A KEY to the COMMON PARASITIC PROTOZOANS

of

NORTH AMERICAN FISHES

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Private, state, and federal fish husbandry industries suffer great losses each year because of disease and parasites. The parasitic protozoans included in this key are the ones most commonly associated with fish mortalities. A total of 23 genera of parasitic protozoans may be identified by use of this key.

The fish protozoan parasites are responsible for a large part of the mortalities that occur at fish hatcheries each year. This is because they are capable of building up tremendous populations within relatively short periods of time, and some are capable of causing extreme damage to fish.

Proper treatment and control of the diseases caused by the various protozoans are impossible without knowing their identity. This key will be helpful to fishery workers in identifying the more common genera. It must be remembered, however, that a microscope and knowledge of its use are absolute prerequisites for identifying protozoans.

Certain parasitic protozoans cannot be identified below the rank of Order without use of special techniques; therefore, all known genera are not included in the herein reported key. Protozoans belonging to such Orders should be sent to a specialist for identification.

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EXAMINATION OF FISH

The majority of protozoans known to cause fish mortalities occur on the gills and body of fish. However, certain of the protozoans which occur internally can also cause mortalities.

Fish should be examined fresh and while the parasites are still alive if possible since their movement aids in locating and identifying them. Dead fish should not be used for the examination because many of the ectoparasitic protozoans tend to drop off soon after the fish dies. Many free-living protozoans, which feed on bacteria and dead organic matter, tend to accumulate on the carcass and may be confused with the pathogen. Preservation of the fish host in formalin or alcohol will cause a distortion of many of the protozoans, making identification difficult if not impossible.

In examining the fish for protozoan parasites, the first step is to remove fins and gills and place them in a drop of water on a microscope slide. However, in the case of large fish only portions of the gills and fins should be used. Next, place a cover slip over the material and examine with a microscope using the low power objective first. The material should then be examined using the high dry and oil immersion objectives. The body of the fish should be scraped with a scalpel and part of the material placed in a drop of water on a microscope slide and examined. Any cysts or nodules noted on fins, body, or gills should be removed, opened, and examined with the microscope since many of the sporozoans are found in cysts at these locations.

There are two techniques used in examining blood for protozoans. The best is that of Strout (1962), in which several drops of whole blood are placed in

a small vial and allowed to clot. A drop of clear serum is then removed by a pipette, placed on a microscope slide, and examined for living, moving specimens using either high dry or oil immersion objective. The second method is use of a drop of fresh whole blood smeared on a microscope slide, allowed to dry, stained with Giemsa's or Wright's stain (Kudo, 1954), and then examined with the oil immersion objective.

Next the fish is opened, the internal organs removed and placed in a dish containing physiological saline. The intestine should be opened, scraped and part of this material placed in a drop of saline or a microscope slide and examined with the high dry objective. Only four protozoans have been reported as occurring in the intestines of fishes: Hexamita, a flagellate; Balantidium, a ciliate; Schizamoeba, an amoeba; and Eimeria, a sporozoan. Schizamoeba has been reported in trout only once (Davis, 1926). Therefore, it has not been included in this key. However, Hoffman (personal communication, 1965) reports that it is found commonly in West Virginia.

The other internal organs should be examined next. Cysts of sporozoans are common in the mesentaries, pyloric caeca, intestine, liver, gonads, kidney, and gall bladder. Some ciliates (Vauchomia) have been found in the urinary bladder and ureters of fish.

CLASSIFICATION OF PROTOZOANS

Phylum Protozoa

The protozoa are single-cell organisms composed of a plasma membrane or pellicle, cytoplasm, one to several nuclei; many have specialized structures that aid in locomotion, food capture, attachment or protection. In protozoans

the functions of ingestion, digestion, gas exchange, and osmotic regulation are performed by various organelles of the cell instead of by separate organs composed of many tissues as occurs in multi-cellular animals.

Protozoans for the most part are microscopic, varying in size, shape, and color. Ichthyophthirius is one of the few protozoans that can be seen with the naked eye. The classificiation used in this report is that of Kudo (1954).

Class Mastigophora (Flagellates)

These are protozoa that possess one to eight flagella, although the majority have one to four. However, a few, which are not parasitic on fish, may have more than eight flagella. The flagella are used both for locomotion and as attachment organelles.

Nutrition is by one of three methods: (1) holozoic - utilize plants and animals as food; (2) holophytic - obtain their nutrients by photosynthesis; and (3) saprozoic - obtain their nurishment by diffusion of organic matter through the body wall. Digestion in the holozoic protozoa occurs in a vacuole that forms around the food particles as they enter the body.

Generally there is one nucleus, but a few are multi-nucleated. Asexual reproduction is generally by longitudinal fission or sometimes by budding. Sexual reproduction may occur in some species.

Class Ciliata

These are protozoa that move by means of cilia, although some become sedentary when mature. Generally they possess two types of nuclei: a large conspicuous macronucleus and a small inconspicuous micronucleus. Asexual reproduction is by binary fission or multiple fission in cysts, and sexual

reproduction is by conjugation.

Nutrition may be either holozoic or saprozoic. The cytostome or mouth usually lies in a depression called the peristome, which is characterized and defined by cilia. The ciliary movement around the cytostome aids in guiding food into the cytostome. In some ciliates the cytopharynx is lined with rod-like supportive structures called trichites and the entire structure is called a "pharyngeal basket".

Class Suctoria

This group of protozoans is characterized by the presence of tentacles and the absence of cilia or other organelles of locomotion in the mature stage. However, cilia are present on young individuals but are lost with the development of tentacles.

The suctorians possess two types of nuclei, a large conspicuous macronucleus and a small inconspicuous micronucleus. Asexual reproduction is by binary fission or by budding, and sexual reproduction is by conjugation.

Capture of food is exclusively by the tentacles, and there is no cytostome. There may be two types of tentacles present; one type is suctorial in function, the other is used in capturing food.

Trichophrya is the only genus in this class that has been reported from fish.

Class Sporozoa

All species within this class are parasitic and all lack organelles of locomotion. However, when in the immature stage, some can move by pseudopodia.

The spore is usually covered with a resistant chitinoid covering that enables it to withstand unfavorable conditions. Reproduction must include both sexual and asexual phases for completion of the life cycle.

HOW TO USE KEY

The key gives two choices, numbered 1a and 1b, 2a and 2b, 3a and 3b, etc., and on the right side of each choice is either a number in parentheses, a name of a group of protozoans or a particular protozoan. If there is a number on the right, go to that couplet with that number and again determine which of the two choices or descriptions most nearly fits the organism being identified. This procedure is repeated until a choice is reached where the name of the protozoan or the group to which it belongs is given. Immediately preceeding the name of the organism is the figure number, which refers to the correct illustration. The illustration should be compared with the organism being identified to verify the correctness of the identification.

KEY TO PARASITIC PROTOZOA

1a. Possess 1 or more flagella. Class Mastigophora ---------- (2)

1b. Lacking flagella ---------- (12)

2a. (1a) Transverse groove present; 2 flagella present; chromatophores present (usually seen without flagella, see 25b).
Order Dinoflagellata ---------- (3)

2b. Transverse groove absent; 1 to 8 flagella present; chromatophores present or absent ---------- (4)

3a. (2a) Attached to gill filaments of marine fish by cytoplasmic processes; stigma present; 12 microns by 8 microns. Fig. 1. ---------- Oodinium ocellatum

3b. Attached to integument of fresh-water fishes; stigma absent; 12 to 20 microns by 7.5 to 13 microns. Fig. 1. ---------- Oodinium limneticum

4a. (2b) Chromatophores absent ---------- (5)

4b. Green Chromatophores present; Order Euglenoidina. Ectoparasitic on gills of Pomoxis spp. Fig. 2. ---- Euglenosoma branchialis

5a. (4a) Flagella 1 to 2; Order Protomonadina. ---------- (6)

5b. Flagella 3 to 8; Order Polymastigina. ---------- (10)

6a. (5a) With one flagellum. ---------- (7)

6b. With two flagella; free flagella may be extremely difficult to see. --- (8)

7a. (6a) Endoparasitic; in circulatory system; undulating membrane present. Fig. 3. ---------- Trypanosoma.

7b. Ectoparasitic; on gills of Pomoxis spp.; 15 to 20 microns long by 3 to 4 microns wide. Fig. 4. ------- Lamellasoma bacillaria.

8a. (6b) Endoparasitic; in circulatory system; with undulating membrane; with one flagellum trailing at posterior end. Family Cryptobiidae. Fig. 5. ---------- Cryptobia.

8b. Ectoparasitic; on body or gills. ---------- (9)

9a. (8b) With rod-shaped blepharoplast; length 6 to 8 microns; a wide ventral groove from anterior end to middle of body. Fig. 6. ------ ---------- Colponema.

9b. Blepharoplast round ---------- (28)

10a. (5b) Ectoparasitic; 4 flagella, 2 long and 2 short. Fig. 8. -------
---------- Costia. (11)

10b. Endoparasitic; 6 flagella, 2 axostyles. Fig. 9. ----------
---------- Hexamita (Octomitus) sp.

11a. (10a) Free-swimming form has been reported only from salmonids; length 9 to 14 microns. Fig. 8.----- Costia pyriformis.

11b. Common on both warm and cold water fishes; length 10 to 20 microns. Fig. 8. ----------Costia necatrix.

12a. (1b) Cilia present throughout life of individual. Class Ciliata ----- (13)

12b. Neither cilia nor flagella present ---------- (25)

13a. (12a) Adoral zone of membranelles a funnel-like arrangement of small cilia leading to mouth)present. ---------- (16)

13b. Adoral (anterior) zone of membranelles (cilia) absent. Order Holotricha. ---------- (14)

14a. (13b) Cytostome at or near anterior end, not visible in large individuals; macronucleus large, horseshoe shaped; covered with short cilia; up to 1 mm in diameter and visible to the naked eye; ectoparasitic. Fig. 10. ----------Ichthyophthirius multifiliis

14b. Cystostome ventral, in anterior half; macronucleus rounded; smaller, never visible to naked eye; ectoparasitic ---------- (15)

15a. (14b) Pharynyeal basket absent; cytostome slit-like, on convex ventral surface; ciliation is uniform and complete. Family Amphileptidae. Fig. 11. ----------Amphileptus

15b. Pharyngeal basket present; cytostome round, on concave ventral surface; ciliation incomplete; parallel rows of cilia on ventral surface. Family Chlamydodontidae. Fig. 12. ---------- ---------- Chilodonella (Chilodon)

16a. (13a) Ciliation incomplete; adoral zone winds counter-clockwise to cytostome. Order Peritricha ---------- (17)

16b. Ciliation complete; adoral zone winds clockwise to cytostome; peristome sunk in funnel-like hollow at anterior end; endoparasitic in intestine. Rare in fish. Order Spirotricha. Fig. 13. ---------- ---------- Balantidium

17a. (16a) Attached to host; ectoparasitic ---------- (18)

17b. Free-swinnimg on body and gills, but with highly developed attaching organelles including denticular ring. Family Urceolariidae --- (22)

18a. (17a) Without stalk. Family Scyphidiidae ---------- (19)

19a. (18a) Macronucleus ribbon-shaped; without large adoral membrane; body usually cross-striated; ciliary ring around adoral end. Fig. 14. ---------- Scyphidia

19b. Macronucleus compact, not ribbon-like; with large adoral membrane; body usually not cross-striated. Fig. 15. ---------- ---------- Glossatella

20a. (18b) Stalk non-contractile; individuals on dichotomous stalk. Family Epistylidae. Fig. 16. ------- Epistylis

20b. Stalk contractile. Family Vorticellidae ---------- (21)

21a. (20b) Individual stalks contract independently ----------
---------- Carchesium

21b. All stalks in a colony expand or contract simultaneously. --------
---------- Zoothamnium

22a. (17b) In urinary bladder; adoral zone of cilia making 2 or more turns (720° of more). ---------- Vauchomia

22b. Not in urinary bladder; all ectoparasitic. Adoral zone of cilia making one and one-half turns or less (less than 720°)---------- (23)

23a. (22b) Adoral zone of cilia making three-quarters of a turn or less (180° to 270°) ---------- (24)

23b. Adoral zone of cilia making three-quarters to one and one-half turns (270° to 540°). Fig. 19. ----------Trichodina

24a. (23a) Inner part of denticles, the thorn, well developed; on gills ---------- Tripartiella

24b. Denticles lack thorn, or if thorn present it forms a small hook. ---
---------- Trichodinella

25a. (12b) Chromatophores absent; without locomotive organelle ------- (26)

25b. Chromatophores present; attachment organelle pseudopodia. Fig. 1. ----------Class Mastigophora Oodinium

26a. (25a) Cilia present in larval stage; possess contractile vacuoles; all ectoparasitic; tentacles present in mature stage. Class Suctoria. Fig. 17. ----------Trichophrya

26b. Lack tentacles and contractile vacuoles; all endoparasitic; spores usually in a cyst. Class Sporozoa ---------- (27)

27a. (26a) Spore shell bivalve; 1 to 4 polar capsules. Usually in cysts in tissue, but some in urinary and gall bladders. Fig. 18. ---------- Order Myxosporidea

27b. Spore shell in one piece; 1 to 2 polar filaments (difficult to see); 4 to 5 microns in diameter --------- Order Microsporidea.

28a. (9b) Length about 12 microns; a wide ventral groove which fades out posteriorly; anterior flagellum easily seen. Fig. 7. ---------- Bodomonas.

28b. Length 9 to 20 microns; attached to skin or gills; wide ventral groove lacking; anterior flagellum difficult to see. Fig. 8. ---------- Costia.

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Drawings shown in Fig. 1, 5, 8, 10, 12, 14-19 are original. Those shown in Fig. 2, 4, 6, 7, and 11 are modified from Davis (1947), and Fig. 9 is modified from Davis (1926). Drawings shown in Fig. 3 and 13 are diagrammatic.

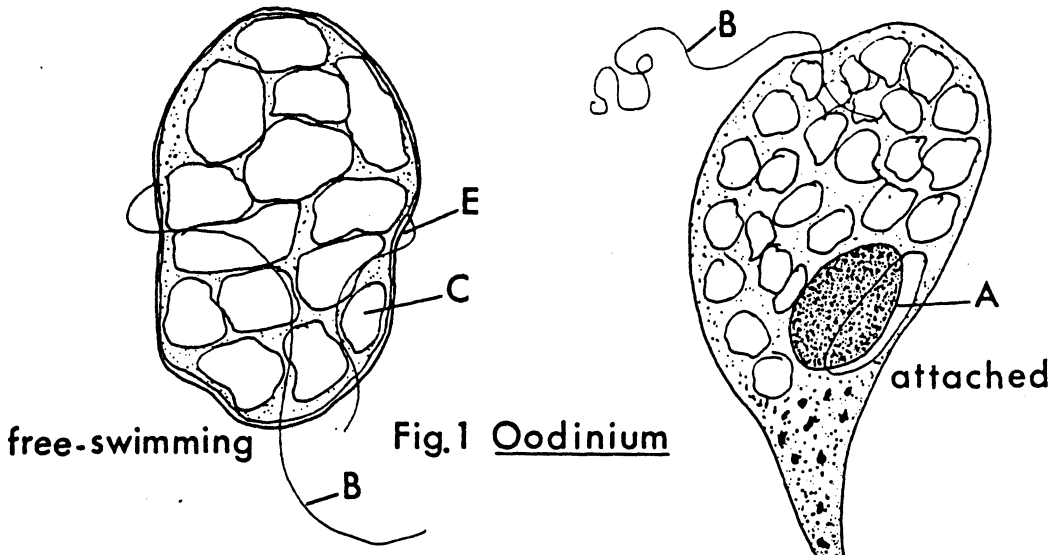

Fig. 1 Oodinium

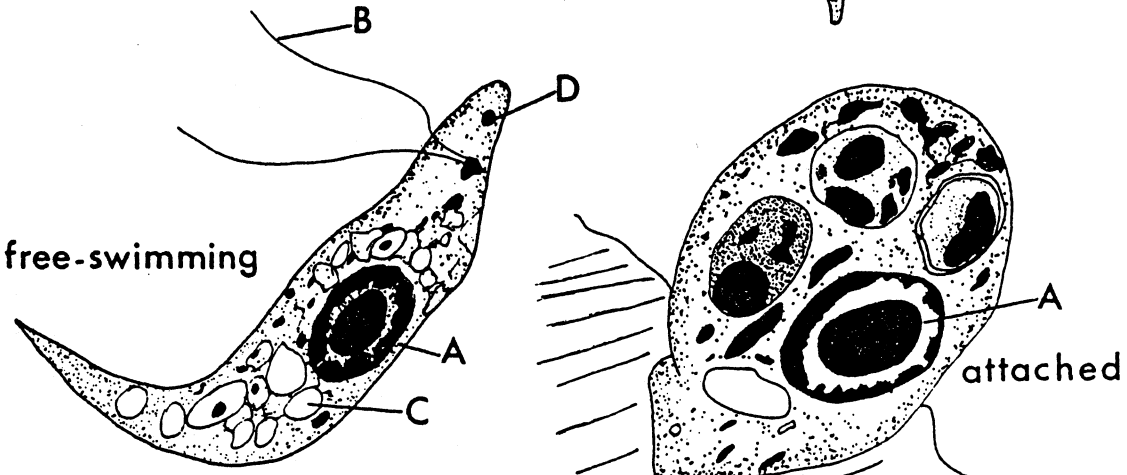

Fig. 2 Euglenosoma

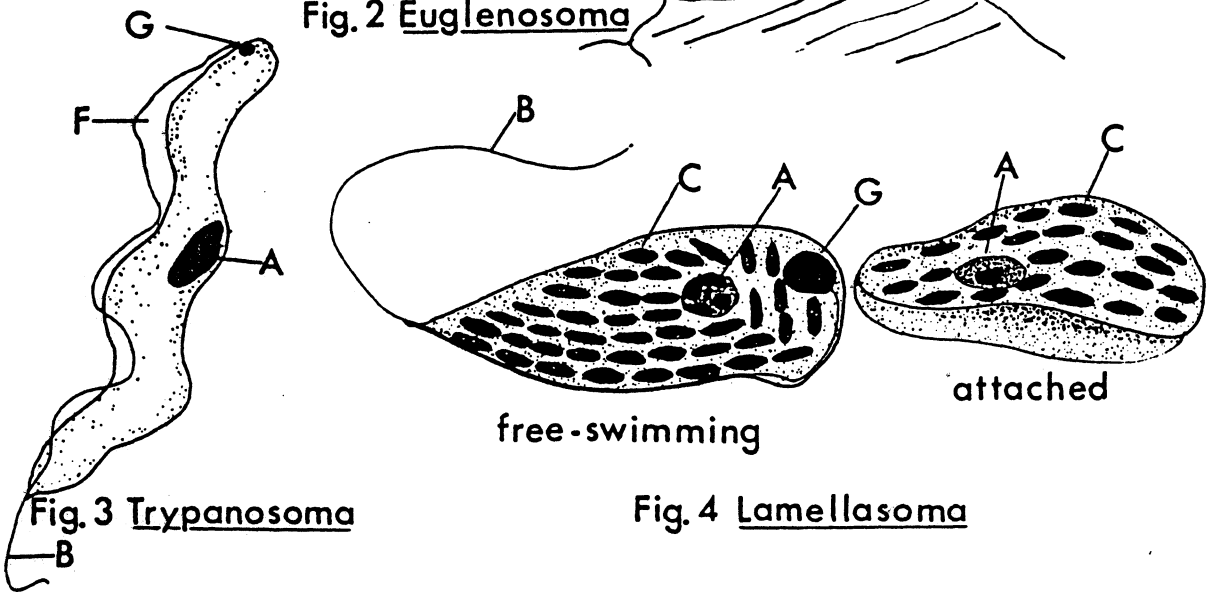

Fig. 3 Trypanosoma

Fig. 4 Lamellasoma

PLATE 1

Legend: A. nucleus B. flagellum C. chromatophores D. stigma
E. transverse groove F. undulating membrane
G. blepharoplast

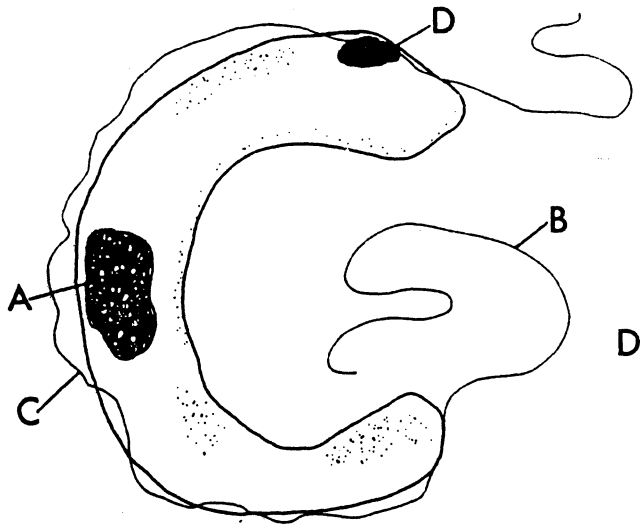

Fig. 5 Cryptobia

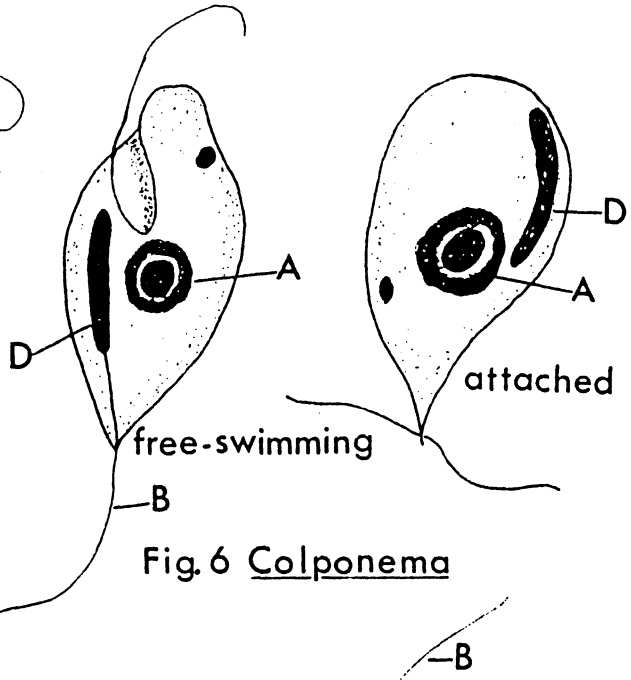

Fig. 6 Colponema

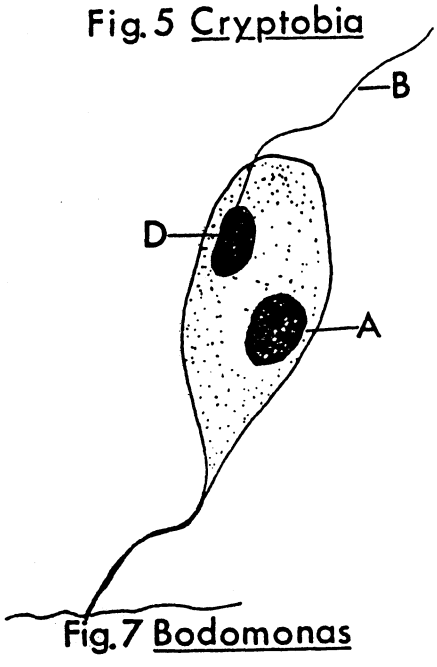

Fig. 7 Bodomonas

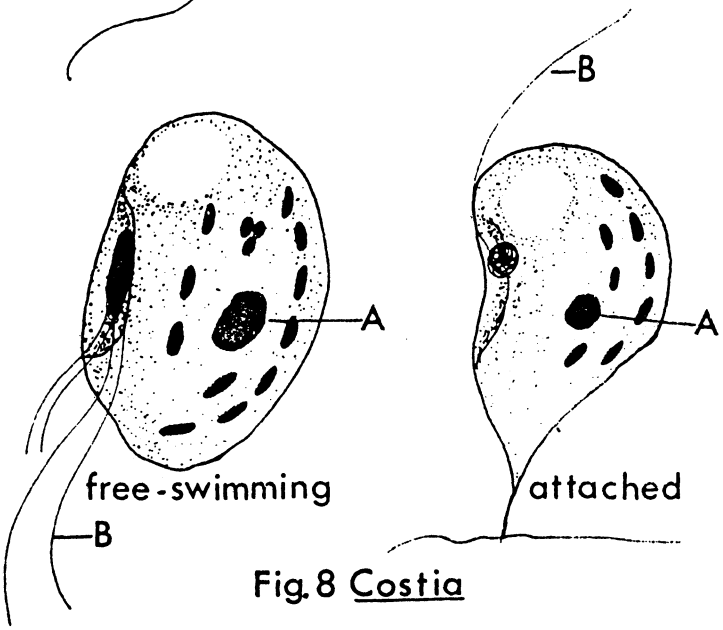

Fig. 8 Costia

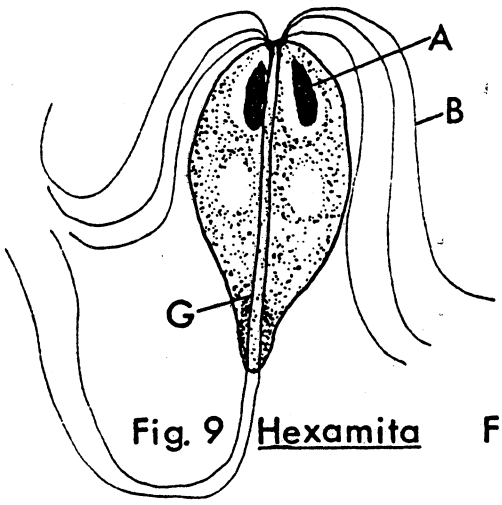

Fig. 9 Hexamita

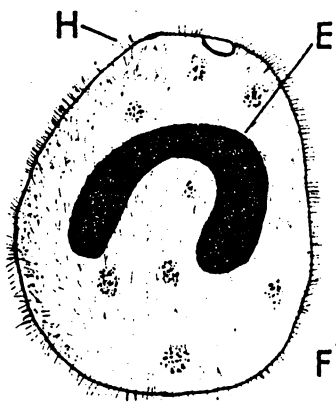

Fig. 10 Ichthyophthirius

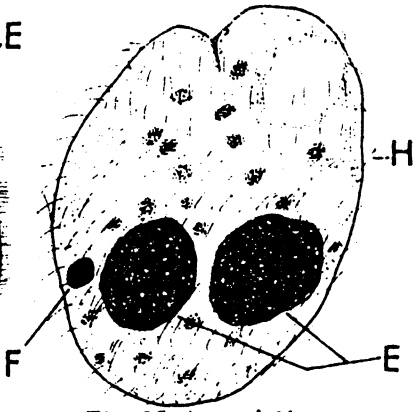

Fig. 11 Amphileptus

PLATE 2

Legend: A. nucleus B. flagellum C. undulating membrane
D. blephoroplast E. macronucleus F. micronucleus
G. axostyle H. cilia

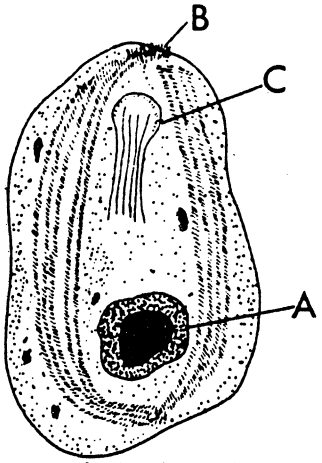

Fig.12 Chilodonella

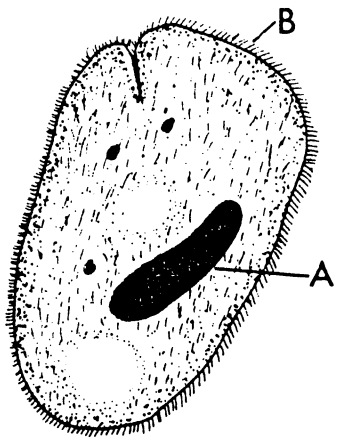

Fig. 13 Balantidium

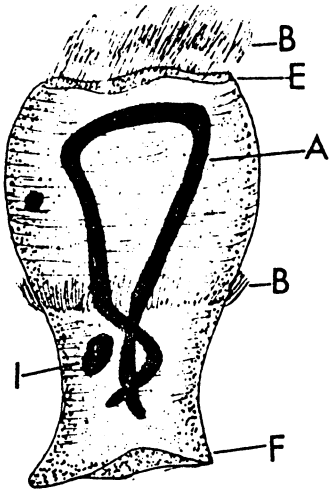

Fig.14 Scyphidia

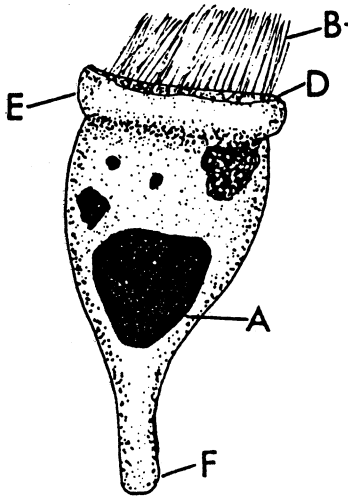

Fig.15 Glossatella

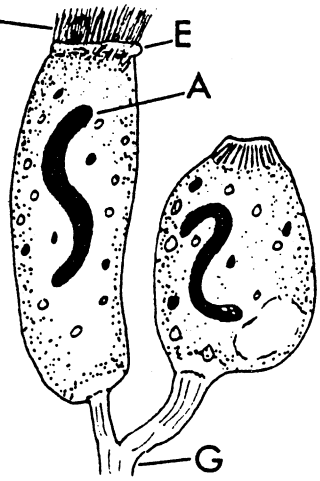

Fig. 16 Epistylis

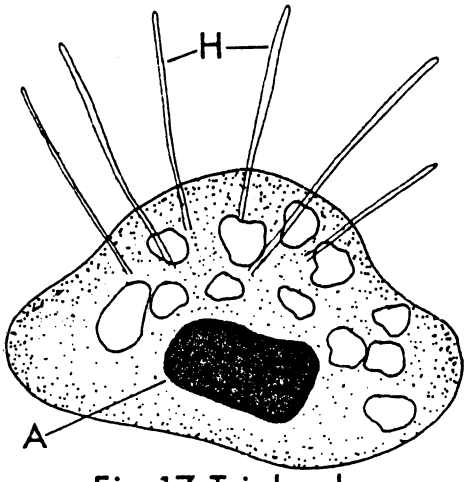

Fig. 17 Trichophrya

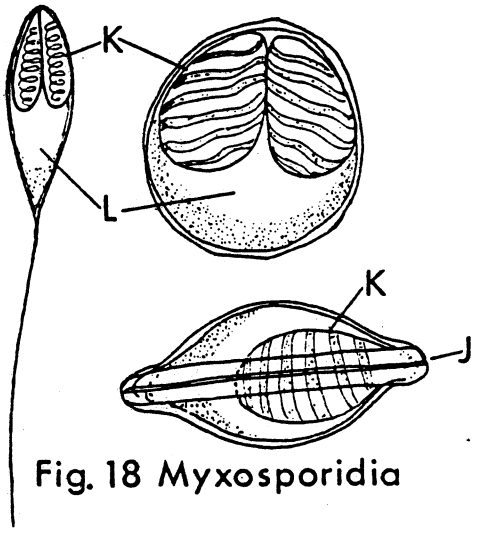

Fig. 18 Myxosporidia

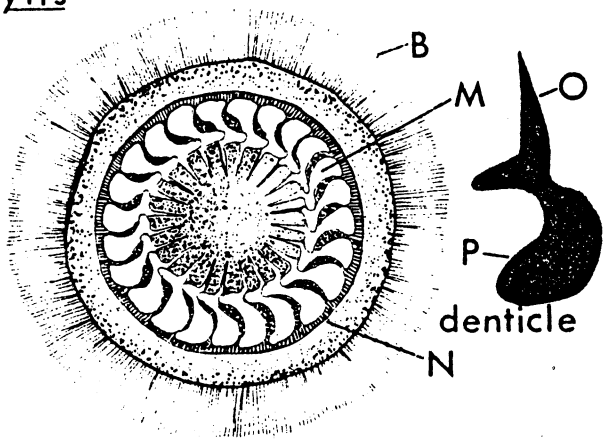

Fig. 19 Trichodina

PLATE 3

Legend: A. macronucleus B. cilia C. pharyngeal basket D. adoral membranelle E. peristome F. scopula G. stalk H. tentacles I. micronucleus J. suture line K. polar capsule with enclosed polar filament L. sporoplast M. denticle N. radial pins O. thorn P. blade

GLOSSARY

Adoral - refers to the area near or around the mouth.

Adoral zone of membranelles - an orderly arranged group or line of membranelles associated with the oral surface. Fig. 15D.

Axostyle - flexible structure, the anterior end of which is near the anterior end of the body and extends lengthwise through the cytostome and ending near the posterior end of the body, or extending beyond the body surface. Fig. 9G.

Chromatophore - a compact mass of pigment granules within the cytoplasm. May be different shapes. Color may be green, brown, yellow, orange, or red.

Cilia - hair-like processes. Organelles of locomotion. Fig. 10H.

Cytostome - the true mouth, present in all holozoic ciliates except those those possessing feeding tentacles.

Denticle - a chitin-like structure composed of an inner thorn, an outer blade, and a center portion, the denticle proper. In trichodinids. Fig. 19M and 19O.

Denticular ring - composed of a number of chitin-like denticles and forming a supporting structure in the trichodinids. Fig. 19.

Ectoparasite - a parasite living on the surface of the body or gills of the host.

Endoparasite - a parasite living within the body or tissues of the host.

Flagellum - a filamentous or thread-like extension of the cytoplasm, an organ of locomotion. Fig. 8B.

Micron - a unit of length equal to 0.001 millimeter or 1/25,000 of an inch. The field of view of a microscope using a 10-power (10X) eyepiece and a 10-power objective is approximately 1600 microns; using a 10-power eyepiece and a 43- to 45-power objective the field of view is approximately 320 to 400 microns; using a 10-power eyepiece and a 96-power objective the field of view is approximately 160 microns.

Peristome - the pouch or depression in the oral area characterized and actually defined by its possession of buccal cilia. Fig. 14E.

Polar capsule - a special "cell" located at the anterior end of a spore (Myxosporidea and Actinomyxidia only) which encloses a polar filament. Fig. 18K.

Polar filament - a long spirally coiled delicate thread enclosed within the polar capsule. Fig. 18K.

Stigma - eye-spot; usually found in anterior region; appears as a reddish or brownish dot or rod. Fig. 2D.

Undulating membrane - in flagellates a delicate membrane extending out from the side of the body with a flagellum bordering the outer margin. In ciliates a ciliary membrane often present as part of the buccal apparatus. Fig. 3F.

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