INDIAN WATER MOULDS—IV

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Fam. *Saprolegniaceæ*

Protoachlya Coker

This genus was established on a species collected at Chapel Hill (America) and previously described as *Achlya*. Coker had first named it as *Achlya paradoxa*, but later changed this to *Isoachlya paradoxa*. Finally in 1923 he established it as a new genus and named it *Protoachlya paradoxa*.

Generic characters.—Hyphæ more delicate than in *Achlya*, sporangia subcylindrical to clavate or flask-shaped, blunt and usually thickest beyond the middle, proliferating like a cyme as in *Achlya*, and also, less frequently, by growth through the empty sporangia as in *Saprolegnia*. Spores diplanetic on emerging ciliate and all or some showing sluggish or less often active motion, some remaining attached in an irregular clump to the tip of the sporangium. Oogonia borne singly, the great majority on short lateral stalks from the main hyphæ and with or without a few pits, eggs usually few, centric. Antheridia androgynous or diclinous, typically pyriform with their tips applied to the oogonium. Gemmæ spherical to pyriform or elongated. Vegetative behaviour not noticeably different from the other genera.

Habitat.—The soil samples along with water were collected from a drain in Lahore and from Hiran Minar tank, Sheikhupura, during the month of November, 1938.

P. paradoxa Coker (Plate I)

Hyphæ slender, spirally twisted, little branched, largest 60 μ at the base, others smaller generally between 29·84–44·76 μ, zoosporangia mostly club-shaped, 508·2–281 $\mu \times$ 62·68–38·5 μ, rounded at the top with a distinct short papilla (Figs. 1–4), secondary zoosporangia usually formed by internal proliferation through the older ones, new zoosporangia formed entirely outside the primary zoosporangia (Figs. 2–4), rarely formed by cymose branching, zoospores biciliate, developed in several rows and all of the same zoosporangium behaving in the same manner, diplanetic. Chlamydospores pyriform or elongated with thick walls. Chlamydospores are developed mostly on the tips of hyphæ, either in chains or terminal (Figs. 8, 9).

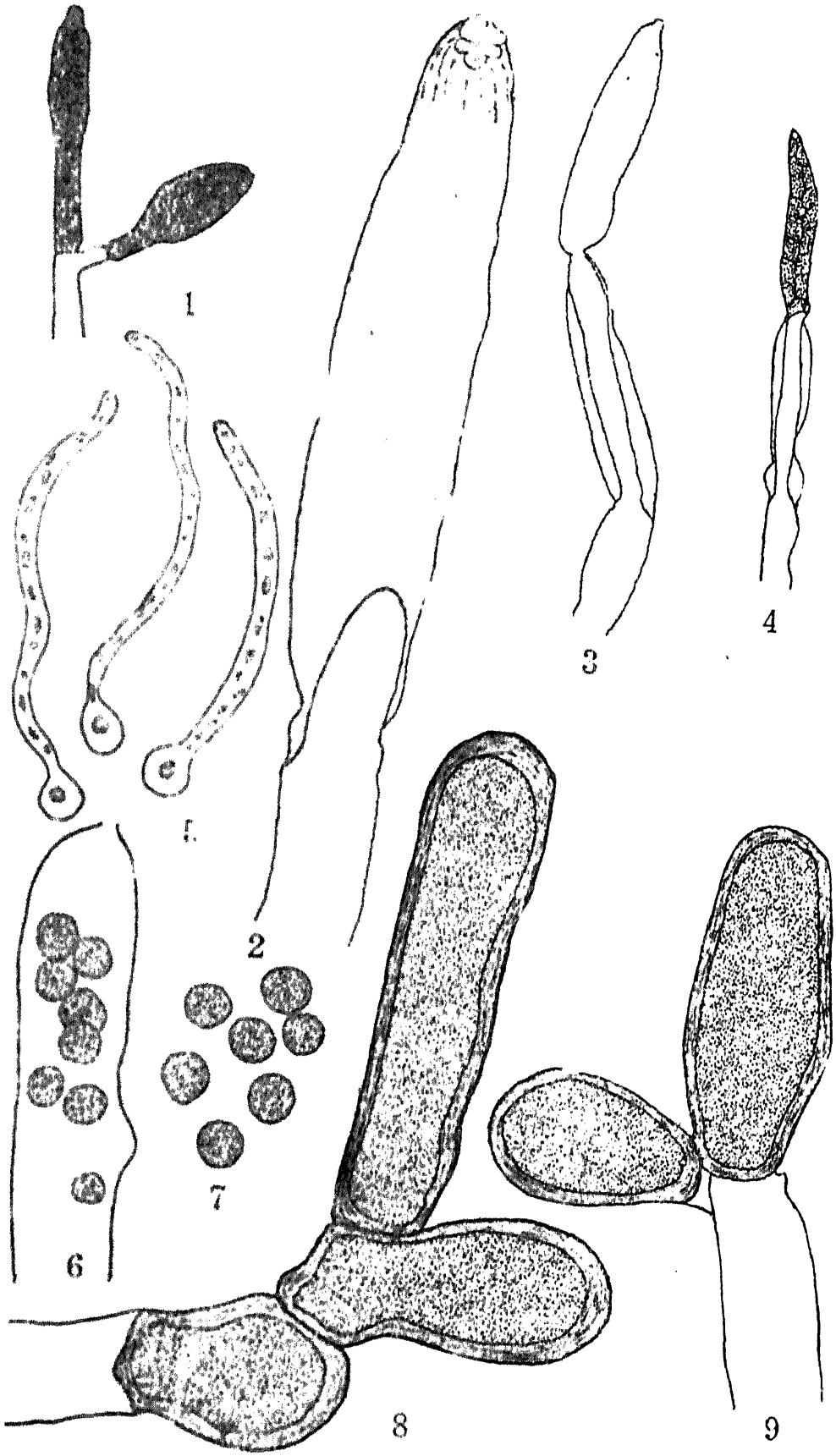

PLATE I. *Protoachlya paradoxa*

FIGS. 1-9. Fig. 1. Cymose branching of zoosporangia. ×105. Fig. 2. Zoosporangium showing ruptured apex. ×525. Fig. 3. Secondary zoosporangium formed through the primary zoosporangium. ×109. Fig. 4. Secondary zoosporangium with mature zoospores. ×109. Fig. 5. Germinating zoospores. ×487. Fig. 6. A portion of zoosporangium showing encysted zoospores. ×487. Fig. 7. Encysted zoospores. ×525. Fig. 8. Three chlamydospores in chain. ×487. Fig. 9. Two chlamydospores. ×487.

They are mostly elongated and rarely globular. They are thick-walled which measure 3·2 μ. A few oogonia and antheridia developed.

Cultural characters.—In pure culture the authors failed to get any oogonial and antheridial formation, but in some contaminated dishes, a few oogonia and antheridia were formed, but these were more or less disorganised.

On egg albumen in sterilised water—growth extensive, reaching a length of 1½ inches from the margins of the bait, plenty of zoosporangia formed; on egg albumen in tap water—growth vigorous, zoosporangia and chlamydospores formed. Later a few disorganised oogonia and antheridia appeared; on egg albumen in 1% potassium phosphate—growth very vigorous with plenty of zoosporangia and chlamydospores; on egg albumen in 1% potassium nitrate—similar growth as above; on egg albumen in 1% asparagin—very little vegetative growth; on boiled house-fly in sterilised water—growth not vigorous, zoosporangia, however, formed; on grams in sterilised water—growth not vigorous, zoosporangia and chlamydospores formed.

When a drop of zoospore-suspension was placed on a slide and covered over with a cover slip in an incubator having a temperature of 35° C., the zoospores germinated after 24 hours (Fig. 5).

Discussion.—This species has already been described by Chaudhuri and Kochhar (1935)[1] when certain variations were observed from the characters given by Coker.[2] In the present specimen further variations have been observed which may be summarised as follows:—

Highest diameter of the hyphæ recorded by Coker is about 37 μ when grown on mushroom-grub, and by Chaudhuri and Kochhar (1935) 18·6 μ and rarely upto 28 μ. But in the present case it was found to be upto 60 μ in rare cases, though usually when grown on egg albumen in water it varied between 29·84–44·76 μ. Zoosporangia are furnished in most cases by an apical papilla. They are club-shaped to globular with all intermediate forms (Figs. 1–4). The size is again variable 508·2–281 μ × 62·68–38·5 μ. Coker (1923) gives the diameter as 20–30 μ and Chaudhuri as 27·6–33·8 μ.

The secondary zoosporangia are formed by internal proliferation through the primary ones (Figs. 3–4). This point has been noted by Coker as a characteristic feature in *Protoachlya*, and thus differing from

[1] *Proc. Ind. Acad. Sci.*, 1935, **2**, No. 2.

[2] Coker, 1923, *The Saprolegniaceæ.*

Saprolegnia. This character has been found to be the most common feature in the present specimen. Cymose branching which has been so amply shown by Coker and Chaudhuri and Kochhar has been found to be of rare occurrence in the present case.

The zoospores are diplanetic, formed in several rows, showing great variation in behaviour. Coker dealing with the behaviour of the zoospores says, "The behaviour of the spores on emerging is remarkable and very variable. The usual behaviour is for some of the spores, perhaps half or a third to swim slowly away on emerging, the other remaining attached to the sporangium mouth and encysting there." The observations made in the present case are quite different. All the zoospores of the same zoosporangium behave alike. Either all are very active and swim far away from the zoosporangium or they show very slow movement settling down away from the mouth of the zoosporangium. In three cases it was noted that the zoospores collected to form irregular clumps at the mouth of the zoosporangium, thus simulating *Achlya* to a certain extent. The normal behaviour was, however, of active movement, and the zoospores charged out with great rapidity and each one dashed rapidly away. All the zoospores were distinctly seen to possess cilia.. The zoospores are oblong to oval inside the zoosporangium but become spherical outside (Fig. 7). The size of the zoospore as given by Chaudhuri and Kochhar is 12·5–13·2 μ while in the present specimen it was 10·0–12·2 μ.

Pythiopsis de Bary, 1888

Morphological characters of the genus as given by Coker and noted by the authors are:—

Hyphæ slender, much or little branched. Sporangia typically short and plump, spherical, oval, pyriform with a distinct apical papilla, or varying to elongated and irregular, primarily borne at the tips of the hyphæ and multiplied from lateral stalks below the older ones to form more or less dense clusters. Spores emerging and swimming as in *Saprolegnia*, pip-shaped with two apical cilia, sprouting after the first encystment-monoplanetic. Gemmæ resembling the sporangia or oogonia, formed plentifully, often in chains, producing zoospores after a rest. Oogonia borne like sporangia and gemmæ and resembling them in youth, typically spherical, oval or pyriform with unpitted walls, smooth or with a few blunt processes. Antheridia short and thick, typically androgynous from the close neighbourhood of the oogonia. Eggs one or few (eccentric with a lunate cap of droplets on one side in *Pythiopsis cymosa*; structure doubtful in *Pythiopsis Humphreyana*).

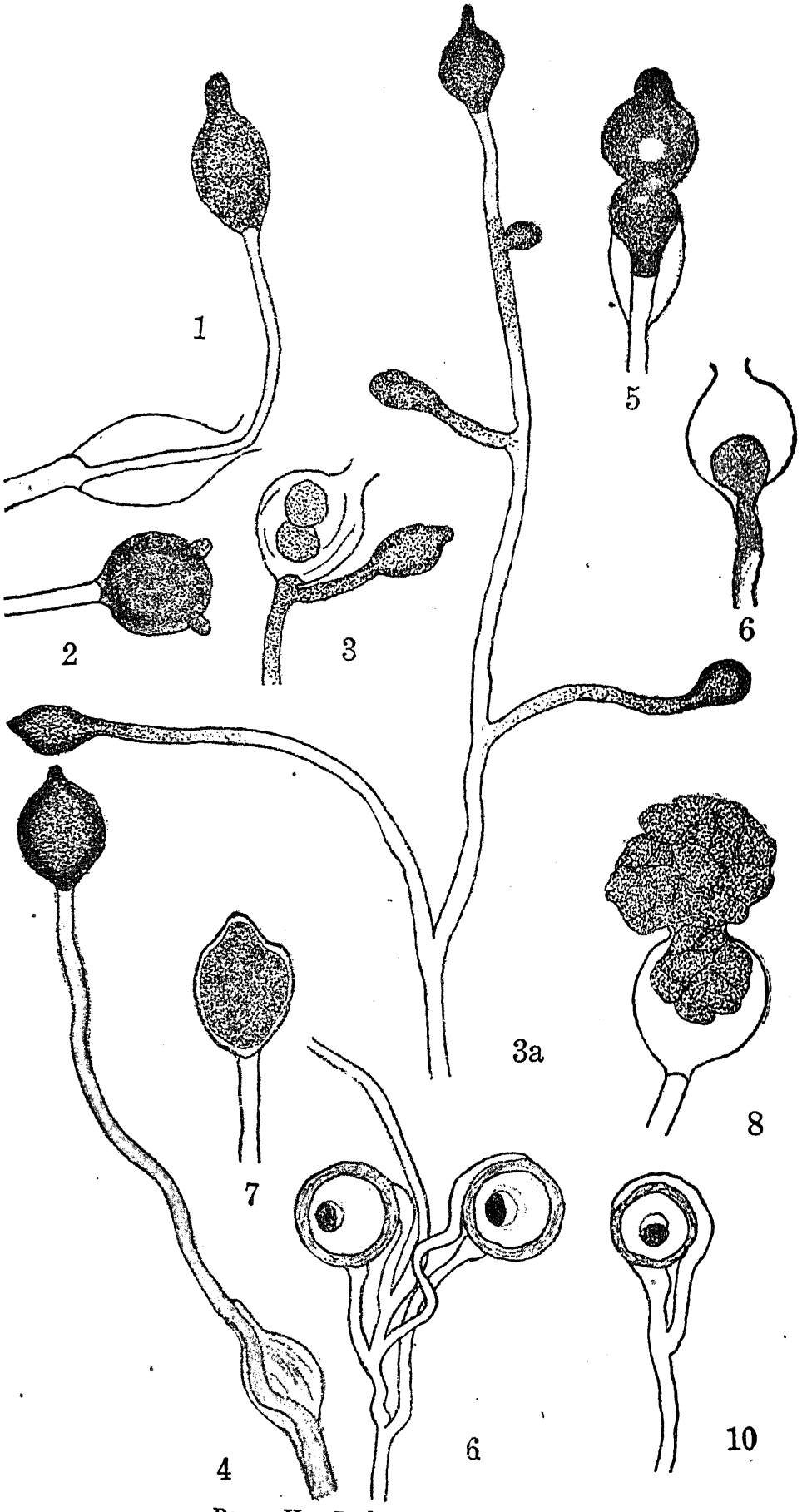

PLATE II. *Pythiopsis intermedia*

FIGS. 1 10. Fig. 1. Internally proliferating zoosporangium. Fig. 2. Zoosporangium with two apical papillæ. Fig. 3. Cymosely borne zoosporangia. 3 *a*. Cymosely borne zoosporangia. Fig. 4. Internal proliferation of zoosporangium. Fig. 5. Abnormal internal proliferation. Fig. 6. Knob formation in internal proliferation. Fig. 7. Zoosporangium half a minute before the liberation of zoospore mass. Fig. 8. Zoospore mass coming out in semi-differentiated state. Fig. 9. Hypha with two oogonia. Fig. 10. Oogonium with androgynous antheridium. Magnification in all figures 487, excepting Fig. 8 where it is 525.

P. intermedia sp. nov. (Plate II)

Hyphæ slender, 4·8–5·5 μ in diameter at base, much branched. Zoosporangia globular or clavate, usually proliferating internally (Figs. 1, 4–6) and borne in a cymose manner. A zoosporangium with two apical papillæ has been seen (Fig. 2). The secondary zoosporangium has been seen to be growing partly inside and partly outside the empty primary zoosporangium (Fig. 5). In cymose branching the secondary zoosporangium is formed either very near the primary zoosporangium or at a fairly long distance away (Figs. 3 and 3 *a*). Zoospores usually 9·5 μ, biciliate, monoplanetic. Oogonia plentifully formed in old cultures, spherical, unpitted, 25·6–35·2 μ in diameter (Figs. 4–5). Eggs 22·4–28·8 μ in diameter, single, eccentric. Antheridial branches long arising far away from the oogonium, one to each oogonium, androgynous (Figs. 9–10), clavate, later curving along the oogonial wall. Gemmæ resembling zoosporangia formed at the tips of the hyphæ but have a long drawn out apex. Gemmæ formed if the mould is kept in stagnant water for a long time or if the acid in the medium is slightly increased.

Pythiopsis intermedia sp. nov.

Hyphæ tenues, basi 4·8–5·5 μ diametro, ramosissimæ. Zoosporangia globosa vel clavata, habitualiter interne proliferentia, in cymis disposita. Zoosporangia cum duabus papillis apicalibus. Aliquando sporangium secundarium ex parte extra et ex parte intra zoosporangium evacuatum crescens. Sporangium secundarium vel proxime a primario formatur vel remote. Zoosporæ habitualiter 9·5 μ, biflagellatæ, monoplaneticæ. Oogonia rotunda efoveolata, diametro 25·6–35·2 μ, in veteribus culturis abunde occurrentia. Ova 22·4–28·8 μ diametro, solitaria, excentrica. Rami antheridiales longi, clavati, procul ab oogonio orientes, unus pro unoquoque oogonio, androgyni, post aliquod tempus jexta oogonii parietem incurvantes. Gemmæ zoosporangiis similes, in summis hyphis um apicibus longe evectis formatæ. Gemmæ habitualiter occurrentes sive in aqua stagnante qua longo tempore hyphæ continentur, sive in medio cujus aciditas leviter augetur.

Cultural characters.—On egg albumen in tap water—growth vigorous and zoosporangia formed in plenty; in eggs albumen on acidulated water—vegetative growth only; very few zoosporangia formed; on egg albumen in 1% potassium phosphates—abundant zoosporangia formed; on egg albumen in 1% potassium nitrate—vigorous vegetative growth; on egg albumen in 0·1% asparagin—very little vegetative growth; on boiled house-fly in 1% potassium phosphate—vegetative growth vigorous, plenty of zoosporangia, oogonia and antheridia also formed; on boiled house-fly in tap

water—growth sparse, a few zoosporangia formed; on corn grains in tap water, acidulated water and 1% potassium phosphate—not much growth in any case; on pea grains in tap water, acidulated water and 1% potassium phosphate—results same as above.

Zoosporangia make their appearance 30 hours after the placing of new baits. After 6 hours all the hyphæ bear zoosporangia. A zoosporangium takes 30–45 minutes to discharge the zoospores. Soon after liberation of the zoospores, multiplication takes place by either cymose branching or internal proliferation. The zoospores swim for 20–25 minutes and then settle down.

Cultured from a soil sample taken from a drain in Lahore in November, 1938.

Discussion.—In this species of *Pythiopsis* the zoosporangia are mostly globular with a dimension of 32·0–41·6 μ (Figs. 2, 7). Some are elongated (Figs. 1, 4, 5) and their dimensions are usually 31·2 × 23·2 μ or 28·8 × 22·4 μ. The hyphæ are much thinner, being 4·8–5·5 μ, while in the other two species (*P. cymosa* and *P. Humphreyana*) the thickness is much greater.

The zoospores come out of the zoosporangium in a semi-differentiated condition by the breaking up of the apical papilla (Figs. 1–6). Outside, in the medium, the zoospores are delimited (Fig. 8) and each swims away.

The size of a zoospore is usually 9·6 μ while in *Pythiopsis cymosa* the zoospores are 8·6–10·8 μ, mostly 9 μ and in *Pythiopsis Humphreyana* they are 8·6 μ. Zoosporangia multiply by cymose branching (Figs. 3, 3 *a*) as in other two species and also by internal proliferation (Figs. 1, 4, 6) which is not found in the other species of *Pythiopsis*.

The oogonium measures 25·6–35·2 μ. In *Pythiopsis cymosa* the oogonium measures 18–30 μ, while in *Pythiopsis Humphreyana* 33–89 μ, average being 43 μ. The oogonium in this new species contains a single egg measuring 22·4–28·8 μ (Figs. 8–9). The egg in *Pythiopsis cymosa* measures 14·8–18·5 μ and in *Pythiopsis Humphreyana* 24–40 μ (average 30 μ). The egg is eccentric as in *Pythiopsis intermedia* and contains a single drop of oil whereas in the egg of *Pythiopsis cymosa* there is a lunate cap of oil droplets. The wall of the oogonium is unpitted as in the other two species but the thickness is 3·2 μ in contrast to 1·4–2 μ of *Pythiopsis Humphreyana*.

As this species differs in above-mentioned characters from the only two species of *Pythiopsis* the authors have described it as a new species.

The most important point is the internal proliferation of the zoosporangia, a character not to be found in either *Pythiopsis cymosa* or *Pythiopsis*

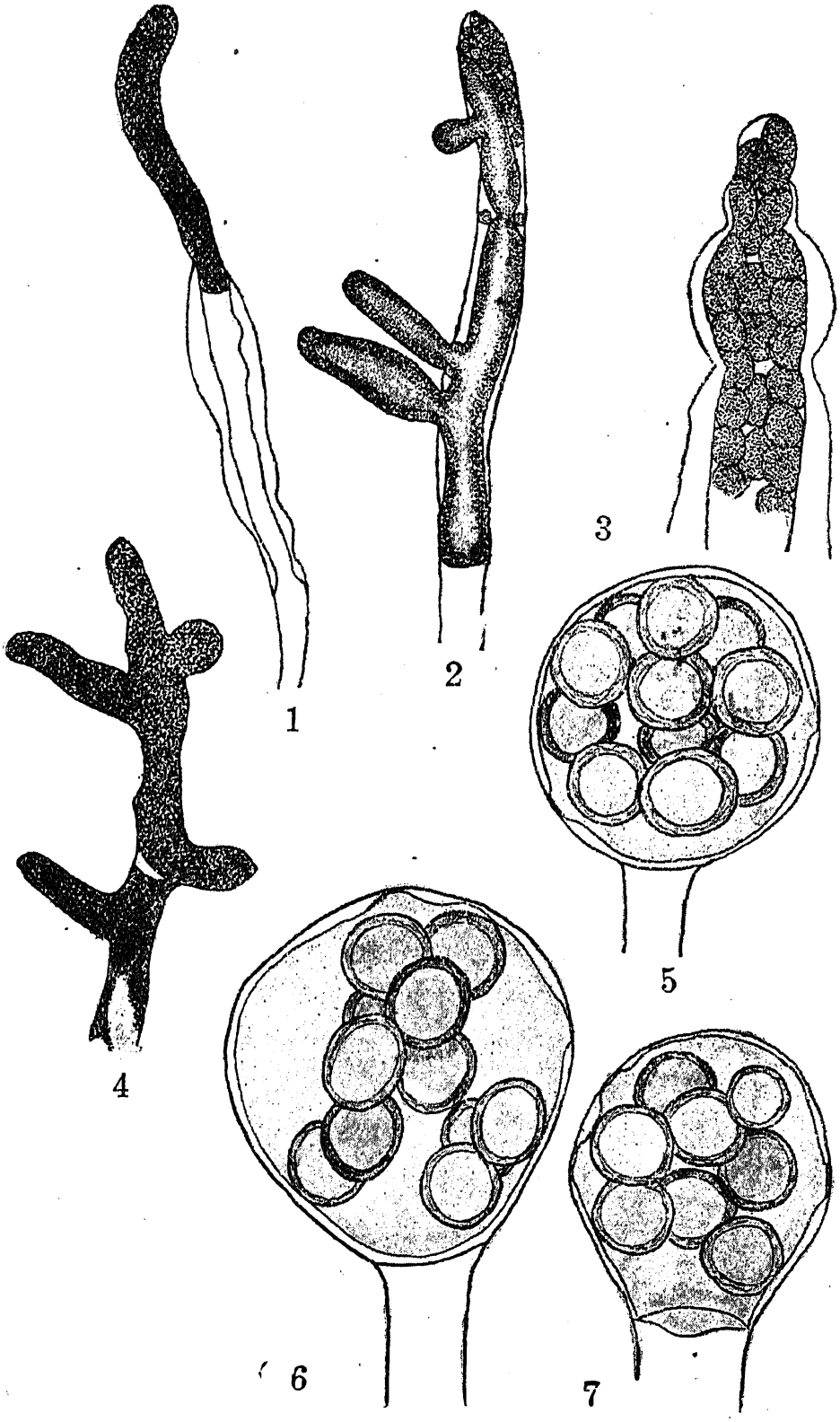

PLATE III. *Saprolegnia rhætica*

FIGS. 1–7. Fig. 1. Internal proliferation of zoosporangium. ×105. Fig. 2. Secondary zoosporangium branching. ×105. Fig. 3. A portion of mature zoosporangium. × 487. Fig. 4. Complicated gemma. ×105. Figs. 5–6. Oogonia with 12 eggs. ×487. Fig. 7. Oogonium with 9 eggs. ×487.

Humphreyana. Differences are also found in such other structures as the hypha, zoosporangium, oogonium, egg and its wall and also the position of the oil drop. Thus it is named as *Pythiopsis intermedia,* because the size of the zoospores, oogonia and eggs is more or less intermediate between the two species already described.

Saprolegnia rhætica Maurizio (Plate III)

Hyphæ branched, 30·4 μ thick. Zoosporangia are at times branched and the branches come out of the empty primary zoosporangia (Fig. 2). Zoospores 8–9·6 μ in diameter. Gemmæ formed, complicated (Fig. 4).

Oogonia 72 × 61·6 μ containing 9–12 eggs, generally 12, 19·2 μ in diameter. Oogonial wall not very thick, or pitted, very few pits (usually 2–3) (Figs. 5–7). Antheridia absent.

Growth in culture.—On house-fly in tap water—growth extensive, reaching a length of 1·5 cm., large number of zoosporangia formed, eggs develop later; on boiled house-fly in 1% potassium phosphate—vegetative growth extensive, zoosporangia formed but no oogonia; on egg albumen in tap water—vegetative growth vigorous, plenty zoosporangia but on egg albumen in ·1% asparagin, very little growth.

The present specimen of *Saprolegnia rhætica* resembles the other two identical species (Coker, 1923; de Bary), *viz., S. torulosa* and *S. variabilis* in the absence of antheridia and few pits on the oogonia walls. It differs from the descriptions given by the authors (Maurizio, Minden and de Bary) who first created these species, namely, *S. rhætica, S. variabilis* and *S. torulosa,* in minor details of measurements of various organs and number of eggs. But on the whole the characters of the specimen are common to all the three species now considered to be identical, hence the inclusion of the specimen under the species *S. rhætica.*

Collected from Lahore in February, 1939.

Summary

In this fourth paper of the series on Indian Water Moulds, the authors have recorded and described three water moulds not so far reported from this country of which, one is a new species. These are *Protoachlya paradoxa, Pythiopsis intermedia* nov. sp. and *Saprolegnia rhætica* Maurizio.

Before concluding, the authors express their sincere thanks to Dr. B. B. Mundkur for various suggestions and help with the literature, to Professor Rapinat for the Latin translation of the diagnosis of the new species and to Mr. A. Hamid for revising the manuscript.