BASIDIOBOLUS MERISTOSPORUS OF TAIWAN

by

BAO-YU YANG

INTRODUCTION

The fungus discussed in this paper was isolated from fish pond water, while the the writer was studying Saprolegnia of fish pond water in the vicinity of the National Taiwan University, Taipei, Taiwan, 1960. Since then it was kept in pure cultures in potato agar slants incubated at 5°C, in the Department of Botany, National Taiwan University.

In respect to its systematic position, three renowned mycologists have been consulted. Both Dr. C. J. Alexopoulos[1] and Dr. John Couch[2] had kindly examined my culture before they sent it to Dr. Charles Drechlser[3] for identification of species. To my presumptive identity, placing the fungus under Basidiobolus, Dr. Drechlser wrote, "The fungus, of course, is a species of Basidiobolus", a genus of the Entomophthoraceae. Coincidentally, the present fungus was previously described by Dr. Drechlser, 1955[4], belonging to the species B. meristosporus which occurs widely in the U. S. A. Nevertheless, it is the first time found in Taiwan.

Emmons, 1959[5] reported that Basidiobolus had caused infection to human disease. It is my attempt to find out whether B. meristosporus would cause any infection to Cyprinus carpio L. and their eggs. The life cycle of B. meristosporus consists of elongate conidia, globose conidia, microconidia and zygospores. On careful scrutiny, the writer observed that it was the elongate conidia that caused damage to Cyprinus carpio and their eggs both dead and living.

MATERIALS AND METHODS

1. The materials for this study were based on the cultures isolated from fish pond water as stated in the foregoing paragraph.
2. The study was approached by the following methods:
   1) Studies made from cultures grown in potato dextrose agar slants. Inoculations to new slants were made every three months or shorter intervals and incubated

(1) Prof. of University of Texas, Austin, Texas, U. S. A.
(2) Prof. of University of North Carolina, N. C., U. S. A.
(3) Myologist, Plant Industry Station, Beltsville, Maryland, U. S. A.
(4) Drechlser. A Southern Basidiobolus forming many sporangia from glonose and elongated adhesive conidia. Journ. of Washington Academy of Sciences, Vol. 45, 49-56 1955.
(5) Emmons and Others, 1959 Basidiobolus and Cercospora from Human Inecrions. Mycologia 48, 1-11 1957.

at first, at 28°C for two days, and then at 5°C until the time for use.

2) Studies made from cultures grown in agar plates which are convenient for microscopic study on the morphology of cells, colony formation and other details of the fungus without disturbing the growing culture. A plate carefully prepared will serve for a few weeks of study, if contaminations of alien are prevented.

3) Studies made from slide cultures- a lump of spores or small fragments from the agar slant were transferred to an agar block (1 cm.×1 cm.) which in turn was placed on a clear slide covered with a clean cover slip. The cultures grown between the slide and the coverslip would be excellent material for detailed microscopic study. The processes of making such slide cultures are outlined in Alexopoulos 1952[6]. The slide cultures should be placed in a moist chamber for periodic examination on their development. The spore germination, the cell structure and divisions and the stages in the formation of zygospores were studied by this device.

3. Potato dextrose agar medium-. It is very satisfactory for the growth of the present fungus.

Peeled, diced potatoes	200 grams.
Dextrose	20 grams.
Agar	15 grams.
Distilled water	1000 mls.

Sucrose (10 grams) may be substituted for dextrose.

4. Infections on the eggs and young ones of Cyprinus carpio Linne'. Eggs 2 days old and young fishes one week old were obtained through the assistance of Mr. Ju-Shey Ho, from the hatchery of the Zoology Department, National Taiwan University. The eggs were collected from the roots and leaves of Eichornia crassipes Solms. (water hyacinth). 50 eggs were selected and placed in a clean petri dish A containing distilled water, 50 in petri dish B containing original pond water from which the eggs were collected. Both dish A and dish B were inoculated with some fragments of B. meristosporus taken from tube cultures. Petri dish C containing the same pond water and the amount of eggs as that of petri dish B but without the fungus served as a controlled plate. This experiment was to find out the infection of the present fungus by comparing the number of eggs hatched from each dish.

Some twenty young fishes one week old were distributed to two glass jars (7 in. in diameter ×3 in. in depth). It was probably due to the sudden change of environment half of the number of fishes in each jar were sickly infected with Achyla oblogata and other microorganisms. The number of fishes left in each jar was 6 and 12 respectively. A few days later, however, they seemed to be

(6) Alexopoulos, Const. J. 1952. Lab. Manual for Intro. Mycology. 9-10.

well adjusted again. So, on May 14 several pieces of fungal culture were transferred to a small stender dish which in turn was set into the jar with only 6 fishes left. It was so arranged that the fishes were in constant contact with the fungus while the latter was kept remained in the stender dish without being destroyed by the former.

OBSERVATIONS

1. By means of the three common ways of fungal study, a life cycle of the present fungus has been completed. A description of asexual and sexual stages with figures is given under the life cycle section below.
2. Infection of Basidiobolus meristosporus on carp eggs was as follows:
   Plate A. (distilled water).....9 hatched, out of 50 eggs.
   Plate B. (pond water).....6 hatched, out of 50 eggs.
   Plate C. (pond water).....2 hatched, out of 50 eggs.
   Plate A and Plate B inoculated with the present fungus while Plate C was not.

   Nearly all the dead eggs (either unfertilized or killed by fungal infections) and egg shells, were badly infected with species of Achyla in one or two days after the inoculation of B. meristosporus. It is obvious that fish pond water is often full of spores of Achyla species which generally infect fish eggs as well as young fishes. There are only two instances shown in Plate IV. that the fungal infection befell on young fishes. In a word, members of Achyla are more susceptible to their infection on fish eggs and young fishes than B. meristosporus.
3. Transferring of B. meristosous to young fishes. Transferr took place on May 14, in the glass jar of 6 fishes. Two comparatively small fishes died immediately on the next day.
   May 18..... first of the larger 4 died (about 1 cm. long)
   May 21..... second of the 4 died, all disorganized except the skeleton left as shown Plate V, fig. 11.
   May 21..... first death occurred in the other jar without inoculation of the fungus. Probably due to starvation or other causes.
   May 24..... the fifth death in the inoculated jar occurred.
   June 21..... the sixth, death, the last of the six fishes of the inoculated jar finally died.

   All the dead fishes were examined under microscope, fungal attack could be noted externally.

   All the fishes of the other jar, not inoculated with B. meristosorus, are free from infection of any fungus, only one death occurred. This might lead one to conclude that the present fungus did cause some damage to the lives of young fishes as shown in the present study. It also showed that the development of the present fungus could be carried out on potato dextrose agar medium as well

as in water though the latter was not as ideal as the former.

LIFE CYCLE OF BASIDIOBOLUS MERISTOSPORUS

The life cycle of B. meristosparus involves the following stages:

1. The resting cells. When fragments of the fungus were transferred from an old slant culture to a slide and examined under the microscope, they showed patches of thick-walled, globose to barrel-shaped spores and no mycelia were present. (Plate II, Fig. 1). As soon as the return of favorable conditions with sufficient moisture and nutrients, they set off cell divisions, longitudinally as well as transversely (Plate 11 Figs. 2 & 3). Each new cell contains a nucleus, centrally located. Then elongation of the cell takes place.
2. The elongate conidia. Shortly after a period of cell division, the new cells begin to elongate, becoming club-shaped, and finally spindle-shaped, by the tapering both ends (Pl. II. Fig. 7, 8). Gradually they enlarge in size and no longer divide. They function as conidia in asexual reproduction, and their vitality is maintained indefinitely. A slide culture 8 months old showed vigorous activity in contacting and forming beaked zygospres between elongate conidia (Pl. II, Figs. 10, 13, 14). The size of these conidia is from 66 u×19 u to 193 u×32 u. In the older conidia the inside of the cells is differentiated into vacuoles and granules of various sizes. However, a distinct nucleus is not demonstrable. Some of the conidia are elongated into coarse thread as to take the place of mycelium (Pl. II, Figs. 4, 5).
3. The conjugation between elongateconidia. The conidia contacting as the beginning of sexual stage if sexuality really occurs, is shown especially in (Pl. II, Figs. 7, 8, 9). Pl. II Figs. 13 & 14 showed three successive stages in the formation of a beaked zygospore. Fig. 13 shows two conidia contacting in the center of the figure, the nucleus has divided into two at the tip of each cell. Fig. 14 shows two distinct stages, the one on the lower right showing the two about to have a nuclear fusion through the formation of protruding beaks, while on the left center is the newly formed zygospore with thick walls and the beaks still seen. The whole process is like this: (1) the beginning of contact between 2 adjacent cells, each has a pair of nuclei as in Fig. 13. (2) the formation of the parallel beaks in contact and (3) one nucleus of each pair remains in the apex at the beak, being cut off by a septum which later forms an opening and the nucleus of one of the two cells passes into the other and they fuse, and consequently, the thick-walled zygospore is formed.(7)
4. The zygospores. They are formed generally, in older cultures where nutrient or

(7) Bessey 1950. Morphology and Taxonomy of Fungi, 174-175.

moisture are not sufficient. Pl. II, Fig. 10 showed a number of zygospores formed. They may be intercalary as Pl. II, Figs. 11, 12.

5. The minute spores and their germination. These minute spores can be seen in many figures, as minute dots in the background (Pl. II, Figs. 8, 15, 11, 10). The germination of these spores is seen in one of the plate cultures (Pl. III, Figs. 1–5) showing various stages of development. Figs. 5 and 7 are two colonies with young elongated gemmae developed from the germinating spores. Pl. III, 8, 9 show older gemmae from the same colonies but were observed at a later date, while figs. 10 and 11, the contacting between conidia. A further development of the culture would be the multiplication of elongated conidia as described before. The life cycle is completed within 2 to 3 weeks depending on the difference in temperature. Wrinkle-surfaced colonies were shown vividly in slant colonies (Pl. I, Figs 2. 3), but none were observed in any of the plate cultures; instead, strings of gemmae formation radiating outwardly as they reach out for new nutrition, are very distinct.

CONCLUSION

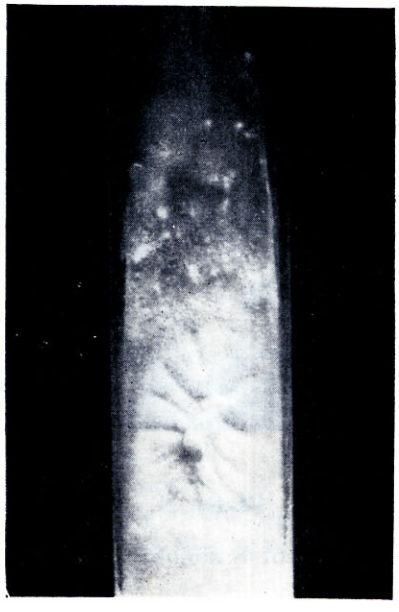
1. Basidiobolus meristosporus is characterized by its vigorous growth of asexual cells by fission and budding occasionally, without the presence of mycelia of any size.
2. Its life cycle shows an indefinite multiplication of asexual globose spores and elongated gemmae. Its sexual stage leading to the formation of zygospores is probably caused by unfavorable conditions that may overtake the fungus. These zygospores are more numerously formed in slide cultures rather than in slants and plate cultures.
3. Its sexual stage is very much like those of Basidiobolus ranarum Eidam as described in Bessey 1950 and Fitzpatrick 1930. Its asexual stage however, differs from that of Basidiobolus, it does not have the conidial formation as decribed in Fitzpatrick. The profuse production of elongated gemmae dominates the asexual phase of life. Besides living on the excrement of frogs as well as their internal organs, Basidiobolus can also grow in aquatic habitat.
4. Finally, fungal infection of Basidiobolus meristosporus did occur on carp fish eggs, both dead and living, and this infection was observed from asexual conidia stage rather than the sexual zygospore stage. In spite of the fact that carp eggs and young fishes are susceptible to the present fungus, still compared with the species of Achyla the former does not bring an immediate infection on the host as that of the latter.

LITERATURE CITED

(1) ALEXOPOULOS, CONSTENTINE JOHN. 1952. Introductory Mcology. 133-139. John Wiley & Sons, Inc., New York. Chapman & Hall, Limited, London.
(2) ———, 1955. Laboratory Manual for Introductory Mycology. 2nd, Printing. 9—10.
(3) BESSEY, ERNST ATHEARN, 1950. Morphology and Taxonomy of Fungi. 174-175. Philadelphia. The Blakiston Co., Toronto.
(4) CLEMENTS, E. & SHEAR, L. 1954. The Genera of Fungi. 239, Pl. 2, Fig. 14 a, b, c, Hafner Publishing Co., N. Y.
(5) COKER, W. C. 1923. The Saprolegniaceae with Notes on other Water Molds. The Univ. Press of North Carolina, Chapel Hill, N. C., U. S. A.
(6) DAVIS, H. S. 1955. Culture and Diseases of Game Fishes. 275-296. (Fungus Diseases) University of California Press, Berkeley and Los Angeles.
(7) DRECHLSER C., 1956. Supplementary Development Stages of Basidiobolus Ranarum and Basidiobolus Heptosorus. Mycologia 48: 457-616, 1956.
(8) EMMONS & Others, 1959. Basidiobolus and Cercospora from Human Infections. Mycologia 48, 1-11 1957.
(9) FITZPATRICK, HARRY MORTON, 1930. The Lower-Fungi Phycomycetes. 283-286. Mc Graw-Hill Book Co., N. Y.
(10) GVYNNE-VAUGHAM & BARNES, 1951. The Fungi. 2nd Edition. 103-110. Cambridge, at the University Press.
(11) RUCKER and OTHERS. 1953. Infectious Diseases of Pacific Salmon. Vol. 83, Transactions of the American Fisheries Society, Published 1954.
(12) SKINNER, EMMONS, TSUCHIYA. 1951. Mold, Yeasts and Actinomycetes. 4th printing. 157. John Wiley and Sons. Inc., Chapman and Hill, Limited. London.

1

2

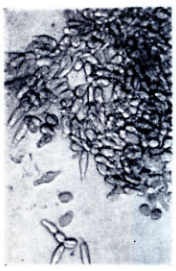


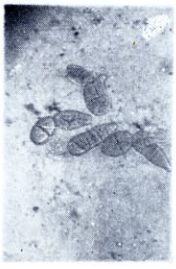
3

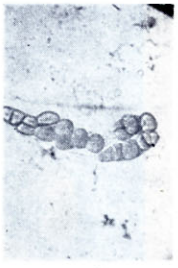
Plate I.

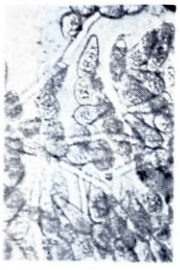
Agar slants of pure cultures of Basidiobolus meristosporus

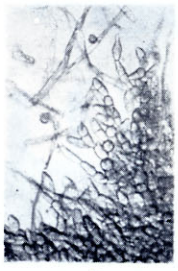
Fig. 1. Colonies in various stages. Fig. 2. Two older colonies at the lower portions of two tubes; the younger ones in scattered spots at the upper portions. Fig. 3. One older colony in natural size showing distinct convoluted surface.

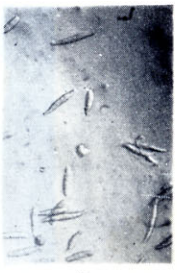

1

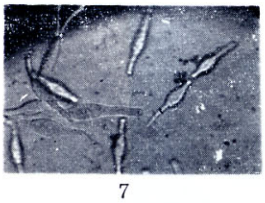

2

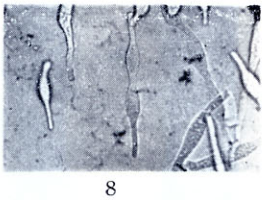

3

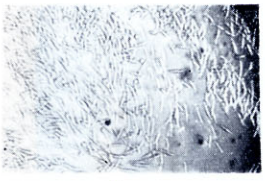

4

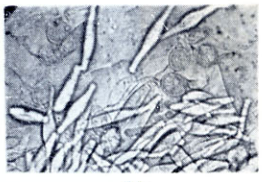

5


6

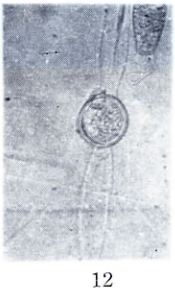

7

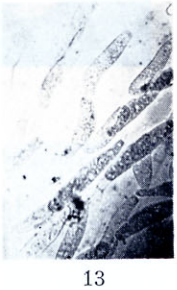

8


9


10

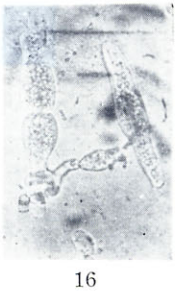

11

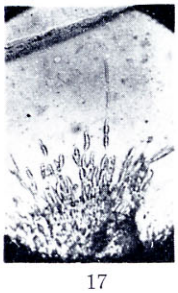

12

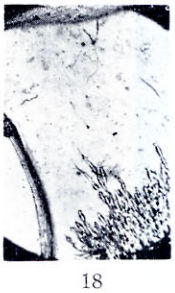

13


14


15

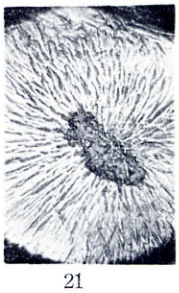

16


17


18


19


20


21

Plates II.

The Life Cycle of Basidiobolus meristosporus

Figs.1-21. 1. Resting cells—transferred from old slant culture to a slide, showing globose and barrel-shaped spores. ×150. 2. & 3. Ditto, showing cell divisions, 5 days later. ×300. 4. Same as 2. 3, but mingled with slender thread-like cells. ×300. 5. Ditto, ×150. 6. Taken at a later date. ×125. 7. Gemmae approaching contacting ×300. 8. Gemmae contacting ×300. 9. Portion of a colony showing elongated gemmae and a few rounded cells ×150. 10. Formation of zygospores after contacting as shown lower right. ×300. 11, 12. Intercalary zygospores ×150, ×300. 13, 14. Three successive stages in zygospore formation. 13. Approaching contact, ×300. 14. Parallel beaks formed, lower right. ×300. Thick-walled zygospore formed after nuclear fusion, beaks still seen. 15. Minute spores scattered at the background. ×300. 16. Ditto, discharging from older gemmae. ×300. 17-21. Formation of new colonies showing different stages, from slide cultures. ×125.

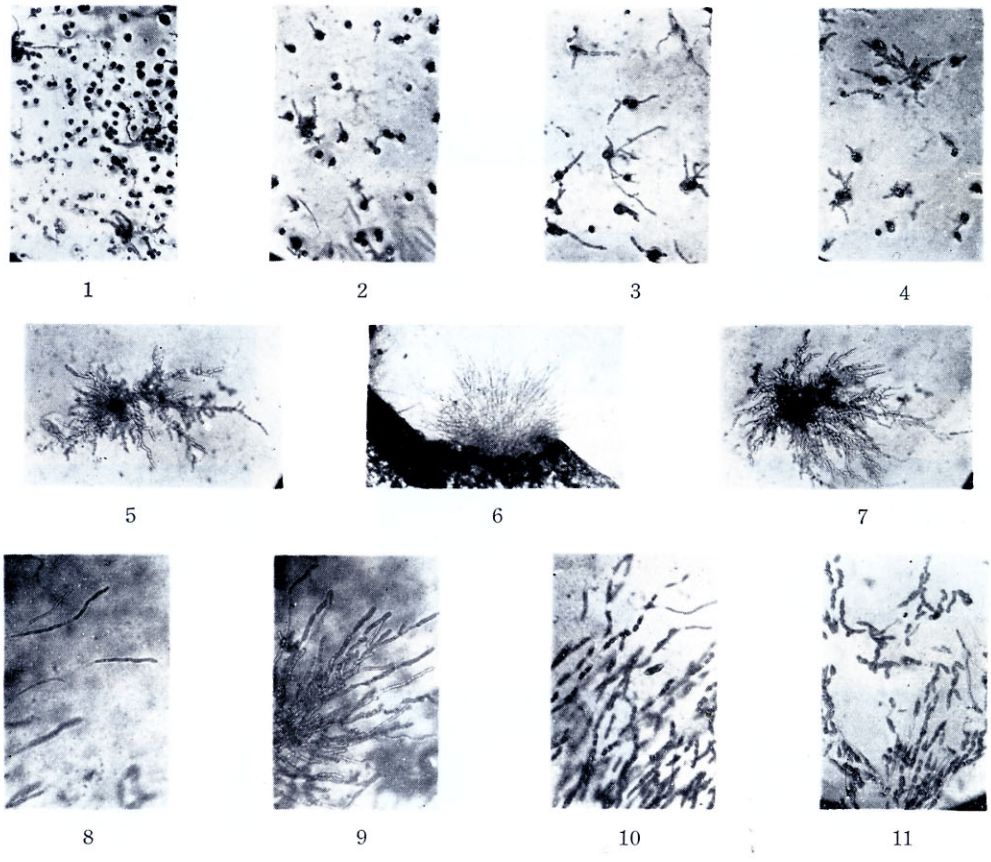


Plate III.

Basidiobolus meristosporus in Plate Culture.

Figs. 1, 2, 3, 4. Various stages in germination. 5, 6, 7. Three newly formed colonies. 8, 9. Elongated gemmae. 10, 11. Contacting between gemmae.
All the above figures (micrographs) are taken approximately at ×125.

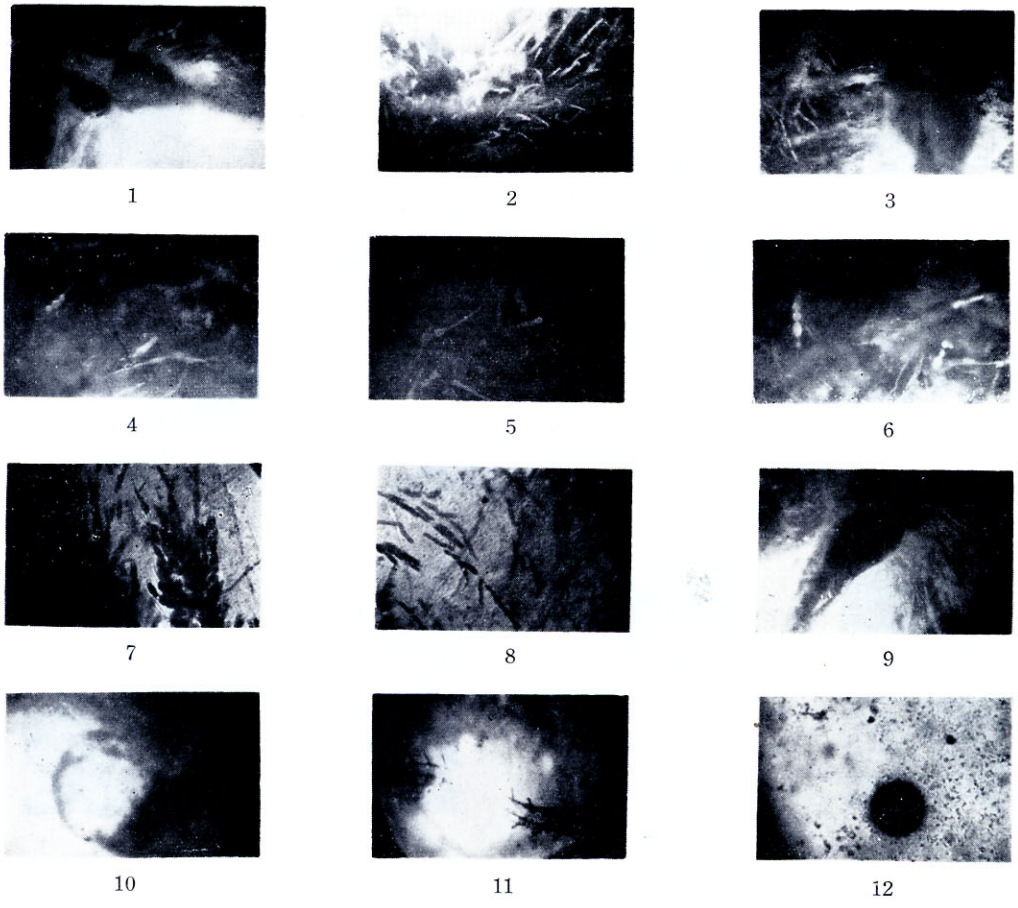
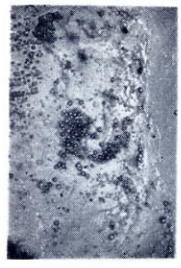
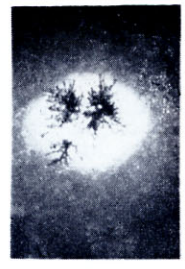


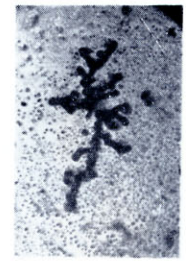
Plate IV.

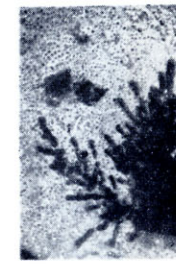
Fungal Infections on Eggs and Young Ones of Cyprinus carpio Linne.

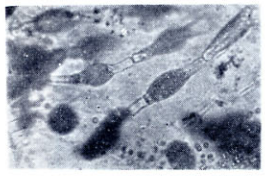
Figs. 1. Embryo fish infected with Achyla oblogata 125×. 2. Ditto, zoosporangia on the posterior of the host. 3. Gemmae extending to infect fish on right 125×. 4-6. Intercalary zygospores- on fish body. 7-8. Older gemmae from a plate culture 150×. 9. Young fish infected with Basidiobolus meristosporus seen in the background. 10. Infected Egg 150×. 11. Remains of fish skeleton after infection. 12. Another infected egg. 150×.

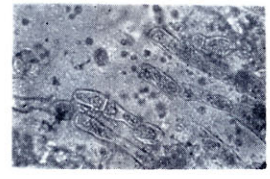

1

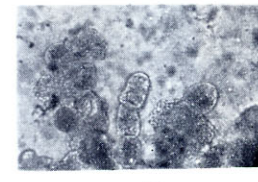

2

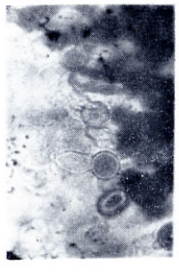

3

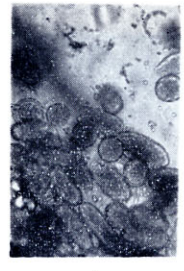

4

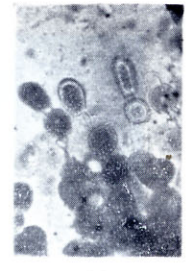

5


6


7


8


9


10


11

Plate V.

Basidiobolus Meristosporus Grown in Agar Medium, in Fish Jar.

Figs. 1-3. Colonies in different sizes grown on agar in fish jar, 250×. 4. Conidia contacting on the same agar, 300×. 5-6. discharging of protoplasts from older conidia 300×. 7. Contents of older conidia drawn to the center of the cell before discharging, 300×. 8-10. The formation of zygospores in chains 300×. 11. Portion of a dead young fish badly infected and disorganized by Achyla and other microorganisms, 125×.