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ON *HORMIDIELLA*, A NEW MEMBER OF THE ULOTRICHACEÆ

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Received for publication on August 12, 1940

THIS alga came up in a culture of some soil algæ at Madras in July 1938. At the time of its appearance there was plenty of *Phormidium* sp. and *Chlorococcum humicolum* (Naeg.) Rabenh. growing in the culture. Soon after its appearance, the alga became very dominant and the *Phormidium* and the *Chlorococcum* gradually became less and less prominent, and, after a month or so, completely disappeared. The alga was sub-cultured into other bottles. It grew well in these cultures and generally occupied the moist sides of the bottles immediately above the level of the culture solution, but a fair quantity of the alga was found at the bottom and also on the surface of the culture solution. The culture solution used was Moore and Karrer's (1919, p. 285) solution which was slightly modified by the addition of 0·005 per cent. of urea. The culture solution had the following composition :—

Ammonium nitrate	0·5 gm.
Potassium acid phosphate	0·2 gm.
Magnesium sulphate	0·2 gm.
Calcium chloride	0·1 gm.
Iron sulphate	trace
Urea	0·05 gm.
Distilled water	1000 c.c.

The detailed development of the alga was followed in hang-drop cultures made from the main cultures.

DESCRIPTION

The alga is filamentous and resembles a *Ulothrix* or a *Hormidium*, but its lowermost cell always possesses a long hyaline thread-like stalk with a knob-like disc at the end by means of which it is attached to the substratum. Its cells are short and cylindrical, often cask-shaped, and are 8–9 μ broad, and 3·2–8 μ long. The thread-like stalk of the basal cell is 3·5–5·26 μ long. Each cell possesses a single nucleus and a plate like parietal chloroplast with a pyrenoid embedded in it (Text-fig. 2). The chloroplast extends the full length of the cell (Text-fig. 4) and, in vertical view, almost completely encircles the cell. The lateral cell wall consists of two

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layers, a thin firm outer layer and a slightly thicker inner layer. The transverse wall is thin, consisting of only a single layer and is continuous with the inner layer of the lateral wall (Text-fig. 3).

Both the cell wall and the thread-like stalk give clear cellulose reaction when tested with iodine and sulphuric acid. They are not stained with ruthenium red showing that there is not much pectic material in them. The apical cell of the filament is broadly rounded at the top and the lowermost cell of the filament is slightly narrowed towards the base. At first, the filaments in the cultures were quite short, and generally did not contain more than about 16 cells, and were either straight or slightly curved (Pl. V, Fig. 2, Text-figs. 1 and 4). But in older cultures, the filaments were very much longer and were moreover very much twisted (Pl. V, Fig. 6). The length of the filament in the younger cultures ranged between 135–168 μ and in the older cultures between 206–250 μ.

Asexual Reproduction

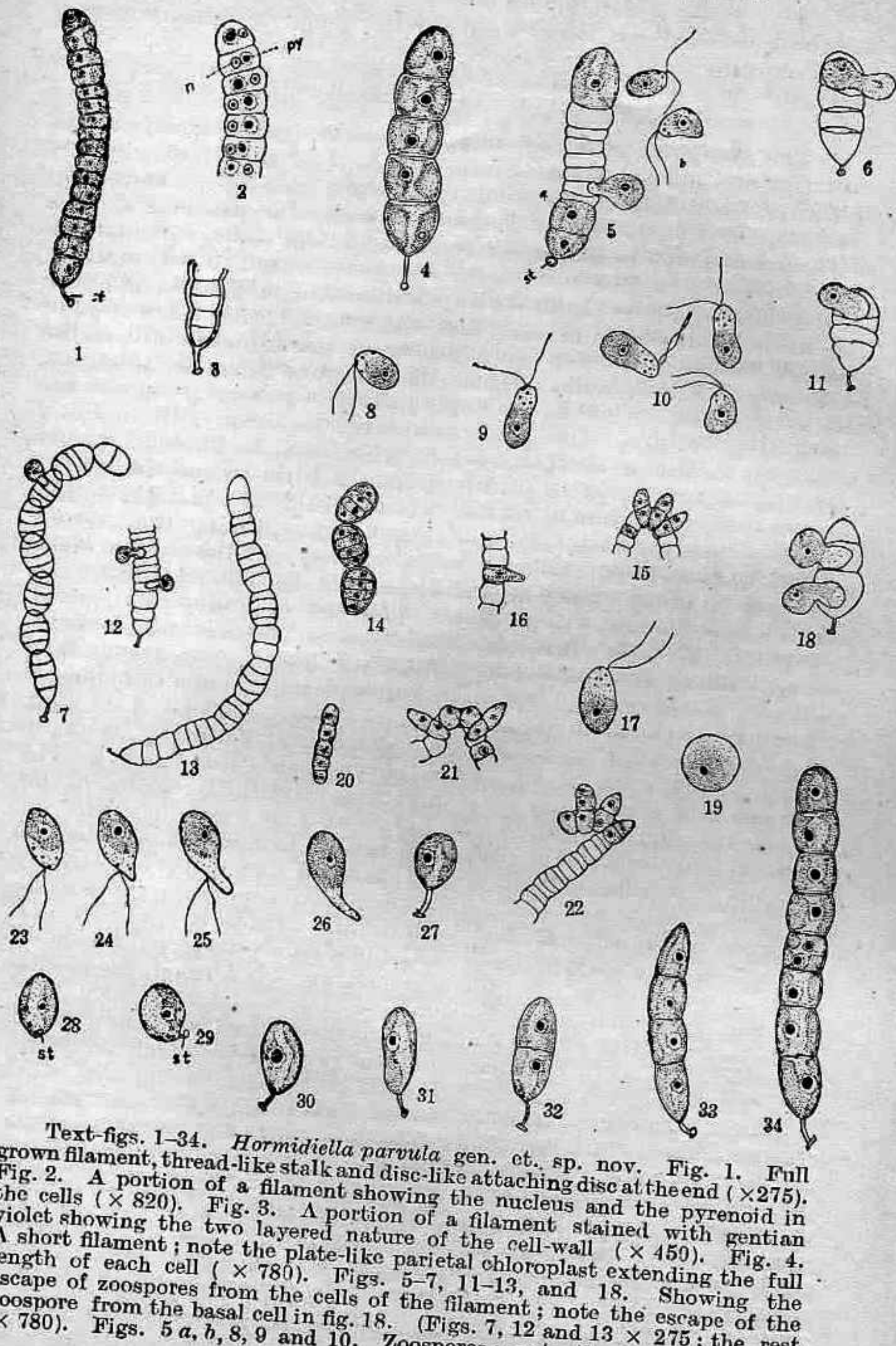
Asexual reproduction takes place by means of zoospores which are formed singly in each cell (Text-figs. 5–7, 10–12, 18). The zoospores are slightly dorsiventral, being somewhat convex on one side and slightly concave or flat on the other (Text-figs. 5 *b*, 10). Each zoospore possesses two cilia which are attached at the anterior end somewhat laterally towards the concave side (Text-fig. 8). The two cilia are equal in length and are placed very close to each other. A small, somewhat thick greenish refractive body could be often seen near the base of the cilia (Text-figs. 8, 9). Whether this represents the blepharoplast or not, it could not be decided, as the body was single and not double and was moreover situated a little to one side of the place of attachment of the cilia. A single, somewhat obliquely oval, plate-like chloroplast in which is imbedded a pyrenoid occupies the posterior portion of the zoospore (Text-figs. 5 *a*, *b*, 8–10). The anterior portion is hyaline and has a somewhat dull refractive appearance. Owing to this refractive appearance, no contractile vacuoles could be seen even when examined very carefully under higher magnifications. A few small granules are present in this portion. No eye-spot could be seen, even though numerous zoospores were carefully examined. In a few zoospores however, a tiny reddish speck was seen very close to the base of the cilia. Whether this represented a rudimentary eye-spot could not be decided. The fact that the swarm spores do not travel very far from the mother-plant, but settles down close to it very soon after liberation may be in some way connected with the poor development of the eye-spot. The zoospores are 5–5·5 μ broad and 6·65–7 μ long. The cilia are about 10·2 μ in length.

The zoospores, in their dorsiventral shape, in the somewhat lateral position of the two cilia and in the absence of an eye-spot, show a very close resemblance to those of *Hormidium* as figured by Klebs (Klebs, 1896, Taf. II, Figs. 23 and 24). Two contractile vacuoles were observed by Klebs in *Hormidium* in the anterior hyaline portion, but as already pointed out, owing to the refractive nature of the anterior portion, no contractile vacuoles could be made out in the present alga. This refractive substance is evidently connected with the formation of the attaching stalk as may be seen later on in the paper (see pp. 161–162).

The zoospores generally come out between 8-30 and 9 A.M. A single zoospore is formed in each cell. It escapes through a round aperture formed in the lateral wall of the cell. Since this aperture is very narrow, the zoospore has to squeeze itself out through it very gradually (Text-figs. 5, 6, 7, 11, 12, 18). At one stage, it looks as though the zoospore would be cut into two (Text-figs. 6, 12, 18). But this never happens and the zoospore escapes from the cell quite alright every time. The method of escape of the swarm-spore through a very narrow opening closely resembles that of *Hormidium flaccidum* as figured by Klebs (Klebs, 1896, Taf. II, fig. 21). Usually zoospores escape out with their posterior end foremost (Text-figs. 11, 18), but frequently they escape with their anterior end foremost also (Text-fig. 6). The time taken for the zoospore to escape from the cell is about 10 to 15 minutes. Very commonly practically whole filaments get nearly or completely empty (Pl. V, Fig. 1, Text-figs. 7, 12, 13). It is very interesting to see the whole of the hang-drop culture full of active motile zoospores. It may be pointed out here that in this alga, unlike in *Ulothrix*, the basal cell also is able to form zoospores (Text-fig. 18). Plenty of zoospores were formed in the young cultures. But in the older cultures zoospore-formation was seen only very occasionally and that only from a few cells of the filaments.

Prior to the formation of the zoospores, the cells of the filaments divide into 2 or 4, or occasionally, 3 smaller cells by one or two successive transverse divisions (Text-figs. 7, 13, 14). And from each one of these smaller cells a single zoospore is formed. It may be pointed out here again that the basal cell, unlike in *Ulothrix*, is able to divide like the rest of the cells of the filament (Text-figs. 7, 18). This division of the mother-cell usually takes place about 3 to 4 A.M. An interesting phenomenon is observable before this cell division takes place. A few hours previous to the cell-division, say about midnight or so (11-30 P.M. to 1 A.M.), the protoplast of the mother-cell begins to show peculiar movements inside the cell. Sometimes the protoplast would get massed together on one side and sometimes on the other and this movement will continue for quite a long time, until finally the cell division takes place.

As soon as the zoospores are liberated, they have a somewhat elongated shape with a slight depression on the concave side (Text-figs. 5 *b*, 10), but they soon become more ellipsoid (Text-figs. 5 *a*, 8, 10). They swim at first with an irregular wobbling forward movement, often also in circles, but a few minutes later, the forward movement is slowed down and the movement becomes more or less rotatory and wobbling and confined to a very narrow area. After about 10 to 15 minutes, the movement becomes very slow and the zoospores finally become completely quiescent or show only slow



Text-figs. 1–34. *Hormidiella parvula* gen. et. sp. nov. Fig. 1. Full grown filament, thread-like stalk and disc-like attaching disc at the end (×275). Fig. 2. A portion of a filament showing the nucleus and the pyrenoid in the cells (× 820). Fig. 3. A portion of a filament stained with gentian violet showing the two layered nature of the cell-wall (× 450). Fig. 4. A short filament; note the plate-like parietal chloroplast extending the full length of each cell (× 780). Figs. 5–7, 11–13, and 18. Showing the escape of zoospores from the cells of the filament; note the escape of the zoospore from the basal cell in fig. 18. (Figs. 7, 12 and 13 × 275; the rest × 780). Figs. 5 *a*, *b*, 8, 9 and 10. Zoospores; note the refractive body oscillatory movements. Their cilia at this stage continue, however, to be vibratile.

GERMINATION OF THE ZOOSPORES

The zoospore, after becoming quiescent, soon forms from its anterior end an elongated thread-like stalk by means of which it attaches itself to the substratum (the cover glass of the hang-drop culture) (Text-figs. 23–27). The way in which the attaching stalk is formed could not be made out for quite a long time. At first we thought that the two cilia of the zoospore became fused together along their length and formed a thread-like attaching stalk, but later on we found that this presumption was wrong. After a few days of careful watching, we were able to observe the actual details of the formation of this stalk. When the zoospore becomes quiescent, its anterior end is close to the lower surface of the cover-glass in the hang-drop culture. The cilia, however, are seen still actively vibratile. After a short time a protuberance is formed rapidly from the anterior portion of the zoospore a little to one side of the region of the attachment of the cilia (Text-fig. 24), and the protuberance soon grows longer and longer, thus forming the narrow elongated thread-like stalk (Text-figs. 25, 26). As the stalk is being formed, the protuberance at first appears to be somewhat short and beaked and hollow with a small quantity of protoplasm inside (Text-figs. 25, 26). But very rapidly, the protuberance becomes longer and at the same time loses its hollow appearance and becomes narrower and solid, its protoplasmic contents evidently receding into the main portion of the zoospore. The end of the stalk becomes somewhat expanded into a disc-like structure by means of which it attaches itself to the cover-glass (Text-fig. 27). The cilia could be seen clearly for a short time after the formation of this protuberance (Text-figs. 24, 25), but, very soon after that, the cilia could not be seen at all. We were unable to find out whether the cilia are discarded outside or are absorbed inside the body of the zoospore. We searched carefully near the zoospore for the possible presence of the discarded cilia, but were unable to find any. It seems therefore very probable that the cilia are retracted inside the body of the zoospore. As the attaching stalk is formed, the hyaline portion of the zoosporic cell decreases in size and at the same time loses its refractive appearance and becomes quite transparent. This is evidently due to the fact that the substance which made the

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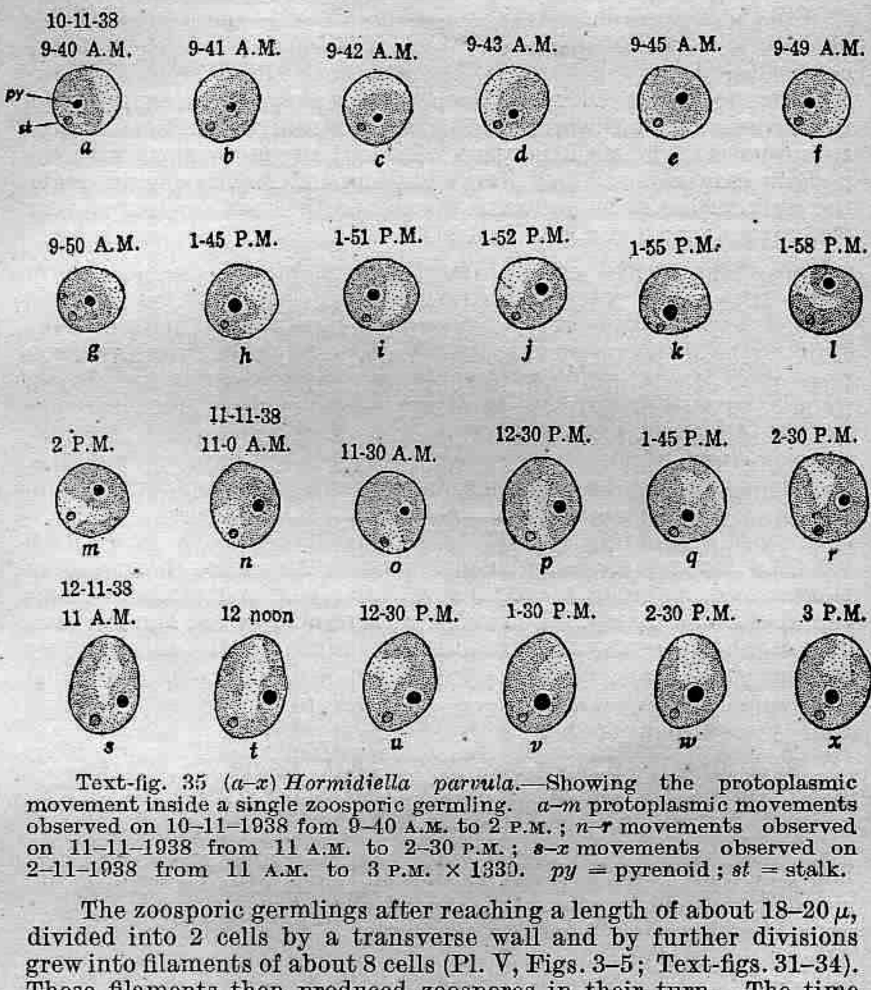
near the base of the cilia (blepharoplast ?) in figs. 8 and 9 (× 780). Fig. 14. Division of the cell into four daughter-cells before the formation of the zoospores (× 275). Figs. 15, 16, 21 and 22. The formation of aplanospores and their germination (× 235). Fig. 20. A short filament formed from a single aplanospore ; note the absence of the stalk in the filament (× 235). Fig. 17. A gamete (?) (× 780). Fig. 19. A gamete (?) which has rounded itself, after losing its cilia (× 780). Figs. 23–27. Showing the stages of formation of the stalk by the zoospore as it settles down (× 780). (Figs. 24–26. Diagrammatic.) Figs. 28–31. Stages showing the development of the zoosporic germling into full grown filament (× 780). n = nucleus ; py = pyrenoid ; st = stalk.

anterior portion of the zoospore very refractive was very probably used up in the formation of the attaching stalk with the result that the refractive anterior portion becomes quite transparent.

In the hang-drop cultures the stalk of the zoospore is not visible at first and the zoosporic germling appears as if attached to the cover-glass directly by a small disc-like knob (Pl. V, Fig. 7; Text-figs. 28, 29). This is because the long stalk is hanging straight from the cover-glass downwards and is therefore not visible when viewed from above. The stalk becomes visible if the cover-glass with the hang-drop is mounted on a glass slide and gently pressed on, when the stalk, owing to the pressure, lies flat on the slide and is then very well seen (Text-figs. 27, 30 and 31).

The thread-like stalk of the alga resembles very much that of *Ophiocytium majus* Naeg. It is very interesting to note that a certain amount of resemblance is seen even in the manner of formation of the stalk in these two algæ. Pascher (1925, p. 73) states regarding the formation of the stalk in *Ophiocytium majus* "Zunachst leigen (immer nach Scherffel) die Schwarmer noch unbeweglich vor der Zelle und schwimmen dann ruhig ohne Rotation davon. Bei der Keimung kugelt sich der vordere Teil ab, wahrend aus dem hinteren, von dichter weiszglanzender Substanz erfullten Teile ein stielformiger Fortsatz herausmodelliert wird, der spater zum Stielchen wird. Die Geisselpartie und das Vorderende sind nach Scherffel bei der Keimung nicht beteiligt." But the stalk in *Ophiocytium majus* is formed by the posterior end of the swarm-spore, whereas, in the present alga, it is formed by the anterior end. This similarity in the formation of the stalk in these two algæ does not of course mean anything more than a mere case of parallel development, since *Ophiocytium* belongs to the Xanthophyceæ, while the present alga belongs to the Chlorophyceæ.

Soon after the formation of the attaching stalk, a wall is formed round the protoplast of the zoospore. This protoplast of the germling is, however, not quiescent, but shows a very peculiar movement inside the wall continuously for quite a long time (up to 48 hours or more). As a result of this movement, the position of the chloroplast could be seen continuously shifted inside the cell. After about 48 hours the movement becomes slower and finally stops. The movements of the protoplast of a single zoospore germling was followed by means of camera lucida drawings for three days and the continuous changes of position of the contents are shown in Text-fig. 35 *a–x*. On the first day, immediately after the zoospore settles down (at about 9 A. M.), the position of the chloroplast was changing almost every five minutes (Text-fig. 35 *a–g*). But towards the evening the change of position of the chloroplast became slower and thereafter still slower. Again the shape of the chloroplast also shows a gradual change. On the morning of the first day the chloroplast was more or less plate-shaped (Text-fig. 35 *a–g*), but towards the evening, it became somewhat bell-shaped (Text-fig. 35 *h–m*). On the third day or so, the cell became somewhat elongated and the chloroplast became a curved plate extending nearly the whole length of the cell, and in vertical view encircling the cell almost completely (Text-fig. 35 *s–x*).



Text-fig. 35 (*a–x*) *Hormidiella parvula*.—Showing the protoplasmic movement inside a single zoosporic germling. *a–m* protoplasmic movements observed on 10–11–1938 fom 9–40 A.M. to 2 P.M.; *n–r* movements observed on 11–11–1938 from 11 A.M. to 2–30 P.M.; *s–x* movements observed on 2–11–1938 from 11 A.M. to 3 P.M. × 1330. *py* = pyrenoid; *st* = stalk.

The zoosporic germlings after reaching a length of about 18–20 μ, divided into 2 cells by a transverse wall and by further divisions grew into filaments of about 8 cells (Pl. V, Figs. 3–5; Text-figs. 31–34). These filaments then produced zoospores in their turn. The time taken for the zoospores to grow into full filaments and form zoospores in their turn was about 17–20 days.

Aplanospores

Some of the zoospores do not escape from the cells, but surround themselves with a thin membrane while still inside the mother wall and become aplanospores. These aplanospores soon grow out into short filaments of 2 or 3 cells (Text-figs. 15, 16, 21, 22). These short filaments grow at right angles to the mother filament and give a false appearance of lateral branching (Text-figs. 15, 16, 21, 22).

But an examination of different stages of these growths clearly shows their real nature. When these short filaments are about 2 or 3 cells long, they become detached from the mother plant and grow into new independent filaments. The filaments which are formed by the aplanospores do not possess an attaching stalk (Text-fig. 20).

Mention must be made here of some peculiar swarm-spores which were formed in some of the older cultures. These swarm-spores resembled in all respects the ordinary zoospores but were slightly larger than the latter (Text-fig. 17). They, unlike the normal zoospores, do not settle down soon after their escape from the mother plants (*i.e.*, within 10–15 minutes), but keep on swimming for quite a long time (two hours or longer) and finally become quiescent. They then lose their cilia and become rounded. They do not form an attaching stalk (Text-fig. 19), nor do they get attached to the substratum (cover-glass) in any other manner. They soon show signs of disintegration and finally die. The significance of these peculiar larger swarm-spores is not clear. The fact that they do not settle down and grow into new plants suggests that they are probably not zoospores. It is just possible that they represent gametes (macrogametes ?) which do not find partners.

Vegetative Reproduction

The filaments, both in the earlier and in the older cultures, often become fragmented into smaller bits of one or more cells. These then by the division of their cells grow into longer filaments. The filaments which grow out of the fragmented bits do not show any attaching stalk.

Discussion

This alga at first sight looks like a *Ulothrix* with a peculiar thread-like attaching stalk, developed from its basal cell. The chloroplast also is like that of *Ulothrix* in being a curved parietal plate and extending the whole length of the cell and also in encircling the cell almost completely. But its swarm-spores are dorsiventral and quite unlike those of *Ulothrix*, but more like those of *Hormidium* (Klebs, 1896, Taf. II, Figs. 24, 28). And its basal cell, unlike in *Ulothrix*, can divide and produce swarm-spores like the other cells of the filament. The present alga differs from *Hormidium* in the following respects. The chloroplast in the latter genus is generally a parietal plate with a circular or elliptical outline and does not extend the full length of the cell, but occupies more or less the centre of it, and moreover, does not encircle more than half of the cell (Printz, 1927, p. 166; Fritsch, 1935, p. 205; Smith, 1933, p. 384; Klebs, 1896, p. 328; Heering, 1914, p. 41). In the present alga, on the other hand, the chloroplast is a curved plate, and extends the full length of the cell and also encircles almost the whole of the cell. Again, in *Hormidium* no rhizoidal cell is formed, and there is no difference between a base and an apex (Klebs, *loc. cit.*, p. 343; Printz, *loc. cit.*, p. 166; Heering, *loc. cit.*, p. 41), whereas in the present alga, a definite stalk is formed by its basal cell, and the differentiation of a base and an apex is a very characteristic feature of the alga.

It may thus be seen that the present alga combines the features of both *Ulothrix* and *Hormidium* and, in addition, possesses a feature peculiar to itself in having a thread-like stalk which is formed in a very characteristic manner. It forms an interesting link between *Ulothrix* and *Hormidium* and, therefore, may be placed in a new genus which may be called *Hormidiella*.

Hormidiella gen. nov.

Thallus filamentous and unbranched and consisting of a row of cells, and attached to the substratum by the basal cell; apical cell broadly rounded at the top; basal cell with a long thread-like stalk below, by means of which the filament is attached to the substratum; the remaining cells, cylindrical or cask-shaped and more or less uniform in appearance; all cells including the basal cell capable of division and of producing swarm-spores. Cells uninucleate; chromatophore parietal, plate-shaped, with a single pyrenoid in it; chromatophore extending the full length of the cell and in vertical view almost completely encircling the cell. Reproduction by means of biciliate zoospores formed singly in each cell; zoospores dorsiventral, with two equal cilia attached somewhat laterally towards the ventral side; eye-spot absent. Aplanospores occasionally formed. Sexual reproduction not known.

Hormidiella parvula sp. nov.

General characters same as those of the genus, cells 8–9 μ broad and 3·2–8 μ long; stalk of the basal cell 3·5–5·26 μ long. Filaments either straight or slightly curved; in older cultures intricately twisted or contorted; zoospores 5–5·5 μ broad and 6·65–7 μ long.

Hab.—In a laboratory culture of soil algæ at Madras.

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EXPLANATION OF PLATE V

FIG. 1. Filaments with plenty of empty cells after the escape of the zoospore ; note the single celled zoosporic germlings. × 140.

FIG. 2. Full grown filaments. × 140.

FIGS. 3–5. Young filaments showing the attaching stalk. Figs. 3 & 4 × 280 : Fig. 5 × 540.

FIG. 6. Filaments from older cultures showing a contorted growth. × 40.

FIG. 7. Zoosporic germlings two hours old attached to the cover glass by means of their stalk. × 510.

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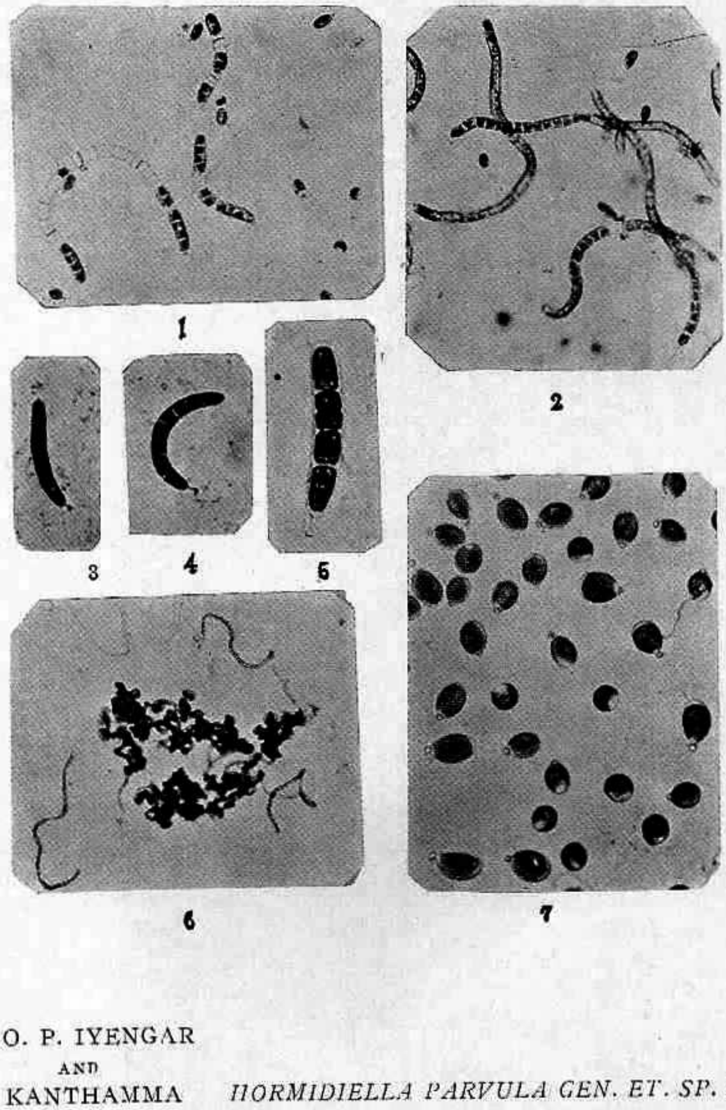

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