

# INDIAN WATER-MOULDSD—I.

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## *Introduction.*

THE majority of the water-moulds belong to *Saprolegniaceæ*, having some 80 species in 14 genera known at present. They live mostly as saprophytes on dead and decaying animal and vegetable matters submerged in water. Some also live as parasites. Most of these water-moulds have been recorded in Europe and America and have been described by de Bary (1881, 1888), Massee (1891), Humphrey (1893), Klebs (1899), Kauffman (1908), Petersen (1910), Von Minden (1912, 1916), Pieters (1915), Coker (1923), Coker and Braxton (1926), Harvey (1925), Kanouse (1927), Fitzpatric (1930) and others. The list of British species has been considerably added to by Ramsbottom (1904-1906), Barnes and Melville (1932), Cook and Forbes (1933) and Forbes (1935). Besides the work of Butler (1907, 1911) describing a few forms, there has been very little done in India. Recently a *Myzocitium* has been described by Chaudhuri (1931).

Apart from systematic studies, the growth of the water-moulds under different cultural conditions has been studied, and inoculation experiments have been made with a pathogenic form. Twenty species, *viz.*, 19 species belonging to 5 genera of *Saprolegniaceæ* and one *chytrid* belonging to *Rhizidiaceæ* have been recorded and described. This paper forms the first of a series of papers it is intended to publish on water-moulds.

## *Method and Technique.*

As the water-moulds reproduce freely by means of zoospores, samples of water were collected in tubes from different localities. A boiled house-fly was placed for 24 hours in each of these tubes after which the fly was washed in several changes of sterilised water and transferred to a Petri dish, containing sterilised distilled water. In a day or two, the fly which is infected by the zoospores, becomes covered by fungal hyphæ which radiate out of the body of the fly. It is then transferred to another Petri dish and fresh transfers are always made every third day. It has been observed that usually a

number of species occur in a sample of water, and the fly gets infected by more than one species of mould. Hence it is necessary to isolate them and grow in pure culture. This was done by picking up, under a binocular microscope, a single hypha bearing a zoosporangium by means of needles and placing that in a Petri dish containing sterilised distilled water, after it had been washed again in several changes of water. In a day or two fungal hyphæ grow around the bits of sterilised egg-yolks placed in those dishes which become infected by the zoospores liberated by the zoosporangium.

Many a time certain seeds and pieces of fruits have been used as baits.

Often cultures get contaminated by bacteria unless the water is slightly acidulated. If an infected fly bearing mature zoosporangia from a bacteria-contaminated dish be washed in several changes of acidulated water, and another fly-bait is gently placed at the opposite end of the same dish in slightly acidulated water and left for about 2 hours, then from the latter a bacteria-free culture may be obtained.

The life-history of the moulds has been studied in the living condition by mounting in water and staining with neutral red. Escape of zoospores and the different stages in the development of the spores could thus be easily followed.

In making permanent mounts, the following procedure was followed. The material was fixed in a solution of formalin (10 c.c.), glacial acetic acid (5 c.c.), and water (85 c.c.) for 24 hours. After washing the material in several changes of water, this was put in 10% glycerine containing .01 % erythro-sine in a watch glass and allowed to dry slowly for two days. When the glycerine thickened, the material was immersed at once in absolute alcohol and then through the usual grades of alcohol and xylol was finally mounted in canada balsam after spreading out the material with a pair of needles. These preparations suffered no shrinkage at all and the stain also did not fade. Mounts were also made directly from the material in thickened glycerine, in either pure glycerine or in glycerine jelly ringed with asphaltum. These latter mounts very soon lost the brilliancy of the stains and after a few months the stain almost faded.

#### *General Characters.*

These fungi consist of cœnocytic threads attached to rhizoid-like system. Hyphæ either branched or more or less simple; sub-hyaline. Septa appear only in connection with reproductive bodies. Asexual reproduction by means of zoospores. Zoosporangia formed at the tips of the hyphæ; of various shape and size; renewed by internal proliferation as in *Saprolegnia* or by cymose branching from below the older ones as in *Achlya* or by both methods

as in *Isoachlya*. Zoospores liberated singly or in groups. Dark coloured resting bodies (gemmæ or chlamydo-spores or conidia) formed sometimes. These either grow into new hyphæ or produce zoospores. Sexual reproduction by means of oogonia and antheridia. Oogonia formed at the tips of main hyphæ or on short lateral branches or sometimes intercalary, singly or in chains. Eggs one to many in an oogonium. Antheridia short or long, simple or branched and either originating from the same thread on which the oogonia are borne (Androgynous), or from other thread (Diclinous). Fertilisation tubes present or may be absent.

*Descriptions of Species and Their Cultural Characters.*

Genus *Saprolegnia* Nees v. Esenbeck, 1823, p. 513.

Hyphæ slender, more or less branched, primary sporangia terminal, long clavate, often slender fusiform, polymorphic in old cultures, secondary ones produced by proliferations through the older ones; zoospores liberated singly and swim away separately.

1. *S. parasitica* Coker. Sap. 1923, p. 57, Pl. II.

Growth rather delicate, dense, short, reaching 0.9 cm. on egg-yellow in distilled water; chlamydo-spores abundant, size and shape variable, often in chains of 2-4, last in the series often spherical or globular. Hyphæ 24.5-36  $\mu$ . Zoosporangia variable, often bent and irregular, cylindrical to long clavate with rounded tips, 45-60  $\times$  280-340  $\mu$ ; occasionally 560  $\mu$  long, proliferating internally through the older ones. Zoospores 9.5-10.8  $\mu$ .

On boiled house-fly in tap water and distilled water—delicate growth, short hyphæ, abundant zoosporangia. On boiled egg-yellow in distilled water—zoosporangia and also chlamydo-spores formed.

2. *S. parasitica* Coker, Var. *Kochhari*, n. var.

Pl. V. Figs. 1-6.

Parasitic on *Belone Cancinula* and other fishes. Abundant hyphæ covering the dead fish. In laboratory *Labio rohita* (8" long) infected by means of zoospores, but *Ophiocephalous punctatus*, though injured at the mouth from which hyphæ grew out, recovered and lived for three months.

Dense long hyphæ, zoosporangia usually very long and stouter; bigger class *S. parasitica*. 50-75  $\times$  400-600  $\mu$  long. Zoospores usually not liberated but germinate inside the zoosporangium when growing on fish.

If zoosporangium is put in distilled water, zoospores are liberated in the usual manner. Microtome sections of the infected fish showed the presence of the fungus throughout the body of the host.

Coll. : S. R. Kashyap, Hiran Minar, Sheikhupura, Punjab.

Genus *Aplanes* de Bary, 1888, p. 613.

Hyphæ slender, very minute, sparingly branched or simple, zoosporangia very rare, almost absent.

3. *A. sp.* Pl. I. Figs. 20-29.

Growth delicate but extensive, reaching upto 2 cm. on coagulated egg-yellow. Hyphæ slender, occasionally branched, 9.2-15.3  $\mu$  in thickness. Zoosporangia very rare, when present cylindrical to long clavate, 28-30.3  $\mu$ ; renewed by internal proliferation, one zoosporangium observed with cymose branching. Chlamydospores abundant, very variable in size and shape, elliptical to globular or irregularly cylindrical, terminal or intercalary, in chains or single, sexuality not observed.

*Growth in culture.*—On house-fly in tap water—only vegetative growth with a few gemmæ, zoosporangia rare. On egg-yellow in distilled water and in boiled corn grain—abundant chlamydospores formed. Old cultures on egg-yellow when transferred to 0.1% leucine solution showed no change in two weeks.

Collected from Lahore, Amritsar and Gujranwala.

Genus *Isoachlya*, Kauffman, 1921, p. 231.

Hyphæ rather stout or slender. Zoosporangia terminal, variable in shape and size, secondary ones arising either by cymose arrangement, as in *Achlya*, or by internal proliferation as in *Saprolegnia*. Zoospores escaping and swarming separately. Oogonia without antheridia in most cases.

4. *I. monilifera* (de Bary) Kauffman, *Amer. Journ. Bot.*, 1921, 8, 231.

Pl. VI. Figs. 1-9.

Vegetative growth rather extensive and stout, reaching 1.5 cm. on egg-yellow in distilled water. Hyphæ 14-27  $\mu$  occasionally upto 33  $\mu$ . Sporangia not abundant, usually cylindrical to clavate, proliferating internally. Zoospores 11.4-12.2  $\mu$ . Chlamydospores variable, terminal or intercalary, usually in chains, the terminal one being globular or spherical. Oogonia very abundant throughout the culture, usually in chains, most of them breaking up easily from the hyphæ and from each other on maturity, typically spherical with a long neck, wall thin, diameter 49-91  $\mu$ . Eggs 1-30, mostly 4-12; 21-25  $\mu$  centric. Antheridia absent.

*Growth in culture.*—House-fly in distilled water—growth rich, zoosporangia developed with zoospores liberated, oogonia not so plentiful. Egg-yellow in distilled water and in boiled corn grain—growth luxuriant, plenty of oogonia of normal shape with mature eggs, no antheridia. On house-fly in 0.1%

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3. *A. sp.* Pl. I. Figs. 20-29.

Growth delicate but extensive, reaching upto 2 cm. on coagulated egg-yellow. Hyphæ slender, occasionally branched,  $9.2-15.3 \mu$  in thickness. Zoosporangia very rare, when present cylindrical to long clavate,  $28-30.3 \mu$ ; renewed by internal proliferation, one zoosporangium observed with cymose branching. Chlamydospores abundant, very variable in size and shape, elliptical to globular or irregularly cylindrical, terminal or intercalary, in chains or single, sexuality not observed.

*Growth in culture.*—On house-fly in tap water—only vegetative growth with a few gemmæ, zoosporangia rare. On egg-yellow in distilled water and in boiled corn grain—abundant chlamydospores formed. Old cultures on egg-yellow when transferred to 0.1% leucine solution showed no change in two weeks.

Collected from Lahore, Amritsar and Gujranwala.

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4. *I. monilifera* (de Bary) Kauffman, *Amer. Journ. Bot.*, 1921, 8, 231.

Pl. VI. Figs. 1-9.

Vegetative growth rather extensive and stout, reaching 1.5 cm. on egg-yellow in distilled water. Hyphæ  $14-27 \mu$  occasionally upto  $33 \mu$ . Sporangia not abundant, usually cylindrical to clavate, proliferating internally. Zoospores  $11.4-12.2 \mu$ . Chlamydospores variable, terminal or intercalary, usually in chains, the terminal one being globular or spherical. Oogonia very abundant throughout the culture, usually in chains, most of them breaking up easily from the hyphæ and from each other on maturity, typically spherical with a long neck, wall thin, diameter  $49-91 \mu$ . Eggs 1-30, mostly  $4-12$ ;  $21-25 \mu$  centric. Antheridia absent.

*Growth in culture.*—House-fly in distilled water—growth rich, zoosporangia developed with zoospores liberated, oogonia not so plentiful. Egg-yellow in distilled water and in boiled corn grain—growth luxuriant, plenty of oogonia of normal shape with mature eggs, no antheridia. On house-fly in 0.1%

solution of potassium phosphate—growth not so thick, hyphæ scattered with very few oogonia, zoosporangia normally developed.

Collected from Batala and Shalamar.

This species differs from Coker's description of *I. monilifera* in having constantly long-necked oogonia and larger number of eggs in the oogonium.

Genus *Protoachlya* Coker. Sap., 1923, p. 90.

Hyphæ rather delicate. Zoosporangia cylindrical to flask-shaped with rounded tips, thickest near the apex. Zoospores diplanetic, some remaining attached in an irregular clump at the tip of the zoosporangium, other swimming away separately.

5. *P. paradoxa* n. comb. (Coker).

Pl. VI. Figs. 10–25.

*Achlya paradoxa* Coker, *Mycologia*, 1914, 6, 285, Pl. 146.

*Isoachlya paradoxa* (Coker) Kauff., *Amer. Journ. Bot.*, 1921, 8, 231.

Growth open, delicate, long reaching 1.75 cm. on egg-yellow in distilled water. Hyphæ 12.3–18.6  $\mu$ , occasionally thicker upto 28  $\mu$ . Zoosporangia very abundant, 27.6–33.8  $\mu$  in diameter, tips rounded or occasionally broad, secondary ones arising usually by cymose branching beneath the older ones. Zoospores diplanetic, formed in several rows, showing great variation in behaviour, some moving away separately after emergence, others forming an irregular group at the tip. Chlamydo-spores terminal, rarely intercalary, mostly in chains, oblong to clavate, sometimes cylindrical, zoospores oblong to oval within the zoosporangium and spherical outside, their long axes parallel to the wall of the zoosporangium during escape, 12.5–13.2  $\mu$ . Oogonia on short lateral branches, rarely terminal or intercalary, spherical, 33–60  $\mu$  in diameter, mostly 43–47  $\mu$ , those near the substratum with many eggs and those outward with few, usually 1–2. Eggs spherical 18–24  $\mu$ . Antheridia present on all oogonia mostly diclinous, occasionally androgynous, sometimes more than one to an oogonium.

*Growth in culture.*—On house-fly and on egg-white in distilled water—growth extensive, delicate, hyphæ thick near the base. Many sporangia with *Achlya*-like proliferation. On egg-yellow in distilled water—as above. Abundant chlamydo-spores formed. Oogonia formed only in old cultures, some disorganising. On corn grain in distilled water—growth vigorous, many zoosporangia and chlamydo-spores, no oogonia. On house-fly in 0.1% solution of leucine—abundant gemmæ and many oogonia with ripe eggs. On house-fly in 0.1% solution of potassium phosphate—growth fairly strong, zoosporangia and chlamydo-spores numerous, zoospores liberated normally.

Collected from Sheikhupura and Atari.

It may be noted that it has smaller-sized eggs than those described by Coker.

Genus *Achlya* Nees v. Esenbeck, 1923, p. 514.

Zoospores from a common mouth, collecting in a group at the tip of the zoosporangia; zoosporangia thicker than the vegetative hyphæ, zoospores not in a single row.

6. *A. americana* Humphrey.

Pl. VII. Figs. 1-11.

Growth dense, rather stout, upto 2 cm. on egg-yellow in distilled water. Hyphæ stout giving out slender branches, upto  $78\mu$  near the base, branches usually  $27.6-48\mu$ , finer branches as small as  $12.3\mu$ , zoosporangia long, cylindrical, with wavy margin,  $24.6-36.9\mu$  in thickness, proliferating frequently from below the older ones. Zoospores liberated in a group,  $8.3-9.1\mu$ . Chlamydo-spores not so frequent, formed by the segmentation of hyphæ into elongated pieces, usually of the same thickness as the zoosporangium. Oogonia plentiful borne on short lateral stalk, rarely in chains, spherical, occasionally flask-shaped, wall pitted,  $40.1-68.7\mu$  in diameter, mostly  $46\mu$ . Oogonial stalk varying from less than to more than twice the diameter of oogonium. Eggs 2-6 sometimes upto 13-15, eggs spherical  $21.5-23.8\mu$ , sub-centric to eccentric. Antheridia mostly androgynous, occasionally diclinous, simple.

*Growth in culture.*—On house-fly in tap water—vegetative growth fairly dense, normal sporangia; chlamydo-spores and oogonia nil. On egg-white in distilled water—growth delicate, open. On egg-yellow in distilled water—growth close, strong, hyphæ branched, few chlamydo-spores, oogonia plentiful, all maturing and forming ripe eggs. On corn grain in distilled water—growth extensive, oogonia few, maturing late. A culture on house-fly transferred to 0.1% solution of leucine formed oogonia with ripe eggs in 5 days.

Collected from Shalamar and Lahore.

It differs from Coker's description in the smaller size of its zoospores and oogonia and in the smaller number of eggs in the normal oogonia. Forbes has also described this species recently from Manchester, and her description shows this species to be closely allied to *A. debaryana* but the latter differs from this in having unpitted oogonial walls and some diclinous antheridia, while the former has pitted oogonial walls and consistently androgynous antheridia.

7. *A. proliferoides* Coker.

P. VII. Figs. 12-19.

Growth open, stout, long, upto 2 cm. on egg-yellow in distilled water. Hyphæ more or less branched, usually wavy, tapering, varying greatly in size,  $135\ \mu$  near the base, ultimate branches as fine as  $15.7\ \mu$ , tips withering. Zoosporangia abundant, long, narrowly cylindrical, sometimes bent,  $30.7-38 \times 210-490\ \mu$ , occasionally  $50\ \mu$  thick, often opening by one or more lateral pores. Zoospores  $10.5-11.2\ \mu$ , coming out in one continuous line and forming an irregular group at the mouth. Oogonia abundant, spherical, rarely irregular,  $45-47\ \mu$  in diameter, on short lateral stalks, which vary from as long as, to  $1\frac{1}{2}$  times as long as the diameter of the oogonium; oogonial wall thin, pitted. Eggs 1-8, spherical  $21-30\ \mu$ , averaging  $25\ \mu$ . Antheridia diclinous, branching a single one sending branches to 2 or more oogonia.

*Growth in culture.*—On house-fly and on boiled egg-white in distilled water—only vegetative growth, zoospores all liberated at maturity. On egg-yellow in distilled water—vegetative growth strong and healthy, few oogonia developed, remaining immature for a long time, all with antheridial branches. Oogonia abundant on corn grain. On corn grain in distilled water—vegetative growth extensive, zoosporangia and zoospores well developed; many mature oogonia with ripe eggs; all with antheridia. A culture on house-fly transferred to 0.1% solution of leucine produced oogonia in 5 days; more than 75% with ripe eggs, antheridia on relatively few oogonia.

Collected from Gujranwala and Lahore.

It will be noted that the size of the eggs is bigger than those described by Coker for the new species.

8. *A. flagellata* Coker. Sap. 1923, p. 116.

Pl. VIII. Figs. 1-8.

Growth somewhat dense, reaching about 2 cm. on egg-yellow in distilled water. Hyphæ branching, tapering gradually,  $30-39\ \mu$  thick, main hyphæ at the base upto  $120\ \mu$ , often dying back at the tip. Zoosporangia plentiful, sub-cylindrical to cylindrical, very variable in length, often long and thick,  $49-54 \times 306-709\ \mu$ , slightly bent and occasionally with more than one lateral opening. Zoospores coming out in a mass and gradually falling to the bottom,  $9.8-11\ \mu$  thick. Gemmæ not so frequent. Oogonia fairly plentiful, usually on lateral stalks, which may be twice as long as the diameter of the oogonium, spherical,  $41-78\ \mu$  in diameter, mostly  $52-58\ \mu$ , wall pitted, not so thick, about  $1.3-1.6\ \mu$ . Eggs 1-9, mostly 3-6, spherical, eccentric,  $21.3-29\ \mu$  in diameter. Antheridia both diclinous and androgynous, more often former, branching attached by means of feet to the oogonial wall.



*Growth in culture.*—On house-fly in distilled water—vegetative growth short but dense; zoosporangia and a few chlamydozoospores formed, no oogonia. On egg-white in distilled water—growth open, hyphæ long, with very few, if any, branches; zoosporangia with zoospores, which are easily liberated. On egg-yellow in distilled water—growth strong and dense; zoosporangia abundant, zoospores germinating within the zoosporangium or outside; chlamydozoospores abundant, oogonia formed late. On a fly in 0.1% solution of Potassium phosphate—few hyphæ coming out and growing to a considerable length, stout. On a fly in 0.1% solution of Potassium nitrate—growth fair, only vegetative, few sporangia, no oogonia or chlamydozoospores.

Collected from Amritsar, Lahore and Gujranwala.

10. *A. imperfecta* Coker. Sap. 1923, p. 118.

Pl. VIII. Figs. 9-20.

Growth dense, not so delicate, reaching 1.75 cm. on egg-yellow. Hyphæ near the substratum stout, giving out delicate branches, branches dying back at the tips; 9.2–30.4  $\mu$ , mostly 12.3–15.3  $\mu$ . Zoosporangia abundant, sub-cylindrical, somewhat irregular in outline, renewed in a cymose manner or formed anew below the older ones, 30.3–39.9  $\times$  520–1205  $\mu$ . Zoospores 10–10.8  $\mu$  thick, dark, spherical. Chlamydozoospores formed by the segmentation of hyphæ, mostly sub-cylindrical and in chains, rarely ovate with lateral branches, often getting loosened and separated from each other and the threads. Oogonia plentiful, spherical or sub-spherical, occasionally irregular in outline and with papillæ on short lateral stalks which are less than, to more than 1½ times the diameter of the oogonium; with or without a neck, 55.2–67.3  $\mu$  in diameter, averaging 59  $\mu$ , wall unpitted. Eggs 2–8 in an oogonium, mostly 3–5, eccentric, 21–28.3  $\mu$  mostly 24.3  $\mu$ , spherical or sometimes elliptical. Antheridia both androgynous and diclinous, more or less branched, a single one sending branches to more than one oogonium.

*Growth in culture.*—On house-fly in distilled water—hyphæ stout, vegetative growth dense, covering the fly; chlamydozoospores few, zoospores developed normally and all liberated. On egg-white and boiled corn grain and egg-yellow—oogonia developed in a week's time, zoospores formed, also a few chlamydozoospores. A culture of egg-yellow in distilled water transferred to 0.1% solution of leucine produced abundant oogonia in 3 to 4 days. Some of these disorganised, zoosporangia short. On house-fly in 0.1% solution of Potassium nitrate—only vegetative growth seen. On house-fly in 0.05% solution of Potassium chloride—only few threads came out and these died in a week.

Collected from Lahore and Amritsar.

10. *A. Kashyapia*, n. sp.

Pl. IX. Figs. 1-10.

Growth rather open, hyphæ stout near the substratum, giving out slender branches,  $12.3-18.4\mu$ , occasionally as wide as  $30\mu$  and as fine as  $9.2\mu$ . Zoosporangia plentiful, elongated, cylindrical or sub-cylindrical, more or less smooth, secondary ones quite common and produced as in other forms of this genus, very variable in size  $32-45 \times 252-873.7\mu$ , occasionally as long as 1 mm., those on finer branches as small as  $15.4 \times 175\mu$ . Chlamydozoospores formed usually by the segmentation of hyphæ and therefore in chains and cylindrical to sub-cylindrical, rarely otherwise, not getting loosened and separated from each other. Zoospores  $9.8-10.5\mu$ . Oogonia not so frequent, when present scattered in groups of three or four, on short lateral stalks, dark coloured, neck short or absent, spherical, occasionally flask-shaped. Wall moderately thick,  $2.8-3.1\mu$ , unpitted. Diameter  $58.2-76.3\mu$ . Eggs 3-8 in number,  $24.6-27.8\mu$ , spherical, sub-centric or centric. Antheridia not on all oogonia, declinuous, branching, a single one sending branches to 2 or more.

*Growth in culture.*—On fly in distilled water—vegetative growth rich; zoosporangia formed and zoospores liberated. Growth poor on egg-white. On egg-yellow in distilled water—vegetative growth very luxuriant, upto 1.5 cm., oogonia plenty, remaining immature for a long time, mature ones degenerating; zoospores not developed in all zoosporangia, liberated early. On corn grain in distilled water—vegetative growth dense and long, upto 2 cm.; oogonia formed but not very abundant, eggs mature; chlamydozoospores seen. A culture on house-fly when transferred to 0.1% solution of leucine produced abundant oogonia in 2 days, eggs ripen. On corn meal agar and corn grain (boiled) in 0.1% solution of Potassium phosphate—growth moderate, few sporangia and gemmæ. On house-fly in 0.1% solution of Potassium nitrate—good growth 1 cm.; zoosporangia and chlamydozoospores developed.

Collected from Lahore and Khasa (near Amritsar).

*A. Kashyapia* approaches *A. prolifera* in having declinuous antheridia but differs from the latter in the unpitted character of the oogonial wall and in its larger eggs. It also resembles *A. imperfecta* except in the size of the oogonia and eggs and in the centric or sub-centric character of its eggs. It may be a synthetic species.

11. *A. Klebsiana* Pieters, *Bot. Gaz.*, 60, 486.

Pl. IX. Figs. 11-17.

Moderately stout, growth on egg-yellow reaching 1.75 cm. Hyphæ  $30-54\mu$ , main threads upto  $84\mu$  at the base, tapering gradually, branched. Zoosporangia  $36-49 \times 429-502\mu$ , occasionally  $71.8\mu$  thick, those at the ends

of fine branches as small as  $15 \times 270 \mu$ . Secondary ones quite common, arising cymosely. Zoospores  $10.7-11.3 \mu$ . Chlamydospores formed by the segmentation of hyphæ, rod-like. Oogonia plentiful, borne on short lateral stalks which are from less than, to  $2\frac{1}{2}$  times the diameter of the oogonium, spherical or sub-spherical, with or without a neck,  $39-60 \mu$ , mostly  $47-55 \mu$ . Oogonial wall thin,  $1.5-2.3 \mu$ . Eggs 1-6, usually 3-4,  $20-26.2 \mu$ , mostly  $23.8 \mu$ ; spherical or irregularly flattened by pressure, centric. Antheridial branches slender, always declinous, androgynous very rare.

*Growth in culture.*—On house-fly in distilled water—vegetative growth good, zoosporangia forming zoospores, some liberating, others retaining them, oogonia after about 8-9 days. On egg-yellow in distilled water—vegetative growth extensive, reaching 1.75 cm., oogonia abundant, all with antheridia. On egg-white in distilled water—vegetative growth delicate, zoosporangia not developing zoospores; oogonia only few. A culture on fly in 0.1% solution of leucine produced abundant oogonia, only 50% forming ripe eggs, no chlamydospores. On house-fly in 0.1% solution of Potassium phosphate—only vegetative growth, zoospores not developed, no chlamydospores.

Collected from Shalamar, Mughalpura and Lahore.

It agrees with Forbes' description of the species.

12. *A. conspicua* Coker, Sap. 1923, p. 131.

Pl. X. Figs. 1-8.

Growth dense, reaching 1.5 cm. on egg-yellow in distilled water. Main hyphæ long and stout, upto  $145 \mu$  thick near the base, fine branches as small as  $24.6 \mu$ ; zoosporangia abundant, broadly cylindrical,  $43-52.2 \times 247-558 \mu$ . Secondary ones abundant, produced in groups or borne in a zigzag manner at long intervals. Zoospores  $8.9-9.9 \mu$ . Oogonia somewhat plentiful, on short lateral stalks, which are at least as long as the diameter of the oogonium, spherical or oval, occasionally cylindrical and notched,  $58.3-64.5 \mu$ , wall yellowish, not thick, slightly pitted. Eggs 3-7, spherical,  $24.6-30.7 \mu$ . Antheridia androgynous, branched.

*Growth in culture.*—On house-fly in distilled water—vegetative growth fair, rather stout; zoosporangia abundant, short, almost oblong. On egg-yellow in distilled water—growth dense, chlamydospores nil. Oogonia with ripe eggs formed after 20 days.

Collected from Amritsar, Atari and Lahore.

13. *A. dubia* Coker, Sap. 1923, p. 135. Pl. XLIX.

Pl. X. Figs. 9-14.

Growth rather dense, 1.5 cm. on egg-yellow. Main threads stout, more or less branched,  $21.3-40.7 \mu$ , thick, tapering gradually. Zoosporangia

abundant, terminal, long cylindrical or somewhat clavate,  $30.7-42.9 \mu$ , often thinner, those on finer branches as short as  $23 \times 270 \mu$ . Zoospores  $10-11.3 \mu$ . Oogonia on short lateral branches, which vary in length from less than, to  $1\frac{1}{2}$  times the diameter of the oogonia,  $61.4-70.5 \mu$ , wall thin, not pitted. Eggs  $2-9$ ,  $24.6-30.8 \mu$ , mostly  $27.6 \mu$ , many without antheridia, others with 2 or more. Antheridia declinous, attached by feet.

*Growth in culture.*—On house-fly in distilled water—vegetative growth fair, about 1 cm., open, hyphæ stout, zoosporangia plentiful, no oogonia. On egg-yellow in distilled water—as above, oogonia formed only in old cultures after one month.

Collected from Lahore, Amritsar and Gujranwala.

14. *A. dubia* Coker, var. *pigmenta*, n. var.

Pl. XI. Figs. 1-12.

Growth slightly dense, hyphæ stout, reaching 1 cm. on egg-yellow. Hyphæ  $27.6-36.8 \mu$ , branched, straight. Zoosporangia thicker,  $36-67 \times 370-720 \mu$ , long cylindrical, outlines regular and straight. Secondary ones arising by cymose branching from below the primary ones. Chlamydospores abundant, produced in groups, of various shapes. Zoospores coming out occasionally in groups and collecting at the tip, mostly retained within the zoosporangium and germinating there. Oogonia plentiful, on short lateral stalks, which are upto as long as the diameter of the oogonium; diameter  $39.2-62 \mu$ , wall smooth, thin, yellowish or dark. Eggs  $2-9$ , usually  $3-4$ ;  $21.4-24.5 \mu$  in diameter. Antheridia declinous or androgynous, mostly former, branched, attached by feet to the oogonial wall.

*Growth in culture.*—On house-fly in distilled water—vegetative growth dense, zoosporangia abundant, only few chlamydospores, no oogonia. Chlamydospores abundant on boiled corn grain. On egg-yellow in distilled water—vegetative growth fair, chlamydospores abundant, oogonia after a week, some without antheridia, zoospores liberated or retained in some. A culture on corn grain transferred to 0.1% solution of leucine produced abundant oogonia; antheridia on all.

Collected from Lahore and Gujranwala.

*A. dubia pigmenta* differs from *A. dubia* in its small-sized oogonial stalks and smaller eggs. It is characterised by its chlamydospores, coloured oogonial wall and constantly occurring declinous antheridia.

15. *A. deBaryana* Humphrey.

Pl. XI. Figs. 9-31.

Growth very dense; 1.75 cm. on egg-yellow. Hyphæ strong,  $19-26 \mu$  thick, more or less branched, or ending in zoosporangia, which are somewhat

elongated or occasionally clavate,  $27.6-30.7\mu$  thick; secondary ones not so common. Zoospores large,  $12.1-14.6\mu$ . Chlamydospores very abundant, of various shapes mostly in chains or branched, each opening by one or more lateral pores. Oogonia abundant, borne on short lateral stalks, or very often intercalary, single or in chains, varying much in form, normally spherical to sub-spherical, very often of abnormal shape, cylindrical or oval, occasionally double, rarely with one or more papillæ.  $47-61.4\mu$  in diameter. Wall thin and smooth. Eggs 2-many, spherical centric,  $21.6-30.7\mu$ ; antheridia not always present, diclinous or androgynous, mostly latter.

*Growth in culture.*—On house-fly and on egg-white in distilled water—growth rather open, zoosporangia and chlamydospores abundant; oogonia formed in cultures, after 15 days. On egg-yellow in distilled water—growth dense and long; zoosporangia not so abundant; chlamydospores plentiful, of various shapes; oogonia developed luxuriantly in 2-3 days; many abnormal oogonia occur, occasionally double and produced in old zoosporangia, cylindrical. A culture on house-fly transferred to 0.1% solution of leucine produced very few oogonia, variously shaped; few chlamydospores here and there. On egg-yellow in 0.1% solution of Potassium phosphate—growth fair, rather open, few chlamydospores; oogonia rare. On fly in 0.1% solution of Potassium nitrate—growth delicate, open, very few hyphæ.

Collected from Amritsar.

This species agrees with Forbes' description of the species fundamentally.

16. *A. aplanes* Maurizio. *Flora*, 1894, **79**, 135, Pl. 4-5.

Pl. XII. Figs. 1-10.

Growth dense, stout, 1.2 cm. on egg-yellow. Hyphæ delicate, fine, scarcely branched  $12.3-27.6\mu$  thick, wavy. Zoosporangia thicker, elongated or narrow clavate,  $31.4-40 \times 222-504\mu$ , occasionally as fine as  $19.1\mu$ . Chlamydospores abundant, cut off from below the zoosporangia by transverse septa, therefore in chains, cylindrical. Oogonia abundant borne on short lateral stalks, which vary from as long as, to more than twice as long as the diameter of the oogonium,  $45.3-67.5\mu$  in diameter, spherical or slightly ovate, wall smooth, thick and yellowish. Eggs 1-6, spherical,  $24.5-27.6\mu$ , eccentric. Antheridia not always present, mostly diclinous, branched.

*Growth in culture.*—On house-fly in distilled water—zoosporangia and chlamydospores only. A few oogonia on egg-white. On egg-yellow in distilled water—dense vegetative growth, 1.5 cm., oogonia plentiful with ripe eggs, many eggs degenerating, zoospores often retained. On a fly in

0.1% solution of Potassium phosphate—only isolated hyphæ here and there, scarcely branched, some bearing zoosporangia at their tips. On house-fly in 0.1% solution of Potassium chloride—no growth.

Collected from Amritsar and Gujranwala.

*Forms without Oogonia.*

17. *A. sp.* No. 1.

Pl. XII. Figs. 12-14.

Growth open, 1 cm. on egg-yellow. Hyphæ delicate, more or less branching, upto  $150\ \mu$  near the sub-stratum, ultimate branches as fine as  $9.5\ \mu$ . Zoosporangia abundant, or narrowly cylindrical,  $39.9 \times 227\ \mu$ , with a long apical papilla; secondary ones rare, no chlamydospores. Sexuality not observed.

*Growth in culture.*—A study of this was made in all the cultures previously used, but no sexual reproduction was observed.

Collected from Lahore.

18. *A. sp.* No. 2.

Pl. XII. Figs. 15-16.

Growth dense near the base, open outwards, 1.5 cm. on egg-yellow in distilled water. Hyphæ moderately stout, slightly branched, upto  $83\ \mu$  near the base, falling to  $30\ \mu$  outwards. Zoosporangia broadly cylindrical or narrowly oblong,  $30-38 \times 402-568\ \mu$ . Chlamydospores abundant formed by the segmentation of hyphæ.

*Growth in culture.*—As in *A. sp.* no. 1.

Collected from Lahore.

19. *A. sp.* No. 3.

Pl. XII. Figs. 17-19.

Growth rather dense, delicate upto 2 cm. on egg-yellow. Hyphæ  $30-51\ \mu$ ; zoosporangia long clavate to cylindrical with pointed ends,  $40-46 \times 70-140\ \mu$ . Chlamydospores abundant, formed by the segmentation of hyphæ, therefore in chains, those lower in the series usually elliptical, the terminal one cylindrical; zoospores germinating within the zoosporangia.

*Growth in culture.*—As in *A. sp.* no. 1. No sexuality induced in any culture medium.

20. *A. sp.* No. 4.

Pl. XII. Figs. 20-23.

Growth open, moderately stout, short, 1 cm. on egg-yellow. Hyphæ strong,  $55.8-70\ \mu$  at the base, upto  $33.5\ \mu$  outwards, profusely branched. Zoosporangia more or less oblong or occasionally cylindrical; secondary

ones few, 1-2 at a point,  $39.9-48 \times 350-440 \mu$ , cylindrical ones as long as  $1050 \mu$ . Chlamydospores abundant, terminal or intercalary, single or in chains, usually short, sub-globose, ovoid or flask-shaped, often elliptic to rectangular. Zoosporangia and chlamydospores sometimes with apical papillæ. Zoospores coming out in mass, spherical,  $9.8-10.2 \mu$ .

*Growth in culture.*—Usual cultural study of this form has been made, but all the media employed in other forms failed to induce sexuality; only on corn grain in distilled water, chlamydospores were more abundant than in others. Zoospores always liberated.

Collected from Amritsar.

Family *Rhizidiaceae*. Genus *Rhizidiomyces* Zopf (1884: 188).

21. *R. apophysatus* Zopf, *Nova Acta Cad, Leop.*, 1884, **47**, 188, Pl. 20, Figs. 1-7.

Pl. XII. Figs. 24-29.

Sporangia spherical or sub-spherical, varying greatly in size, from  $9 \mu$  to  $22 \mu$ , seated on the surface of the oogonia of *A. Klebsiana* and sending fine tube-like bodies into the interior of the host. Spores  $3.5-3.7 \mu$  thick. Disorganises the eggs, which break into pieces, and prevents their further development.

Found parasitic on the oogonia of a culture of *A. Klebsiana*, developed from sample No. 66.

#### *Summary and List of Species Noted.*

An examination has been made of the waters from various pools and ditches of the Lahore district of the Punjab and 20 species of water-moulds, most of which have not been noted before in this country and some new forms, have been recorded. These have been grown in various culture media and their cultural characteristics studied. Full diagnoses of the species have been given and differences, if any, from the older descriptions have also been noted. All the types described in this paper are fully illustrated. Inoculation experiments with a parasitic form—*Saprolegnia parasitica* var. *Kocchhari*, n. var.—have been made. The following types have been described:—

*Saprolegnia parasitica*; *S. parasitica* var. *Kocchhari*; *Aplanes* sp.; *Isoachlya monilifera*; *Protoachlya paradoxa*; *Achlya americana*; *A. proliferoides*; *A. flagellata*; *A. imperfecta*; *A. Kashyapii* n. sp.; *A. Klebsiana*; *A. conspicua*; *A. dubia*; *A. dubia* var. *pigmenta*; *A. deBaryana*; *A. aplanes*; *A. sp.* Nos. 1 to 4 (without oogonia) and *Rhizidiomyces apophysatus*.

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EXPLANATION OF PLATES.

(N.B.—All figures reduced to 7/16 in reproduction.)

PLATE VI.

- FIGS. 1-6.—*Saprolegnia parasitica*, var. *Kochhari*.  
 FIGS. 1-2.—Different stages in zoospore formation. × 140.  
 FIGS. 3-4.—Germination of zoospores inside zoosporangium. × 140.  
 FIG. 5.—*Belone cancinula* naturally infected. Reduced ¾.  
 FIG. 6.—Photos of 3 other fishes, artificially infected. Reduced ½.  
 FIGS. 7-19.—*Saprolegnia parasitica*.  
 FIGS. 7, 8, 9 & 10.—Various forms of zoosporangia showing internal proliferation. × 140.  
 FIG. 11.—Zoospores. × 610.  
 FIG. 12.—A part of zoosporangia showing internal proliferation. × 372.  
 FIGS. 13, 14, 15, 16, 17 & 18.—Chlamydozoospores. × 140.  
 FIG. 19.—Zoosporangia proliferating. × 140.  
 FIGS. 20-29.—*Aplanes* Sp.  
 FIGS. 20-21.—Zoosporangia, (1) showing both internal and lateral proliferation; (2) showing internal proliferation. × 140.  
 FIG. 22.—Zoosporangia. × 610.  
 FIG. 23.—Intercalary chlamydozoospore. × 140.  
 FIG. 24.—Intercalary and terminal chlamydozoospores. × 140.  
 FIGS. 25, 26, 27 & 28.—Various forms of chlamydozoospores. × 140.  
 FIG. 29.—Chlamydozoospores metamorphosed into zoosporangium. × 282.

PLATE VI.

- FIGS. 1-9.—*Isoachlya monilifera*.  
 FIG. 1.—Internally proliferating zoosporangium with zoospores. × 372.



- FIG. 2.—Chlamydozoospores, one with zoospores, some germinating within.  $\times 372$ .  
 FIGS. 3-4.—Zoosporangia (3)  $\times 83$ ; (4)  $\times 140$ .  
 FIG. 5.—Chlamydozoospore.  $\times 83$ .  
 FIG. 6.—Habit of oogonia with ripe eggs.  $\times 372$ .  
 FIG. 7.—An oogonium on a zoosporangium.  $\times 140$ .  
 FIG. 8.—A double oogonium with small eggs.  $\times 372$ .  
 FIG. 9.—An oogonium with ripe eggs.  $\times 610$ .  
 FIGS. 10-25.—*Protoachlya paradoxa*.  
 FIG. 10.—Habit of zoosporangia.  $\times 140$ .  
 FIG. 11.—Zoosporangium liberating zoospores.  $\times 140$ .  
 FIG. 12.—A part of zoosporangium showing elliptical zoospores.  $\times 610$ .  
 FIG. 13.—Zoospores, one germinating.  $\times 610$ .  
 FIG. 14.—Part of zoosporangium, zoospores spherical.  $\times 610$ .  
 FIGS. 15-16.—Habit of chlamydozoospores.  $\times 372$ .  
 FIG. 17.—Oogonium.  $\times 610$ .  
 FIG. 18.—Oogonia with antheridia.  $\times 372$ .  
 FIGS. 19-24.—Chlamydozoospores of various types.  $\times 140$ .  
 FIG. 25.—Chlamydozoospore metamorphosed to zoosporangium.  $\times 610$ .

## PLATE VII.

- FIGS. 1-11.—*Achlya americana*.  
 FIG. 1.—Oogonium with declinuous antheridia.  $\times 645$ .  
 FIGS. 2-3.—Habit of zoosporangia.  $\times 140$ .  
 FIG. 4.—Zoospores.  $\times 610$ .  
 FIG. 5.—Habit of chlamydozoospores.  $\times 140$ .  
 FIG. 6.—Habit of oogonia.  $\times 140$ .  
 FIG. 7.—Oogonium with androgynous antheridia.  $\times 645$ .  
 FIG. 8.—Cylindrical oogonium with ripe eggs.  $\times 280$ .  
 FIGS. 9-10.—Oogonia with zoosporangia.  $\times 140$ .  
 FIG. 11.—Large oogonium with ripe eggs.  $\times 390$ .  
 FIGS. 12-19.—*Achlya proliferoides*.  
 FIGS. 12-13.—Habit of zoosporangia.  $\times 145$ .  
 FIGS. 14-15.—Habit of chlamydozoospore.  $\times 145$ .  
 FIG. 16.—Habit of oogonia.  $\times 145$ .  
 FIG. 17.—Oogonia with declinuous antheridia.  $\times 390$ .  
 FIG. 18.—Oogonia with zoosporangium.  $\times 145$ .  
 FIG. 19.—Young oogonium with antheridium.  $\times 645$ .

## PLATE VIII.

- FIGS. 1-8.—*Achlya flagellata*.  
 FIGS. 1-2.—Habit of zoosporangium.  $\times 146$ .  
 FIG. 3.—Zoospores.  $\times 610$ .  
 FIG. 4.—Zoosporangia with lateral openings.  $\times 83$ .  
 FIG. 5.—Habit of oogonia.  $\times 140$ .  
 FIG. 6.—Oogonia on zoosporangia.  $\times 83$ .  
 FIG. 7.—Mature oogonium with ripe eggs.  $\times 372$ .  
 FIG. 8.—Young oogonium with antheridia.  $\times 372$ .  
 FIGS. 9-20.—*Achlya imperfecta*.  
 FIG. 9.—Photograph of a culture on egg yolk.  
 FIGS. 10-11.—Habit of zoosporangia.  $\times 145$ .  
 FIG. 12.—Zoospores.  $\times 610$ .  
 FIGS. 13-14.—Chlamydozoospores (4)  $\times 88$ ; (5)  $\times 145$ .

- FIG. 15.—Habit of oogonia.  $\times$  140.  
 FIGS. 16-17.—Peculiarly notched double oogonia.  $\times$  390.  
 FIG. 18.—Chlamydo-spores.  $\times$  390.  
 FIGS. 19-20.—Oogonia with antheridia.  $\times$  390.

PLATE IX.

- FIGS. 1-10.—*Achlya Kashyapia*. n. sp.  
 FIGS. 1-3.—Habit of zoosporangia too with lateral openings.  $\times$  140.  
 FIG. 4.—Habit of oogonia.  $\times$  140.  
 FIG. 5.—Oogonia with declinuous antheridia.  $\times$  372.  
 FIG. 6.—Chlamydo-spores.  $\times$  83.  
 FIG. 7.—Double oogonium, the central egg shaped as the oogonium.  $\times$  610.  
 FIGS. 8-10.—Oogonia with antheridia. (3, 5)  $\times$  610; (4)  $\times$  372.  
 FIGS. 11-17.—*Achlya Klebsiana*.  
 FIGS. 11-13.—Habit of zoosporangia.  $\times$  145.  
 FIG. 14.—Zoospores.  $\times$  610.  
 FIG. 15.—Habit of oogonia.  $\times$  145.  
 FIGS. 16-17.—Oogonia with antheridia.  $\times$  390.

PLATE X.

- FIGS. 1-8.—*Achlya conspicua*.  
 FIG. 1.—Dichtyuchus-like zoosporangia.  $\times$  83.  
 FIG. 2.—Habit of oogonia.  $\times$  88.  
 FIG. 3.—Zoosporangia with peculiar oogonium.  $\times$  145.  
 FIGS. 4-5.—Oogonia of abnormal shapes.  $\times$  390.  
 FIG. 6.—Notched oogonium with antheridia.  $\times$  390.  
 FIG. 7.—Zoosporangia showing branching.  $\times$  140.  
 FIG. 8.—Typical habit of zoosporangia.  $\times$  140.  
 FIGS. 9-14.—*Achlya dubia*.  
 FIGS. 9-10.—Habit of zoosporangia.  $\times$  140.  
 FIG. 11.—Zoospore formation.  $\times$  610.  
 FIG. 12.—Habit of oogonia.  $\times$  140.  
 FIG. 13.—Oogonium with declinuous antheridia.  $\times$  372.  
 FIG. 14.—An oogonium with mature eggs.  $\times$  610.

PLATE XI.

- FIGS. 1-12.—*Achlya dubia* var. *pigmenta* n. var.  
 FIGS. 1-2.—Normal habit of zoosporangia.  $\times$  140.  
 FIG. 3.—Zoosporangium.  $\times$  372.  
 FIG. 4.—Zoospores.  $\times$  610.  
 FIG. 5.—Habit of oogonia.  $\times$  610.  
 FIG. 6.—Two oogonia in chains.  $\times$  610.  
 FIG. 7.—A large oogonium with ripe eggs.  $\times$  610.  
 FIG. 8.—Oogonium with declinuous antheridia.  $\times$  610.  
 FIGS. 9-31.—*Achlya de Baryana*.  
 FIG. 9.—Zoosporangia.  $\times$  140.  
 FIGS. 10-11 & 13-16.—Chlamydo-spores of various types.  $\times$  140.  
 FIG. 12.—Two types of oogonia borne on a hypha.  $\times$  83.  
 FIG. 17.—Intercalary oogonia in chains.  $\times$  372.  
 FIG. 18.—An oogonium with a long papilla.  $\times$  610.  
 FIGS. 19-21.—Abnormal types of oogonia (11, 12)  $\times$  372; (13)  $\times$  610.

- FIG. 22.—An oval oogonium with an antheridium.  $\times$  610.  
 FIG. 23.—A cylindrical oogonium.  $\times$  372.  
 FIG. 24.—Oogonium in an old zoosporangium.  $\times$  140.  
 FIGS. 25–26.—Abnormal oogonia.  $\times$  372.  
 FIG. 27.—Oogonium at the tip of chlamydozoospores.  $\times$  372.  
 FIG. 28.—Triangular oogonium with ripe eggs.  $\times$  372.  
 FIG. 29.—An abnormal oogonium.  $\times$  140.  
 FIGS. 30–31.—Oogonia with zoosporangia.  $\times$  140.

## PLATE XII.

- FIGS. 1–10.—*Achlya aplanca*.  
 FIGS. 1, 2, 7, 8, 11.—Chlamydozoospores of various forms. (7)  $\times$  88; others  $\times$  145.  
 FIGS. 3, 4.—Habit of zoosporangia. (3)  $\times$  88; (4)  $\times$  145.  
 FIGS. 5, 6.—An oogonia with zoosporangia.  $\times$  145.  
 FIG. 9.—Habit of oogonia.  $\times$  145.  
 FIG. 10.—An oogonium with declinuous antheridia.  $\times$  372.  
 FIGS. 12–14.—*Achlya sp.* No. 1.  
 FIGS. 12–14.—Various types of zoosporangia.  $\times$  140.  
 FIGS. 15–16.—*Achlya sp.* No. 2.  
 FIG. 15.—Zoosporangia.  $\times$  83.  
 FIG. 16.—Zoosporangia.  $\times$  140.  
 FIGS. 17–19.—*Achlya sp.* No. 3.  
 FIGS. 17, 19.—Habit of zoosporangia.  $\times$  140.  
 FIG. 18.—Habit of chlamydozoospores.  $\times$  83.  
 FIGS. 20–23.—*Achlya sp.* No. 4.  
 FIGS. 20, 23.—Chlamydozoospores formed intercalary.  $\times$  83.  
 FIGS. 21, 22.—Zoosporangia with apical papilla.  $\times$  140.  
 FIGS. 24–29.—*Rhizidiomyces apophysatus*.  
 FIGS. 24–25.—Sporangia of the parasite on oogonia of *A. Klebsiana*.  $\times$  610.  
 FIG. 26.—Spores resting on the oogonium.  $\times$  610.  
 FIG. 27.—Parasite sending rhizoids in the interior of the host.  
 FIG. 28.—Showing two empty zoosporangia of the parasite at (A).  
 FIG. 29.—A photomicrograph showing the parasite on the oogonia.

















