A Monadine parasitic on Saprolegnieae[1].

BY

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With Plate XXII. B.

IN my cultures of Saprolegnieae I was for a long time perplexed by structures which looked like abnormal spores with a gigantic nucleus, and which I at first really regarded as such. However, on making cover-glass cultures of 'cuttings' (fragments of mycelium removed with a blunt knife) with young oogonia, I found to my cost that they were parasitic organisms, which demanded careful study. They soon turned out to be members of Cienkowski's group 'Monadineae,' now regarded as the close allies of Myxomycetes; and, so far as can be judged from the vegetative conditions and zoocysts, are referable to the well-named genus *Pseudospora* Cienk.[2], created in 1865, to receive very similar parasites on the green Conjugates and Desmids. The organism is so abundant in cultures of Saprolegnieae that it could not escape the notice of previous observers. Pringsheim[3] first saw the zoocysts in *Saprolegnia* (*Leptomitus*) *lactea*, and figured them in company with the undestroyed cellulin-corpuscles in an empty hypha. He describes them as 'Eine grosse Anzahl stark mit Inhalt erfüllter kugeliger Zellen . . . die offenbar keine Schwärmsporen sind. Ihre Bedeutung ist mir noch unbekannt.'

Lindstedt in 1872, in his 'Synopsis Saprolegniacearum,' recognised their true nature. 'In älteren Fäden deren fand

[1] Read at the British Association, Newcastle-on-Tyne, 1889.

[2] Beiträge zur Kenntniss der Monadinen, in Schultze's Arch. f. Micr. Anat. i. 213.

[3] Jahrbücher, II. 234, t. xxiii. f. 6.

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ich mehrmals die von Pringsheim bei *Leptomitus lacteus* erwähnten kugeligen Zellen. . . . Diese Zellen sind von verschiedener Grösse, aber gleichartiger kugeliger Anstalt; die kleineren sehen wie homogene Protoplasmatropfen aus, die von einer sehr zarten Membran begränzt scheinen; in der grösseren befinden sich in der Mitte mehrere dichte Körperchen von unbestimmter Anzahl, doch nicht 4 übersteigend. In einem einzigen Falle bemerkte ich zwischen solchen ruhenden Zellen auch bewegliche, die sich in Form und Grösse von diesen nicht unterschieden. Diese Erscheinung deutet darauf hin, dass die kleinen Organismen Monaden sind. . . . Identificirt mit einer der von Cienkowski aufgestellten Arten konnte diese Monade nicht sein, sie entzog sich bald ganz der Beobachtung.' It is obvious that neither the proper flagellate nor the amoeboid stage was seen by our author; but his instinctive recognition of its systematic position merits that I should attach his name to the species.

For another decade the organism remained unnoticed, so far as I know, till Pringsheim [1] described and figured certain amoeboid structures as differentiated in the antheridial tubes of *Achlya*, and escaping into the oogonia presumably to fertilize the oospores; he therefore termed them 'sperm-amöben.' Zopf [2] at once took up the cudgels in favour of the parasitic nature of the spermamoebae, and in connection therewith referred to what are certainly the monadines we have to deal with; but, though he promised a fuller paper, it has not appeared to the best of my knowledge, though in his monograph, 'Die Pilzthiere oder Schleimpilze,' published in Schenk's 'Handbuch der Botanik' in 1887, he makes a further allusion to the controversy. Pringsheim answered the challenge [3], but an account of the discussion, so far as it bears on our species, is best deferred till we have studied it in detail.

[1] Neue Beobachtungen über den Befruchtungsact der Gattungen *Achlya* und *Saprolegnia* in Sitzungsber. d. k. Akad. d. Wiss. zu Berlin, 1882, 855, t. xiv.

[2] Ueber Parasiten in den Antheridien Oogonien und Oosporen der Saprolegnieen, in Botan. Centralblatt, xii. 356.

[3] Ueber die vermeintlichen Amöben in den Schläuchen und Oogonien der Saprolegnieen, in Bot. Centr. xiv. 378.

The first stage I shall describe is the mastigopod swarmer, or zoospore, which may frequently be found in and about the infested hyphae of old cultures about 9 o'clock in the evening, or later. It is oblong (7–10 μ long), acute in front (Figs. 1, 2), with one or two anterior flagella, blunt behind or acute with posterior tractellum; in this variability it resembles the type-species *P. parasitica*, as indeed in almost every respect. Each has a nucleus of the rhizopod or myxomycete type, i.e. vesicular with the nuclein in a spherical central mass; and there is at least one contractile vacuole anterior to the nucleus. They swarm hither and thither in the hypha for a long time before escaping, and then swim about freely in all directions. Being usually formed in closed hyphae they must escape by boring through the wall by the emission of a pseudopodium, but the actual escape has eluded my observations. After a prolonged period of active swimming, less rapid and peculiar than that of Chytridian swarmers, they settle down on the walls of living hyphae, glide along them amoeba-fashion, and finally penetrate into them. I have not seen the full process of penetration; but in several cases I have seen the amoeboids emit a long pseudopodium through the cell-wall and parietal protoplasmic investment of the living hypha and wave for some time in the lumen (Figs. 7, 8). We can scarcely doubt but that the rest of the body follows suit after the fashion of a white blood-corpuscle in diapedesis, but, of course, inversely. The aperture must be elastic and closes completely, possibly by secretion of cellulose from the fungal protoplasm; for there is no loss of turgescence in the hypha so attacked. The stage we are at now is usually termed 'amoeboid'; but '*Heliozooid*' would be the better term, since the pseudopodia are always radiate and stiffish, as in most of the *Monadineae*.

The amoeboids may exist in large quantities in the hyphae, probably from simultaneous or consecutive attacks of numerous swarmers. Without denying the possibility of multiplication by fission in this stage (as occurs in *P. parasitica*, according to Zopf) I must state that I have never seen any indications of it. From the figures (4–8) may be seen the very characteristic

forms assumed at this stage. From theoretical considerations I had not anticipated that the contractile vacuoles would be retained as such in the cell-sap of the living fungus; but this was not confirmed, for they persist, and may be seen to arise afresh after systole by the confluence of two or three very minute vacuoles on their previous site (Fig. 3).

The changes in the infested hyphae are very noteworthy. First of all, the microsomes diminish rapidly in number, so that the protoplasm becomes much more hyaline; granules, with rapid Brownian motion, appear in the hitherto limpid cell-sap. During this stage the nuclei are unaffected, and owing to the decrease of the microsomes (which normally mask them from view) may be readily studied in the living state, as I have stated elsewhere [1]; the protoplasmic currents persist on the walls and in the threads traversing the lumen. The protoplasm is gradually reduced to a granular *débris*, or finally disappears entirely. The cellulin-corpuscles are completely unaffected, and remain to the last, which is not the case in the normal formation of zoospores or oospores in the Saprolegnieae, nor in hyphae of *Achlya* affected by the Chytridian *Woronina polycystis*. In some cases I have seen dumbell-aggregates of minute needle-shaped crystals in such exhausted hyphae. It not unfrequently happens that when a hypha is attacked in one part, the unaffected end protects itself by a transverse wall of protoplasm, which usually bulges out; and the healthy part very frequently emits a narrow hypha, which grows thyllus-fashion into the cavity of the affected part.

During their growth the amoeboids can migrate, leaving one hypha to enter another; that figured in 7 and 8 is obviously, from its size, not the direct transformation of a young zoospore.

After the nutrient protoplasm is used up, that of the parasites has become coarsely granular. Soon the pseudopodia are retracted, and the granules become collected into a highly refractive excrementitious mass, surrounded by a clear vacuole,

[1] Recherches sur la Structure des Saprolegniées, in Comptes Rendus, April 5, 1889.

and simulating a gigantic nucleus. These granules stain deeply with nuclear stains (borax carmine, haematoxylin, nigrosin), and are obviously nitrogenous (can they be the undigested nuclein derived from the nuclei of the host?). The pseudopodia are retracted, and the body rounds off and becomes spherical. At this and subsequent stages of the zoocyst a long flagellum may be protruded, giving rise to the sluggish movements of the body (Figs. 14, 15), seen by Lindstedt. The spherical mass soon becomes infested by a membrane, which is probably chitinous, as it neither swells nor stains blue in the Schultze solution of iodine. It is in this stage, that of the 'zoocyst,' that the species has been noted by my predecessors. Zoocysts may be found lying free in the *débris* of old cultures as well as inside the hyphae. In my balsam mounts of Saprolegnieae these zoocysts frequently occur slightly or not at all stained. The stain I use is borax carmine and nigrosine, followed by treatment with acid alcohol for differentiation, so that the parasite is possibly more readily decolourised than the fungus. When well-stained the excrementitious mass is often even darker than the nucleus, which still retains the characteristic rhizopod type. From comparison of successive stages (Figs. 9–13) in stained preparations we find that the nucleus then undergoes complete bipartition to form from four to sixteen—usually eight—daughter nuclei; which soon become regularly distributed through the protoplasm. The latter then divides according to one of two ways: either the vacuole around the fæcal mass sends directly radiating processes outwards, or else radiating vacuoles appear in the plasma and open first into that surrounding the fæcal mass before they extend to the periphery, and so divide the plasma into wedge-shaped masses. These are the zoospores. One of these bores through the cyst-wall, and the others follow through the same hole; no discharging process is formed as in Chytridieae and Saprolegnieae themselves. The zoocyst-wall persists; but the excrementitious mass left within soon disintegrates and disappears (Figs. 14, 15). Hyphae attacked by *Woronina polycystis*, may also be attacked by our parasite, which lives

and forms its zoocyst unaffected by the presence of the Chytridian.

As stated above, the species I have described differs little so far from Cienkowski's type *P. parasitica*; and I should not have ventured to separate it but for one reason: the type-species preys on Desmids and Conjugates; ours never attacks green Algae (nor Phycochromaceae) unless they be previously injured: this point I have made out by observation.

As to its systematic position. Following Cienkowski and Zopf, this must belong to the Monadineae Zoosporeae, possessing mastigopod zoospores. This is divided by Zopf into three orders: Plasmodiophoraceae, Gymnococcaceae, and Pseudosporaceae. In the absence of resting-spores we can only affirm certainly that it does not belong to the first; but from its extreme likeness to *Pseudospora parasitica* C. it is almost certainly a member of the same order and probably the same genus. I define it thus:

'*Pseudospora* (?) *Lindstedtii* mihi. Monadinea Zoosporea, zoosporis oblongatis 1–3 flagellatis, postea Heliozoi habitu nec in plasmodia coalitis; Zoocystis massâ faecali excentricâ vacuolo spherico circumdatâ praeditis, 4–16 (plerumque 8–) paris; in hyphis Saprolegniearum diversarum (*Leptomiti*, *Saprolegniae*, *Achlyae*), nec in algis viridis v. cyaneis parasitica; sporis "quiescentibus" dictis adhuc ignotis.'

We may now revert to the position it has taken in the Saprolegnia-fertilisation controversy.

Pringsheim, as mentioned above, states that certain portions of the antheridial protoplasm of Saprolegnieae become specialised and escape as spermamoebae to fertilize the oospheres. Zopf in a series of theses gives the following account. Numerous minute amoebae appear in the antheridium of Saprolegnieae at the time of fertilization, pass into the antheridial tubes and apparently disappear on arriving at the oospheres. The oospore shows however changes due to the infesting parasite, manifested in the appearance of numerous fat-globules finally coalescing into a single lateral drop. The protoplasm and wall of the oospore thus affected show

changes indicating its 'pseudomorphism' into a parasite-spore. Besides these, *other parasitic swarmers are found which become somewhat amoeboid* ('schwach amöboid werden') *and bore into the vegetative hyphae; these are larger than the former and possess a pale nucleus.* They may enter the antheridium and pass down the fertilizing tubes into the oogonium, and according to Zopf form zoocysts therein, but do not prey on the spores. The smaller amoebae, and to some extent the larger, he regards as identical with Pringsheim's spermamoebae. Now while it is obvious that in many respects Zopf's larger amoebae correspond with my species, he refers them in his monograph to the genus *Vampyrellidium*[1], and species *V. vorax* Z., which differs in that the zoospores do not pass through a mastigopod stage, and, from his figures (Fig. 37), in the characters of the zoocyst which has granular peripheral plasma, with a central nucleus surrounded by hyaloplasma; and finally in preying on green Algae as well as on Saprolegnieae. This species I have not found; but I cannot help thinking that he must have overlooked the differences between it and mine, which is unmistakably that figured by Pringsheim and recognised by Lindstedt, and which I have never found absent from old cultures. While in his paper he says they do not attack the oospores, in his monograph he says they wander 'in die vegetativen Schläuche sowohl als in die Oogonien und Antheridien hinein, nähren sich vom Inhalt dieser Organe, und bilden in ihnen schliesslich auch Dauersporen.' I have repeatedly seen my organisms destroy the young oospores before encysting in the oogonium, so that the same oogonium may contain zoocysts of the parasite and oospores of the Fungus; and they sometimes even attack the ripe oospores despite the protection of the thick cell-wall. I think it very possible that Zopf may have confused two species here. His phrase 'Schwärmer, welche schwach amöboid werden' would seem to imply that they were previously flagellate, and hence could not belong to *Vampyrellidium.*

[1] Die Pilzthiere oder Schleimpilze, 101.

Pringsheim in his reply denies that the large amoebae can have anything to do with his spermamoebae. He admits that amoebae may be seen in empty organs which are no longer closed, and that Chytrideae and their allies can, of course, in the swarming stage penetrate healthy normal organs, but observes, justly, that these have nothing to do with Zopf's amoebae. He denies that the amoebae so often seen crawling over the hyphae ever penetrate them, and refers Zopf's observations to a confusion with the cellulin-corpuscles, which by the way I may note he was the first to correctly describe and name in a full study in a later paper[1]. His words are worth quoting: 'Es sind frei im Zellumen der Saprolegnieen-Schläuche und in den Oogonien niedergeschlagene und im Alter geschichtete Körner aus einer Art Pilzcellulose, oder eine verwandte Modification derselben. . . . Es sind diese körnige Niederschläge des Zellinhalts, welche derselbe [Zopf] für zu Ruhe gekommene Amöben und Spermamöben angesehen hat.' On this identification I may note that it is obviously wrong, as the cellulin-granules always disappear in the formation of spores, and it is only in hyphae emptied by the action of parasites that I have ever found them persist. Pringsheim's own old figure of *Leptomitus* with a zoocyst of the Monadine side by side with a laminated cellulin-body, is ample proof of the inaccuracy of the last conclusion. I have frequently seen an amoeboid of the form of an acute isosceles triangle, with the angles rounded and the base anterior in locomotion ('*Amoeba limax*' form), crawling about my cultures; but this is quite distinct from the amoeboids of any monadine[2].

It is strange to find that Pringsheim should have fallen into this confusion, and that neither he nor possibly Zopf appears to have seen the mastigopod stage. I can only account for it by the hour at which swarming takes place, an hour not likely

[1] Berichte d. Deutsch. Bot. Gesellschaft, 1883.

[2] Pringsheim also answered Zopf in a paper in his own Jahrbücher (vol. xiv. 1884, p. 111, Nachträgliche Bemerkungen z. d. Befruchtungsact von *Achlya*), but he gives no further account of the larger amoebae which alone concern us here, nor does he even refer to his previous reply to Zopf in the Centralblatt.

to be chosen by a man who has leisure for original research in the daytime.

It is obvious that, as my organism is probably identical with Zopf's larger amoebae, while his smaller ones are equivalent to Pringsheim's spermamoebae, the foregoing study does not directly solve the question as to the fertilization or apogamy of the Saprolegnieae. Yet in so far as it proves that Pringsheim has confused a parasitic with a normal structure in the one case, it raises a presumption that he may have made the same mistake in the other; and I have a strong body of evidence from a totally different source tending to prove that his theory is erroneous. But this belongs of right to my nearly completed study of the protoplasmic structures of the Saprolegnieae and will be fully discussed therein.

EXPLANATION OF FIGURES IN PLATE XXII. B.

Illustrating Prof. Hartog's paper on a Monadine parasitic on Saprolegnieae.

Figs. 1, 2 (× 750 E). Two young swarmers as drawn in the infested hyphae which contains also mature zoocysts; nucleus seen.

Fig. 3 (× 750 E). Young amoeboids in the living hyphae of *Achyla*. Two contractile vacuoles shown, one in the moment of reconstitution after systole by the confluence of three minute ones. *n* = nucleus; *co* = contractile vacuoles.

Figs. 4, 5, 6 (× 750 E). Older amoeboids from within a hypha; 5 and 6 are successive stages of the same; a few bright (faecal?) granules present.

Figs. 7, 8 (× 750 E). A migrating amoeboid inserting a pseudopodium into a living hypha; two successive stages.

Figs. 9–13 (2 mm. apochr., 12 Comp. Oc. × 1000; owing to their being balsam specimens this is equivalent to a magnification of 666 × the living organism). 9, young zoocyst with faecal mass (*e*) and nucleus; 10, same, with seven daughter nuclei visible (eight in all); 11, 12, further stages of spore-formation, the spore-origins somewhat unduly separated by shrinkage; 13, the spores completely separated.

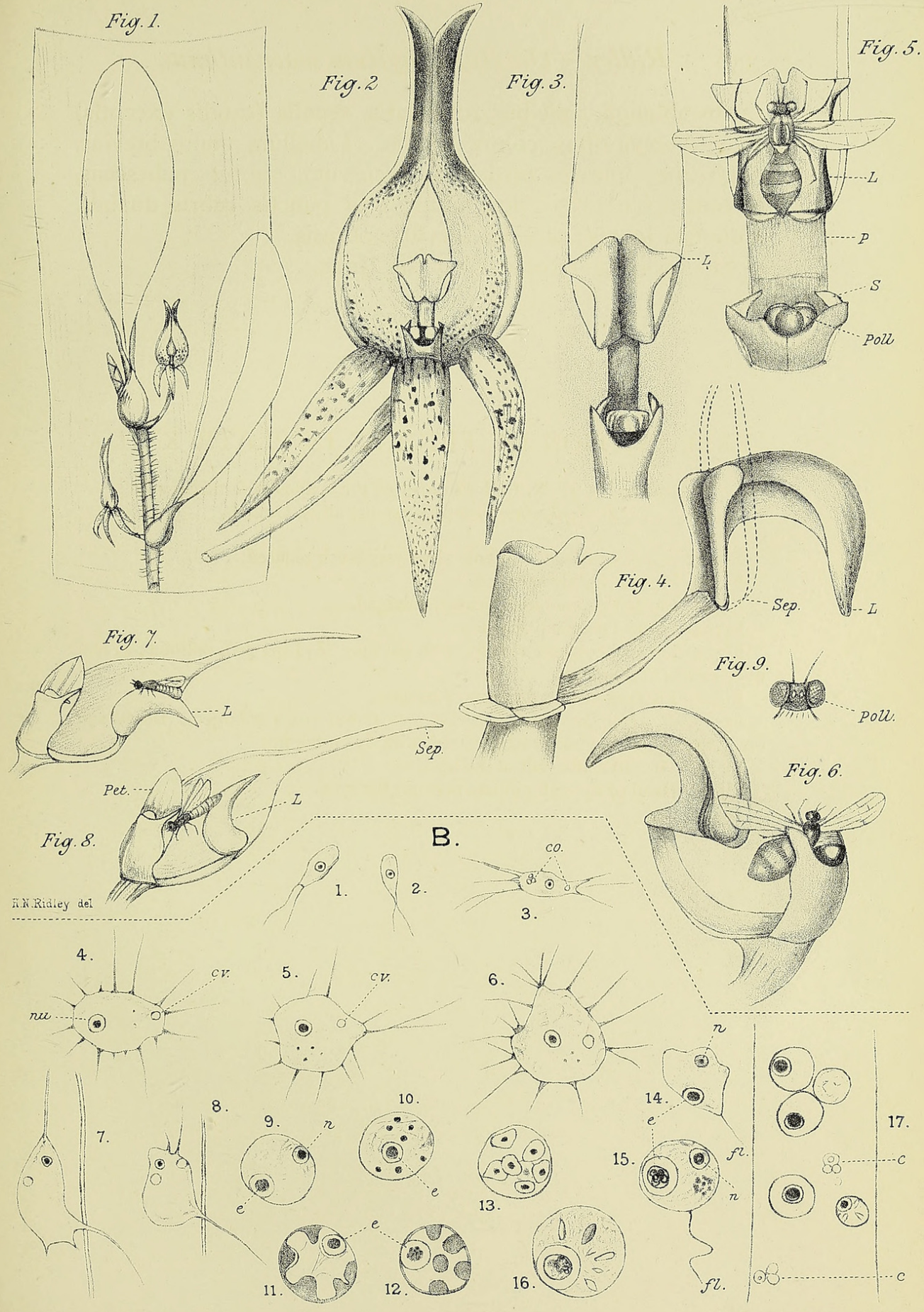
Fig. 14 (same magnification). Balsam specimen at commencement of zoocyst-formation, with flagellum protruded.

Fig. 15 (× 750 E). Same stage living; some of the faecal granules still in the protoplasm.

Fig. 16 (× 750 E). Live zoocyst, divided partially into spore-origins by radiating vacuoles.

Fig. 17 (× 350 E). Portion of infested hypha of *Achyla* with zoocysts and cellulin-bodies (*c*).

Besides the magnifications, the objective of Zeiss under which the drawing was made is given.



M.M.Hartog del.

University Press, Oxford.

A. RIDLEY.—ON FERTILISATION IN BOLBOPHYLLUM.

B. HARTOG.—ON A PARASITIC MONADINE.