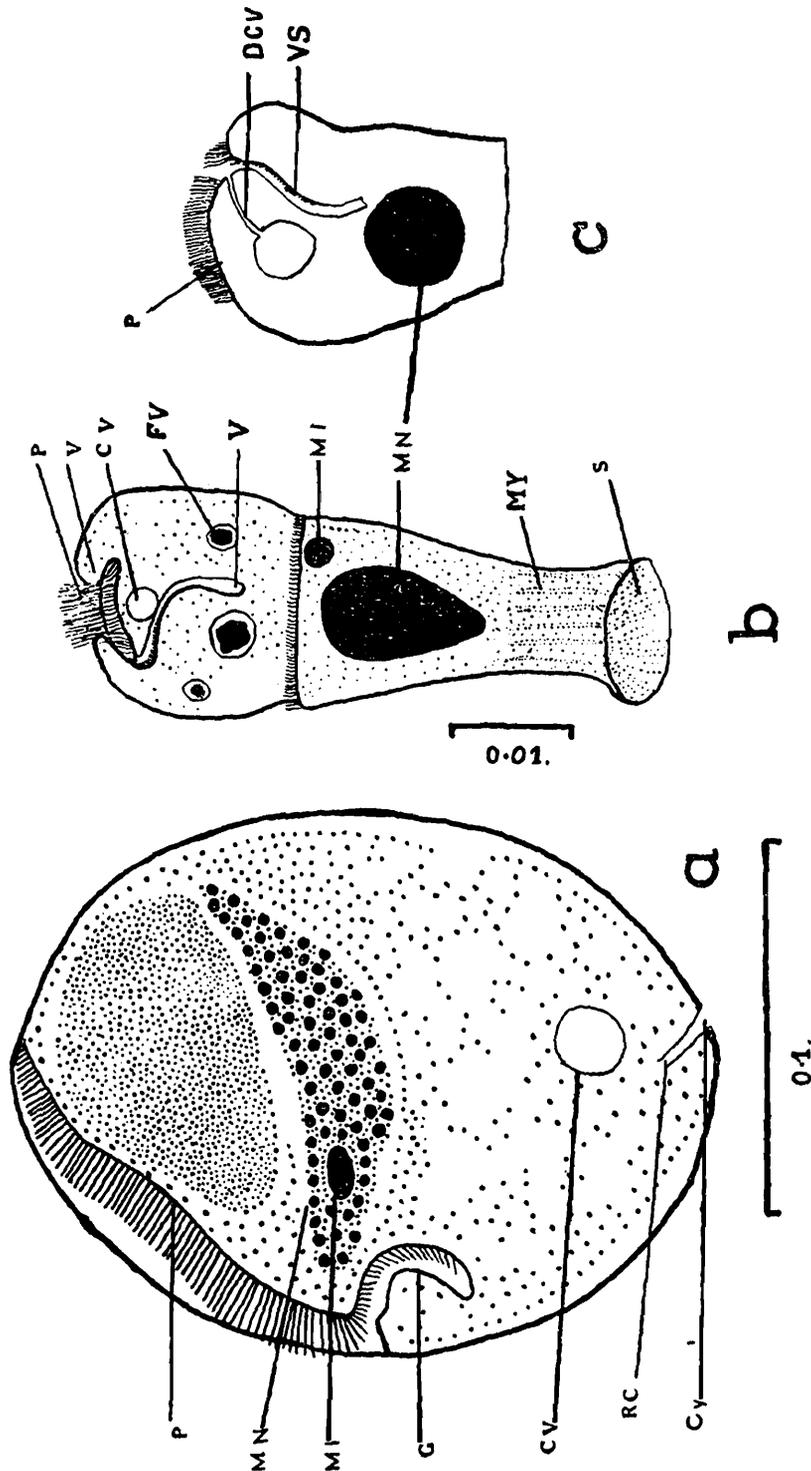


TEXT FIG. 1.—*Bodomonas rebæ*, sp. nov. (a) & (b) Stained with neutral red in living condition. (c) Stained with methylene blue showing nucleus, in living condition. AC, Anterior flagellum; B, Blepheroplast; CV, Contractile vacuole; G, Cytopharynx; HT, Host-tissue; N, Nucleus; PC, Posterior flagellum; RG, Refringent granule; VC, Vacuole.

The anterior part of the body wriggles vigorously when attached to the host tissue, gyrating with the anterior end forward,

The effect of this parasite is masked as it is always in association with *Trichodina indica*, sp. nov. or *Scyphidia pyriformis*, sp. nov. (see page 223).

Reproductive stages were not observed.



TEXT-FIG. 2(a).—*Nyctotherus pangasi*, sp. nov. (b) & (c) *Scyphidia pyriformis*, sp. nov. CV, Contractile vacuole; CY, Cytopyge; DVC, Duct of contractile vacuole; FV, Food vacuole; G, Cytopharynx; MI, Micronucleus; MN, Macronucleus; MY, Myonemes; P, Peristome; RC, Rectum; S, Scopula; V, Velum; VC, Vacuole VS, Vestibule.

*Measurements.**—Length (including the posterior flagellum) 15.0-18.0, breadth, 4.5-6.0; anterior flagellum, 5.0-6.0; contractile vacuole, 1.5-2.

* All measurements are in microns.

Remarks.—The only other species of the genus is *B. concava* Davis, 1947, found on the gills of *Pomoxis annularis*, *P. sparoides*, and *Lepomis macrochirus* in the U. S. A. These two species differ in the shape and size of the blepharoplast which in *B. rebœ* is elliptical and smaller than in *B. concava*.

Class CILIOPHORA

Order SPIROTRICHA Butschli.

Family SPIROSTOMIDÆ Khal.

Genus *Nyctotherus* Leidy.

Nyctotherus pangasia, sp. nov.

(Tex-fig. 2a)

This ciliate parasite was found in the intestine of *Pangasius pangasius* from the river Ganga at Buxar in January 1953 and from the river Cauvery at Mettur Dam in June 1953. The infected fishes were 41.3—60.9 cms. long. Smaller specimens 16.8-30.5 cms. long bought from the local fish market were free from the infection. Although infection was heavy no pathological effects were observed on the intestinal wall.

Body oval to round, Peristome reaching to middle of body, its outer margin lined with long stiff cilia. Cytopharynx arched, nearly one-fifth as long as body and extending posteriorly beyond the middle of body. A long thin cytogut visible in some specimens between the cytopharynx and the rectum. Cytopyge at the posterior end $\frac{2}{3}$ the body. Spherical contractile vacuole lateral to rectum. Protoplasm granular. Pre-nuclear part of body dark brown due to glycogen granules. Macronucleus elliptical to oval, granular in texture. Micronucleus embedded in or just posterior to the macronucleus.

Measurements.—Length, 150.8-262.2; breadth, 114-197.6; length of cytopharynx, 38.0.

Remarks.—Bhatia (1936) has given a key to nine species of *Nyctotherus* from Anuran and invertebrate hosts from India. The present species is the first record of *Nyctotherus* from an Indian fish. It is characterised by the ovoid body, the cytopharynx reaching beyond the middle of the body, and nearly a quarter of the body width in length. In these characters it resembles *N. kempi* Ghose, and *N. cordiformis* (Ehrb.) but all the three species differ from one another in the shape of the nucleus and the curvature of the cytopharynx.

Recently Sandon (1950) has recorded without description some Opa-linids, *Balantidium* and *Nyctotherus* from the Nile fishes belonging to the families Siluridæ, Mochocidæ and Citharinidæ. He did not find any relation between the feeding habit and the infection in these fishes. *Pangasius pangasius* feeds on Lamellibranch and Gastropod molluscus from the bottom of the river. Other Siluroids and Carps examined from these sources were free from the infection, therefore it is not possible to make any general comment on the relationships between the food and feeding habits and parasitisation of these fishes. However, it may be noted that only large-sized *P. pangasius* are infected.

Order PERITRICHA Stein.

Family URCEOLARIDAE Stein.

Genus *Trichodina* Ehrenberg.*Trichodina indica*, sp. nov.

(Tex-fig. 3, a-c)

This is the commonest Ciliate parasite infecting the pond fishes and their fry and fingerlings. The following table gives the incidence of infection.

Species.	No. examined.	No. infected.	Per cent infection.
1. <i>Labeo rohita</i>	2,000	1,828	91.4
2. <i>Labeo calbasu</i>	40	5	12.5
3. <i>Cirrhina mrigala</i>	2,000	1,453	76.5
4. <i>Cirrhina reba</i>	1,500	840	56.0
5. <i>Catla catla</i>	2,000	937	46.85
6. <i>Amblypharyngodon mola</i>	20	4	20.0
7. <i>Chela bacaila</i>	10	1	10.0
8. <i>Ophicephalus gachua</i>	9	2	22.22
9. <i>O. punctatus</i>	15	6	40.0
10. <i>Ambassis nama</i>	10	3	33.33
11. <i>A. ranga</i>	10	4	40.0

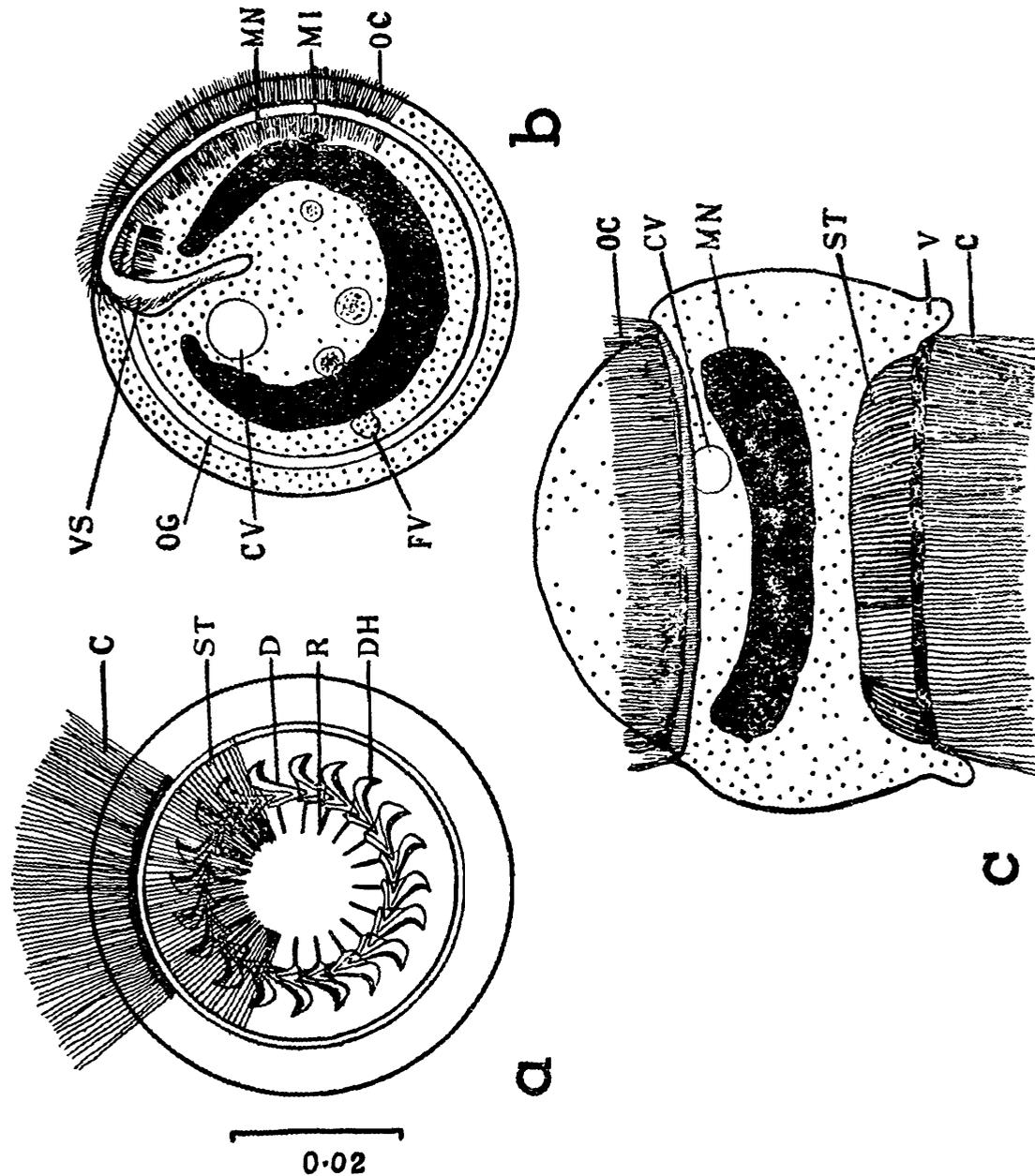
The percentage of infection in the last six species (nos. 6-11), though low and erratic, varies between 10-40. Amongst the carp fry and fingerlings the intensity of infection was generally high, being highest in *L. rohita*.

Body semicircular or sub-spherical in side view and circular in anterior view. Anterior end arched, surrounded by the oral ciliary groove which makes one anti-clockwise turn and then descends obliquely to form the vestibulum. Inner and outer margins of groove lined with cilia. Outer ciliary ring continued into the vestibule, posterior part of which is non-ciliated and forms the gut. Food vacuoles arise first near the gut and then move away towards the periphery. Contractile vacuole in anterior half of the body opening in the vestibule by a small duct. It pulsates every 4 or 5 seconds.

Aboral end with skeletal ring consisting of an outer striated ring and an inner denticulate ring, the latter consisting of 20-22 cone-shaped denticles, fitting into one another to form an interlocking ring. Each denticle with an outer winged hook and an inner ray. Striated ring formed by striæ slightly thicker at the outer end and 7-9 striæ to each denticle.

Aboral ciliary ring outer to the skeletal ring, formed by long cilia fused at their base to form a small membranelle velum, a thin protoplasmic pellicle variable in shape, outer to the aboral ciliary ring.

Macronucleus horse-shoe shaped, thicker in the centre and slightly tapering at the open ends. Micronucleus elliptical and outer to macronucleus.



TEXT-FIG. 3.—*Trichodina indica*, sp. nov. (a) Aboral side. (b) Oral side. (c) Side view; C, Aboral cilia; CV, Contractile vacuole; D, Denticle; DH, Denticle-hook; FV, Food-vacuole; MI, Micronucleus; MN, Macronucleus; OC, Oral cilia; OG, Oral groove; R, Denticle ray; ST, Striated ring; V, Velum; VS, Vestibule.

Reproduction by binary fission as in other species of the genus. Fission is more frequent during July to September (temp. 25-32°C.), decreasing during winter when the temperature falls below 20°C. Examination of individuals on two occasions during July and August 1952 showed that 30 per cent and 27 per cent of the population consisted of daughter individuals.

The parasite attaches itself to the tissue of the host by the aboral end. By an upward movement of the skeletal ring a slight vacuum is created between its body and the host tissue, thus ensuring firm fixation,

The parasite moves freely now and then on the body of the host or is sometimes found swimming about in the water as part of the plankton. When the host dies the parasite attaches itself to another host. It feeds on the desquamated epithelial cells and bacteria.

Measurements.—Total diameter, 21·9-28·4 ; height, 18·25-20·07 ; diameter of striated ring, 18·25-20·07 ; diameter of denticulate ring, 7·3-11·9.

Remarks.—There are 39 species of *Trichodina* including the present, eight of which belong to the sub-genus *Cyclochæta*. The present new species belongs to the sub-genus *Trichodina* and differs from all the other species of this sub-genus in the size of the body and the number of its denticles.

Family SCYPHIDIDÆ Khal.

Genus *Scyphidia* Dujardin.

Scyphidia pyriformis, sp. nov.

(Text-fig. 2, *b* & *c*)

This parasite was first observed infecting the fry of *L. rohita* (2·5-3·8 cms. long) on 26-viii-1952 in a nursery pond at Serampore (W. Bengal). The fry of other carps, *i.e.*, *C. catla*, *Cirrhina mrigala* and *C. reba* were not infected in this lot. The parasite was usually present on the skin, but sometimes more were present on the fins than on the gills. The infected fishes were weak and sluggish. These fry were kept in tap water for eight hours and then despatched by air, in sealed oxygen tin carriers to Jorhat (Assam) where, on arrival, the mortality was only two per cent. Subsequently this parasite has been found on fry and fingerlings of *catla*, *mrigala* and *bata* (local name for *C. reba*.). The following table gives the incidence of infection on carp fry for the year 1952-53.

Species.	No. examined.	No. infected.	Per cent infection.
<i>Labeo rohita</i>	1,200	743	61·91
<i>Cirrhina mrigala</i>	756	339	43·51
<i>C. reba</i>	946	10	1·05
<i>Catla catla</i>	800	3	0·375

Body urn-shaped or conical, anterior end round, posterior end elongate and truncate with broad frill-like 'scopula' for attachment. Single row of cilia girdling the middle of body. Peristome arched, with ciliated circular oral groove which descends obliquely to form vestibule. Velum covers the peristome completely in contracted forms. Contractile vacuole in the anterior part of body, pulsates every 3 or 4 seconds and is connected with vestibule by a small duct. Vestibule sigmoid, reaching to near the macronucleus. Food vacuoles contain desquamated epithelial cells from epidermis of the host. Macronucleus pyriform, pointing posteriorly. Micronucleus spherical, anterior to macronucleus. Protoplasm granular. Posterior part of body contractile due to presence of myonemes in protoplasm.

Reproduction by binary fission as in other peritrichous ciliates. In one specimen in a stage prior to fission, the macronucleus was divided into two, and the micronucleus into four.

The parasite attaches itself by the scopula to the host-tissue, but does not move as freely in the water as species of *Trichodina*.

Measurements.—Length, 38.5-46.9 ; breadth, 18.0-20.36 ; oral cilia, 7.8 long ; contractile vacuole, 5.0-6.0 in diameter ; macronucleus length, 8.9 ; scopula breadth, 6.0-9.0.

Remarks.—The posterior elongation of the body puts the species in the sub-genus *Scyphidia*. The pyriform nucleus, the two rings of cilia on the body and the arched peristome are the characteristic features of this species. In the shape of the nucleus it resembles *S. micropteri* Surber, but the latter species lacks the middle ring of cilia. *S. pyriformis* differs from the other two Indian species *S(S). indica* Bhatia and *S. (Gerda) purniensis* Ghose, in the shape of the nucleus and the peristome. Hirshfield (1949) has given the size and other characters of 23 species of *Scyphidia*. The present is the twenty-fourth species known, and the third to be described from India.

DISCUSSION.

From the foregoing, it would seem clear that the fry of major carps are more susceptible to infection by *T. indica* than by *S. pyriformis* or *B. rebæ*. The incidence and intensity of infection is higher in *L. rohita* than in other carps. This may be due to the biochemical properties of its mucus which forms the microhabitat for the parasite. The gills of fry below 1.8 cms. in length are not infected with any of the above parasites, though they may be present on the body surface. The density of population of the parasites specially of *T. indica* is higher between July and August (temp. 25°-32°C.) when the rate of its binary fission is also higher. Nigrelli and Atz (1943) also report higher rate of reproduction in *Trichodina* sp. from *Spheroides maculatus* between July to September when the temperature was 70°F. (23.75°C.).

As transmission of parasites from one fish to another is due to proximity, the chances of infection are greater in crowded ponds. The higher incidence of *Trichodina* infection is probably due to its freer movement in the water which enables the species to infect a larger number of fishes. The intensity of parasitisation on fry obtained from the river is less than on those from the ponds. A similar observation was made by the present author for those obtained from the river Ganga at Futwah (Bihar) in July 1951.

The presence of these parasites on the body and the gills, causes irritation, resulting in hyper-secretion of the mucus. In the case of gills, the effective respiratory area is decreased and the fish cannot breathe properly. It has been observed that in the ponds where mortality of fish is due to deficiency of oxygen, such infected fishes are the first to succumb. In the case of the heavy infection on the body, the free movements of the fins are hampered by the excess of mucous secretion,

and the fish consequently become sluggish and emaciated. Up to now, no case of mass mortality of fish in ponds, due to excessive infection by these parasites, has been recorded. But when such fishes are kept in the aquaria or in small earthen vessels (*handis*) some mortality has been observed. Nigrelli (1943) reports that *T. spheroides* and *T. halli* were responsible for 10 per cent, 4 per cent, and 12 per cent of death of fish in New York aquarium during 1939, 1940 and 1941 respectively. The focus of infection in the above cases was *Spheroides maculatus* and he suggests that fishes should not be haphazardly introduced in the aquaria. It is a well known fact that fishes and their parasites in a state of nature establish a physiological balance which is normal to them. Parasites introduced into new areas may infect new hosts with greater intensity, resulting in greater harm to them than to the accustomed hosts. It is therefore advisable to exercise due care to introduce only parasite-free fishes from one part of India to another. The introduction of exotic fishes into India is fraught with even more danger of new infection by parasites from other countries.

CONTROL.

The three species of ectoparasites are killed in 5 to 10 minutes in 2-3 per cent salt solution. The time and concentration may be varied depending on the physical condition of the fish and the intensity of infection. In some cases, more than one treatment in a saline bath may be necessary. Davis (1947) has suggested the use of 3 per cent salt solution or 1 : 500 acetic acid solution, or 1 : 4000 formalin (40 per cent) solution. The two latter chemicals have been tried with success in the laboratory in proportions as follow : 1 : 1000 acetic acid solution, and 1 : 5000 to 1 : 6000 formalin solution. A salt bath is usually preferred and recommended for field use under Indian conditions on account of its low cost and ease of application, particularly when handling sick fry.

SUMMARY.

Nyctotherus pungasi, sp. nov. is described from the intestine of *Pangasius pangasius*. *Trichodina indica*, sp. nov., *Scyphidia pyriformis*, sp. nov., and *Bodomonas rebæ*, sp. nov., are described from the gills and skin of fry and fingerlings of Indian major carps. Their pathological effects and methods of control are also given.

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REFERENCES.

- BHATIA, B. L., 1936.—Faun. Brit. Ind., *Ciliophora*, p. 493. London, Taylor and Francis.
- DEMELLO, I. F. & VALES, C., 1936.—On a Trypanosome found in the blood of the Indian freshwater fish *Clarias batrachus* Linn. *Proc. Ind. Acad. Sci. B.* 6, pp. 403-404.

- DAVIS, H. S., 1947.—Studies on Protozoan parasites of Freshwater Fishes. *Fishery Bull., Fish and Wildlife Service.* **51**, No. 41, pp. 1-29.
- HIRSHFIELD, H., 1949.—*Urceolaria karyolabia*, sp. nov., *Trichodina tegula*, sp. nov. and *Scyphidia ubiquita*, sp. nov., three new ciliates from Southern Californian limpets and turbens. *Journ. Morph.* **85**, pp. 1-34.
- JONES, S. & JOB, T. J., 1938.—An interesting case of animal association (Synoecy?) between a brackish water fish *Acentrogobius neilli* (Day) and *Zoothamnium* sp. *Curr. Sci.* **6**, pp. 558-559.
- KHAJURIA, H. & PILLAY, T. V R., 1952.—On a new species of *Zoothamnium* Stein, (Protozoa, Vorticellidæ) from the grey Mullet *Mugil-tade* Forsk. *Rec. Ind. Mus.* **48**, pp. 55-58.
- NIGRELLI, R. F., 1940.—Mortality statistics for specimens in New York aquarium, 1939. *Zoologica*, N. Y. **25**, pp. 525-552.
- , 1943.—Cases of disease and death of fish in captivity. *Zoologica*, N. Y. **28**, pp. 203-216.
- & ATZ, J. W., 1943.—Biometry of Puffers and their parasites. *Zoologica*, N. Y. **28**, pp. 1-8.
- PAI, K. T., 1950.—Fibrillar system of *Trichodina pedunculatus* Ehrb. and *Trichodina bulbosa* Davis. *Sinensia*, N. S. **1**, pp. 90-111.
- & WANG, C. C., 1948.—The variation of *Nyctotherus ovalis* Leidy and its fibrillar system. *Sinensia.* **18**, pp. 43-58.
- SANDON, H., 1949.—Opalinids from Nile fish. *Nature.* pp. 164, 410.
- SURBER, E. W., 1940.—*Scyphidia micropteri*, a new Protozoan parasite of largemouth and smallmouth black bass. *Trans. Amer. Fish Soc.* **62**, pp. 169-175.
- , 1942.—*Scyphidia tholiformis*, a Peritrichous Protozoan found on the gills and external surfaces of *Micropterus dolomieu* and *Micropterus salmoides*. *Trans. Amer. Fish Soc.* **72**, pp. 197-203.
- SUZUKI, S., 1950.—Studies of Urceolarid ciliates of Japan. *Bull. Yamagata Univ. (Natural Science).* **2**, pp. 182-217
- THOMSON, S., KIRKEGAARD, D. & JAHN, T., 1947.—*Scyphidia ameyri*, a Peritrichous ciliate from bullhead. *Trans. Amer. Micro. Soc.* **62**, pp. 315-317.
- TRIPATHI, Y. R., 1948.—A new species of ciliate, *Trichodina branchicola*, from some fishes at Plymouth. *J., Mar. Biol. Assoc., U.K.*, **27**, pp. 440-449.