On a Fungus Allied to the Saprolegniaceæ found in the Pea-crab *Pinnotheres.*

By

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With 13 Figures in the Text.

INTRODUCTION.

In the course of work on *Pinnotheres*, a fungus, which from its general characters would appear to be allied to the Saprolegniaceæ, was noticed in pea-crabs taken from mussels (*Mytilus edulis*) coming from beds in the estuary of the Camel near St. Issey Cliff (Padstow), from the estuary of the Yealm, and from near the junction of the Tamar and Tavy (Weir Point). Although the pea-crab lives in estuarine situations where the water is brackish, yet it may be taken in bivalves dredged in rather deep water on the coast (**1**, p. 123) ; those in the Laboratory were kept in the sea-water in ordinary circulation in the tanks and it was under these conditions that the fungus was seen to grow and produce reproductive elements. This fungus therefore attacks a marine invertebrate and, as far as I have been able to ascertain, it is the first member of the Saprolegniaceæ which has been so described. Some members of the family which occur on living freshwater fish are *Saprolegnia parasitica* Coker* on salmon, trout, etc.—stated by Patterson (**13**, p. 5) to be unable to grow in sea-water—*Achlya Hoferi* Harz on Bohemian mirror carp (**8**, p. 201 ; **2**, p. 145), *A. Nowickii* Raciborski on a sink carp (**2**, p. 147), and *A. polyandra* and *A. prolifera* on various fish (**9**, p. 108), while two which are found on freshwater plankton-crustaceans are *Leptolegnia caudata* de Bary on *Leptodora Kindtii* in Denmark (**14**, p. 511) and *Pythiopsis cymosa* de Bary on *Holopedium gibberum* in Lake Malmagen in Sweden (**14**, p. 511).

The mycelium of the *Pinnotheres* fungus may penetrate deeply into the body of the pea-crab, surrounding the organs, and may occasionally extend into the appendages, mouth parts, and even the eye-stalks, while it is generally found in the gills (Fig. 1). Such deep penetration of the tissue of the host would appear to be somewhat unusual among the Saprolegniaceæ, but it may also be effected by the salmon fungus where

* Coker (**2**, p. 58) considers that the fungus described from salmon and certain other fish as *Saprolegnia ferax* is distinct from that species, and he names it *S. parasitica.*

the way is prepared by *Bacillus salmonis pestis*, the bacteria breaking down the tissue which the fungus is then able to invade (**13**, p. 7), and by *A. Hoferi* of the Bohemian mirror carp, which is also probably preceded by bacteria (**2**, p. 145 ; **8**, p. 201). In these cases the fungus may penetrate the entire dermis with the exception of the muscles. The mycelium of *L. caudata* which attacks and kills *Leptodora* in large numbers, sometimes almost exterminating them from certain lakes in
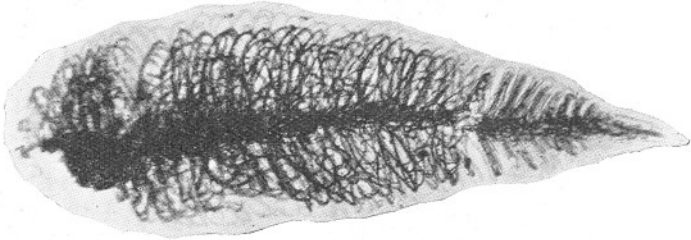

Fig. 1.—Photograph of a gill infested with the fungus, fixed in Flemming's fluid.
× ca. 29.

Denmark, is said by P. E. Müller to envelop the organs. Petersen (**14**, p. 512) thinks that the mycelium usually enters round the mouth opening and, spreading rapidly, kills the animal, while it finally envelops both the mother individual and the eggs with a thick meshwork of hyphæ.

DESCRIPTION OF THE FUNGUS.

Mussels were obtained from Padstow on the following consecutive dates, November 25/27, February 24/28, April 25/28, August 1/28, and September 14/28, and a certain number of the pea-crabs taken from them on arrival at the Station either showed signs of the disease at the time or developed it later, but crabs obtained from mussels from the same place on October 11/28 were apparently free, for they did not develop it although many were kept together in bowls for about 6 weeks. Crabs which also developed the disease were obtained from near the junction of the Tamar and Tavy on March 22/28, April 10 and 20/28, and from the Yealm on July 21/28. The fungus would therefore seem to attack crabs during the greater part of the year in the comparatively mild climate of the south-west of England and is not restricted to the period of July to about the middle of September as are apparently the attacks of *Leptolegnia* on *Leptodora* in Denmark (**14**, p. 512). In some few instances it is certain that the crabs were already infected when taken from the mussels on arrival from the beds, and against the possibility of their acquiring the infection from the water circulating in the tanks in the Laboratory is the fact that crabs kept for over a year separately in mussels for rearing experiments have not developed the disease.

The presence of the fungus is mostly indicated some days before the death of the crab either by opaque white patches showing through the chitin of its body and abdomen, or else more rarely by the opaqueness of the gills, though a crab may die of the disease without any outward indication.   The patches in the body generally occur over the gill chamber, especially the posterior region, though as the disease progresses the whole of the roof of the gill chamber may become involved.   A white line is sometimes seen along the junction of the carapace with the
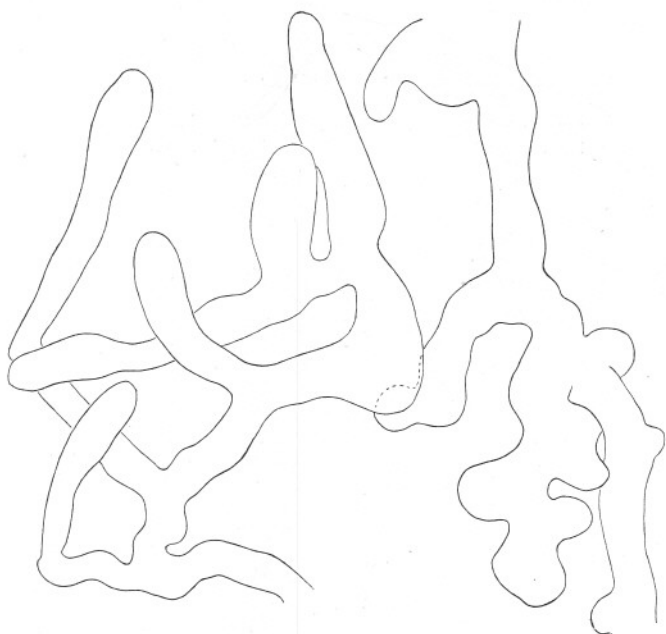

Fig. 2.—Broad, uncrowded hyphæ from roof of gill chamber.   The protoplasmic contents are not shown, they were very finely granular with indication of vacuoles.   × ca. 253.

This figure and all following ones were drawn from living material.

abdomen, and patches may also occur in the abdomen, where they are generally to be found round the junction of two segments.   The white appearance due to the presence of the fungus can readily be distinguished from that due to parasitisation by a Sarcosporidian identified by Dr. Pixell Goodrich ; in the latter case the muscles only become an opaque white.   The white patches of the fungus probably surround the original point of infection, and from their position it will be realised that the fungus enters the crab either where the chitin is extremely thin or along the fine chitinous membrane which unites the segments of the abdomen, and the posterior border of the carapace to the abdomen.   The white patches are

an intricately branched felt-like mycelium of fine hyphæ averaging about
9μ in width ; occasionally very broad, uncrowded hyphæ up to about
32μ in width are found in the roof of the gill chamber (Fig. 2).

It appears that when the fungus enters through the exceedingly fine
chitin roofing the gill chamber, it spreads for some distance in the tissue
between it and the thicker chitin of the dorsal surface of the carapace,
while it sends branches into the gills and sometimes the mouth parts.
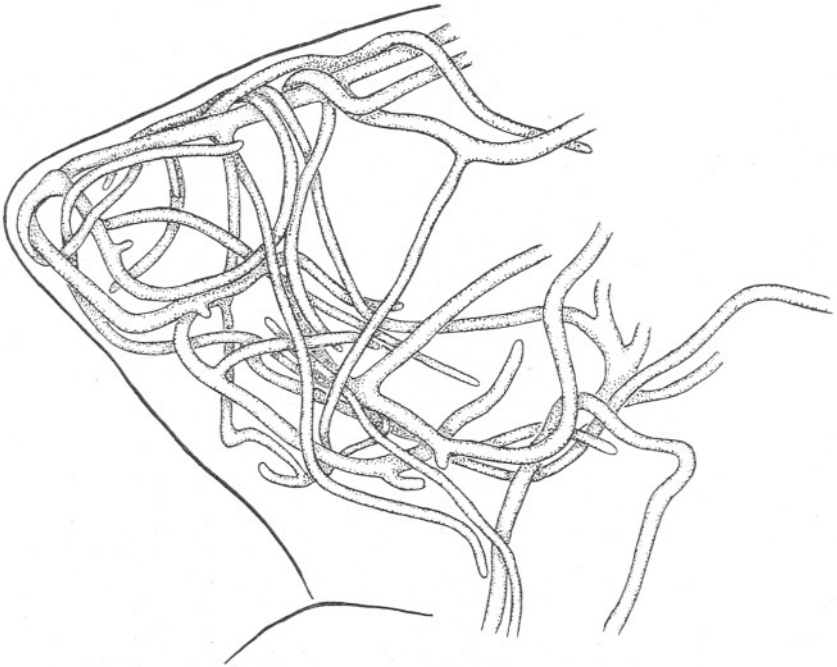The fungus is most easily seen in these situations, but probably in some,

FIG. 3.—A rather heavy growth of the fungus in one of the larger leaflets near
the base of a gill.   × ca. 114.

if not all cases, it also extends into the body of the crab. Perhaps the
easiest way of following the hyphæ, but only possible if the crab is small
and thin-shelled, is to lift off the carapace, puncture the abdomen in
several places, and fix the animal in Flemming's fluid. The oil globules,
often present in great numbers in the hyphæ, are blackened by the osmic
acid, and the branches of the fungus can be traced after clearing the crab
in glycerine. By this means the fungus was seen without dissection in
the body of three small crabs.

When the fungus has once entered the gills it soon absorbs the tissue
and death of the pea-crab is rapid. It is an interesting possibility that
in this case death may be due to asphyxiation.

In examining crabs for the presence of the fungus, when the white patches are absent, an inspection of the gills only has usually been relied on. A crab may die, however, when only one gill out of the six is infected, and therefore it would appear that in such a case there must be a considerable growth of the fungus in the body, unless laboratory conditions, which possibly included for these crabs shortage of food, markedly weakened them.

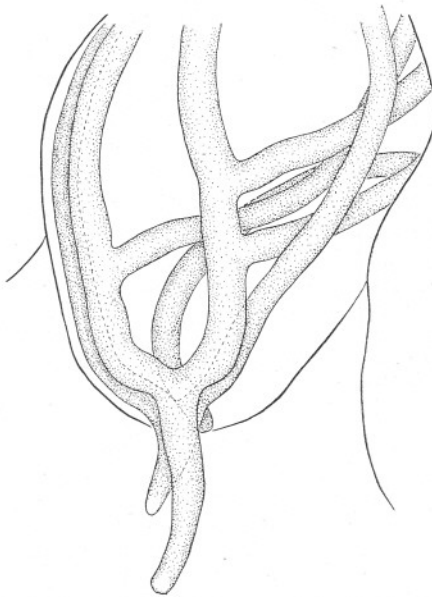The fungus is most easily studied in the gills where the delicate chitinous

Fig. 4.—Sketch to show the very regular mode of growth of the hyphæ within the gill leaflet. The papillæ for discharging the spores are well developed but intact. The protoplasm is indicated by stippling, but no attempt has been made to show its structure. The thick lines show the outline of the gill leaflets. × ca. 253.

covering is so thin as to be transparent (Fig. 1). The hyphæ would seem to enter through the base of the gill from the infection area in the roof of the gill chamber and pass down the axis of the gill, giving off branches into the gill leaflets, both the axis and the leaflets soon becoming crowded with richly branched hyphæ (Fig. 3). At least in the young stages of the fungus the hyphæ keep within the leaflet, passing up one edge, curving very regularly round the tip, and passing down the other edge and so into the main axis, giving off branches on the way.

The whole of the contents of the hyphæ in the gills of the host gradually

divide into zoospores and no marked sporangia are formed. This fungus resembles *Aphanomyces* (**2**, p. 160) and *Leptolegnia* (**14**, p. 522) in that the sporangia are formed from unchanged hyphæ. Cross walls arise, but the portion cut off may be of considerable length with several branches ; it may include a length running along the axis of the gill with loops into the gill leaflets, but apparently with only one exit. When the abdomen of
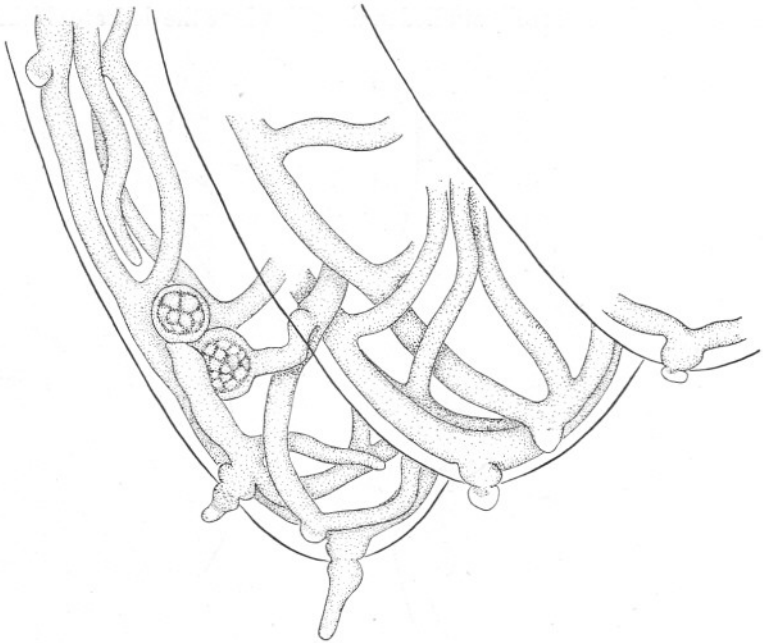

Fig. 5.—Sketch to show the fungus in three overlapping gill leaflets. Discharging papillæ are developing and show a bulge at the point of penetration of the chitin of the gill. The sketch was made at 8 p.m.; the next morning the filaments were empty, as was also the larger of the two reproductive bodies ; the smaller, which had a wall dividing it from the hypha, was still full. The protoplasm is indicated by stippling, but no attempt has been made to show its structure, except in the two round reproductive bodies. The thick lines show the outline of the gill leaflets.   × ca. 253.

the crab has areas of infection, hyphæ may pass into the pleopods and there become sporangia. A characteristic of this fungus is that the spore-producing filaments occur within the crab, though they would appear to be restricted to the gills and pleopods, the hyphæ in the roof of the gill chamber and in the body being purely vegetative. The reproductive hyphæ are generally stouter than the vegetative and are remarkably regular, tapering gradually to the tip with very clear and definite outline (Fig. 4). They vary in width from about 10μ to 20μ. Gills and pleopods

may be seen with a network of hyphæ empty except for a comparatively few spores which, unable to escape for some reason, have encysted there. The tissue of such gills and pleopods generally has disappeared entirely. It would seem that the filaments which enter these structures are entirely reproductive, though where infection takes place directly on the gill there is a tiny opaque patch of vegetative hyphæ. It may be that in the comparatively confined space of the gill or pleopod the tissue is soon consumed, and under these conditions spore formation naturally follows.
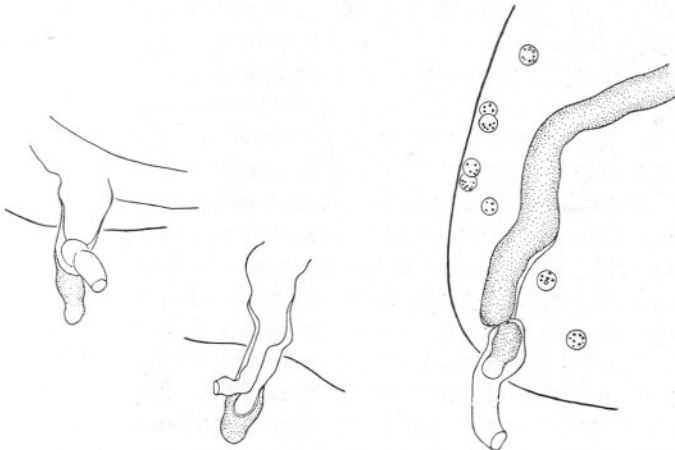

FIG. 6.—Sketches to show the development of a secondary discharging papilla when the tip of the first one has not given way. × ca. 342½.
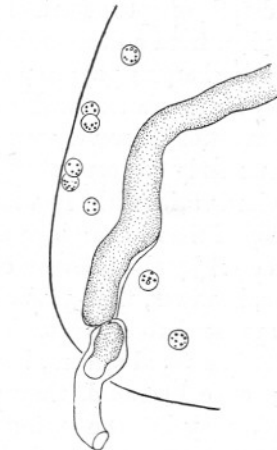
FIG. 7.—Hypha growing up within an old sporangium after the Saprolegnia type of proliferation; it is much reduced in size where it penetrates the gill chitin. The tip of the hypha is of almost clear protoplasm. A few spores are lying free on the surface of the gill leaflet, the outline of which is shown by a thick line. × ca. 342½.

Klebs in 1899 showed that sporangia are formed only when the food-supply is quickly and markedly decreased (15, p. 484).

When spore formation is about to take place a small branch or papilla arises either from the end of a hypha (Figs. 7, 8A) or along its length (Figs. 4, 5, 9, 12) and the tip becomes applied to and penetrates the chitin. There is a slight bulging of the tip of the papilla, before the chitin is penetrated (Fig. 5). The branches to the exterior are mostly quite short, ranging between 0·03 mm. to 0·12 mm. It is by these short branches or papillæ that the zoospores pass to the exterior and, with very few exceptions, this is the only part of the fungus which extends into the sea-water. In one interesting case where the long blade-like exopodite of the second

pleopod of one side was infected, the long hairs were absent towards the tip, only their circular bases remaining, and the tips of the hyphæ were protruding through the centre of these cup-like bases. Branched discharging papillæ may occur where the tip of the original papilla has apparently failed to give way and a lateral branch has arisen through which discharge has taken place (Fig. 6).

Occasionally a new sporangium may grow up within an old one, as sometimes occurs in *Leptolegnia* (**2**, p. 157) and is typical in *Saprolegnia*, but the most that has been seen is one within another (Fig. 7).

The details of spore formation have not been observed. A gill which when pulled from a dead crab at night shows no sign of spore formation or of the discharging papillæ will the next morning be seen with a quantity of empty sporangia and with others in the process of discharging spores ; spore formation, however, occurs in gills in situ. Spore discharge seems most commonly to occur at night. The number of spores in a row in a sporangium varies with the width, from several to one or two.

The tip of the discharging papilla is mostly rounded ; it would seem to be forced off by the pressure of the zoospores packed behind it ; in one instance, however, the tip was flattened, with a fine membrane across it which appeared to dissolve. When the tip first gives way the zoospores pour out in a rapid stream, but when pressure is relieved they swim out singly ; once liberated, they swim off rapidly. Zoospores within the sporangia may be seen trying the various branches until they find the exit. If the exit should be blocked by an encysted spore, or if the sporangium is very long and branched, a considerable number of spores may be retained, becoming encysted.

The time during which the first zoospores may remain active varies considerably. They are apparently very susceptible to changes in the environment, for the placing of a cover-slip over a drop containing motile spores, just out of a sporangium, causes rapid encystment. Both within the sporangium and outside, zoospores may come to rest while still irregular in shape ; this is probably due to sudden change in the environment, such as increasing salinity on the slide. On the other hand, zoospores seen coming from a sporangium at 9.30 p.m. were taken up in a fine pipette and put on a cover-slip as a hanging drop ; many were still active 50 minutes later. The length of time during which the zoospores may remain active seems unusually long for the first zoospores, which in the Saprolegniaceæ apparently encyst after 5 to 15 minutes.

The first zoospores are of two sizes, large and small, as in *S. anisospora* de Bary (**2**, p. 33) ; the small are, however, comparatively rare. The small ones are about 8μ in length, the large about 14μ. Fig. 8 A and B shows sporangia from different parts of the same gill, one containing a few small zoospores, the other containing large ones ; the smaller appear to be more

regular in shape. Zoospores of intermediate size occur. The zoospores within the sporangia vary considerably in shape, especially in different sporangia. They may be oval, pear-shaped, or very elongated, almost rod-shaped, and may be rather irregular in outline, while some have a distinct protoplasmic tail. The zoospores appear capable of changing their shape to some extent, are slightly amœboid (Fig. 9). They are considerably flattened, as may be seen when they turn up an edge in swimming. It is probable that when free in the water they become a
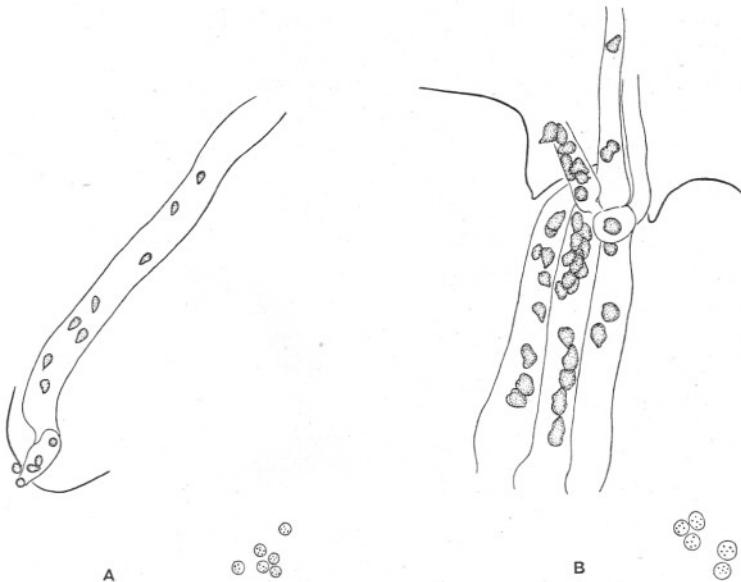

FIG. 8,—A. Zoosporangium with small zoospores. In the lower right-hand corner are cysts formed from some of these spores within the sporangium. B. Zoosporangium with large zoospores. In the lower right-hand corner are cysts formed from some of the spores within the sporangium. × ca. 253.

more regular pear-shape. Zoospores swimming in the sporangia always keep the same end foremost, but in some cases the fore end is blunt, in others rather pointed, though generally all in the same sporangium swim with the same end foremost. The free zoospores have many shining droplets, and a small vacuole near the apex ; they are biciliate but swim rapidly enough to make the number of the cilia and their attachment very difficult to discern. They move with the pointed end foremost and tend to swim in wide circles. The smaller zoospores, which seem to have a more regular shape, swim in a more straightforward manner and more rapidly. They often swing round in their tracks ; this *volte-face* could be more easily understood if there were an anteriorly and a

posteriorly directed cilium. One apically attached cilium can be seen
clearly, a second is sometimes glimpsed but is much more difficult to
distinguish. A possible explanation is that this one, though also apically
attached, is normally posteriorly directed and therefore often hidden by
the body of the zoospore. In an attempt to make certain of the point of
attachment of the cilia, zoospores were fixed in vapour of glacial acetic
acid, then treated with iodine, but drops of sea-water containing the
zoospores were also swarming with bacteria, which were particularly
thick round the spores and often tended to arrange themselves on fixing,
end to end in crinkled lines, sometimes touching the spores, so that any
cilia were entirely hidden.

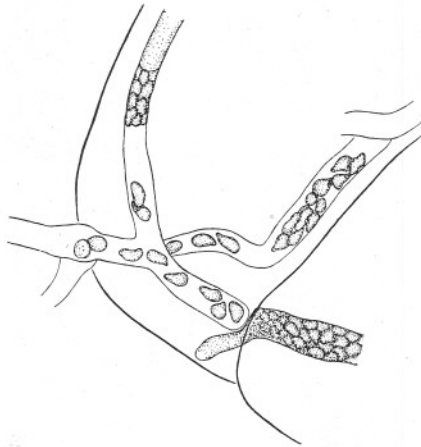An interesting case was noted of spores emerging from a sporangium

Fig. 9.—Sketch showing slightly amœboid zoospores
within sporangia. A spore has encysted in the
discharging papilla of one sporangium making it
difficult for the zoospores to pass. × ca. 342½.

by way of a tiny discharging papilla which was markedly constricted
where it penetrated the chitin of the gill. The emerging zoospores took
a considerable time to pass through, they were almost pinched in half and
detained there for perhaps 30 seconds. After passing through they halted
at the exit for a few seconds as though held up by a posterior cilium or
protoplasmic process adhering to the wall of the papilla ; they would
fling themselves sideways in an effort to become free.

As mentioned previously the length of time before encystment varies
considerably, as does also the time taken actually to encyst. They may
take several minutes in the process, swinging round and round in small
circles and becoming more and more rounded until at last movement
ceases ; or a zoospore may stop abruptly when swimming rapidly, and,

as it were, tucking in the pointed end, become rounded in a second. When rounded the spore often swings round for a short time, and at least one cilium can be seen lashing. The spore comes to rest, a shining droplet appears on the tip of the cilium as it gradually shortens, and while still of some length it breaks off and floats away. This process of events was seen very clearly perhaps a dozen times and the path of the drifting cilium followed for a second or so. Generally only one cilium has been clearly seen during this process. The shortening of the cilia with the appearing of a droplet was noted by Butler in the encysting zoospores of *Pythium de Baryanum* Hesse (Pythiaceæ) (**6**, p. 94). On one occasion a zoospore of the *Pinnotheres* fungus, seen when about to encyst, had two
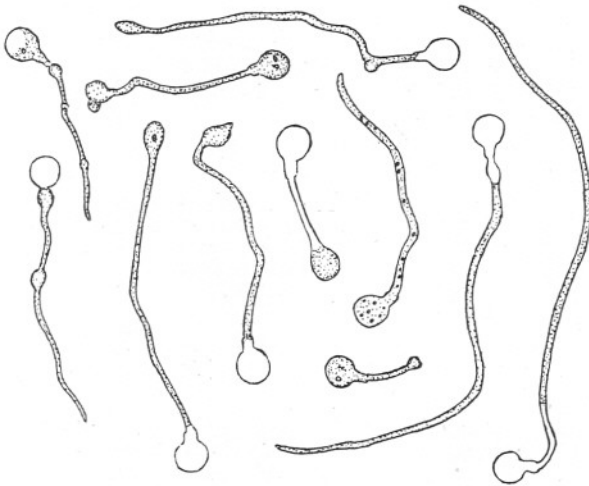

FIG. 10.—Germinating cysts. Some have small swellings along the length of the germ tube and one seems to be attempting to re-encyst. × ca. 685.

apical cilia which curled in towards each other, then broke off and floated away. The cysts vary from about 6μ to 10 or 11μ in diameter; they contain several shining globules. A fine germ tube is put out, but since no nutritive medium was supplied, as the tubes grew in length the part next the cysts was gradually left empty and after a time the plant died. Small swollen structures occur along the germ tube (Fig. 10), some nearly as large as the original cyst, as though there was an attempt at re-encystment (**3**, p. 283).

The question of the structure of the zoospores and as to whether there are two motile phases has had to be left, at least for the present, in a very unsatisfactory state. Owing to lack of time no attempt was made to obtain pure cultures and, as already mentioned, it was found impossible otherwise to obtain a drop containing zoospores that was not swarming

with bacteria, hiding the cilia. A second zoospore has not been observed emerging from a cyst, all that can be brought forward in favour of the existence of two motile phases in this fungus is, firstly, the finding of empty cyst cases with tiny exit tubes : the empty cases showed up quite clearly owing to the thick zone of bacteria collected round them. Secondly, the occurrence of a few zoospores of a definite bean-shape with two cilia which appeared to be laterally inserted, answering to the description of the second zoospores of the Saprolegniaceæ. These came from a sporangium, but may have emerged from spores encysted there.

Difficulty was experienced with growing the fungus in hanging drops of sea-water ; it was found that the development of the fungus in the
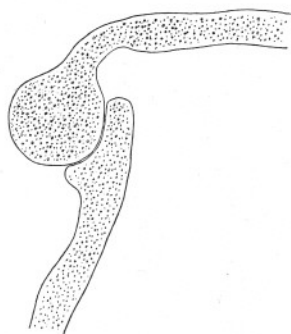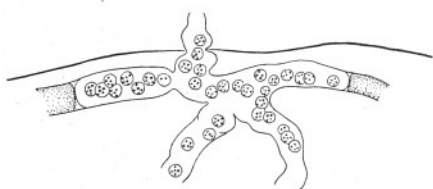

Fig. 12.—Sketch to show part of a branched sporangium with a single aperture ; a number of zoospores have encysted within it. The thick line shows the outline of the gill leaflet. × ca. 253.

Fig. 11.—Young oogonium with applied antheridium (?). × 400.

Fig. 13.—Sketch to show the highly vacuolated nature of the protoplasm in hyphæ which very occasionally grow out from the gill into the sea-water. × ca. 342½.

gills in such drops was arrested after a day or two, but that development would go forward and spores form if they were changed into a bowl of sea-water, even if growth had been at a standstill for 14 or so days. A somewhat similar state of affairs has been described by Lechmere (11, p. 318) for a species of *Saprolegnia*. The cysts formed in some of the hanging drops, probably owing to artificial conditions, did not develop and after a few days became enlarged, while their contents shrunk away from the wall, in some cases stretching in a band across the cell.

The infection and death was observed of a tiny nematode worm which had accidentally become fixed by its posterior extremity near the edge of a hanging drop made with an infected gill. The spores settled in the first place on the motionless tail of the worm ; the rest of the body was writhing vigorously for over 16 hours.

Sexual organs have not been definitely identified. Fig. 11 shows what

appears to be a young oogonium about 32μ in diameter with an applied antheridium of diclinous origin. In gills fixed in Flemming's fluid a very few round bodies containing many black globules of varying size have been seen. Similar bodies are shown in Fig. 5 which was sketched from fresh material. They appeared as shown at night, the next morning the larger one and the hyphæ were empty, the smaller still full. On two or three occasions two round bodies have been seen in proximity as in this figure. There is a possibility that they may be oogonia or gemmæ, but in any case they are exceedingly rare and are formed on spore-producing hyphæ.

LIFE-HISTORY (SO FAR AS KNOWN).

The mycelium enters the pea-crab in places where the chitin is extremely thin, and round the point of infection the hyphæ form a densely woven patch. In cases where the entrance is effected through the roof of the gill chamber, some strands pass into the body while hyphæ invade the gills and mouth parts. When the tissue of the gills is partly absorbed and the death of the crab is imminent, the hyphæ in these parts turn into zoosporangia, swarms of zoospores being liberated. These remain active under laboratory conditions for a period of a few up to at least 50 minutes, when they encyst. After encystment a second motile period may occur. Infection probably occurs during one or other of the motile phases. Organs which have been recognised as possibly sexual would appear to be rare.

DISCUSSION OF RELATIONSHIPS.

The *Pinnotheres* fungus has the general characters of the Saprolegniaceæ, the hyphæ, which are freely branched and unconstricted, are aseptate until the approach of the reproductive stages, and the asexual spores are biciliate. The zoosporangia are formed from unchanged hyphæ, as in *Aphanomyces* (**2**, p. 160) and *Leptolegnia* (**14**, p. 522) ; they are mostly branched (Fig. 12) as Coker (**2**, p. 159) observed for the sporangia in old cultures of *Leptolegnia* which may become very complex from the extension of a single sporangium into a number of adjoining branches. In both *Aphanomyces* and *Leptolegnia*, however, the spores are typically formed in a single row, while in the *Pinnotheres* fungus the number varies with the width of the sporangium. Very occasionally a second sporangium is formed within an empty one, a feeble development of the " nested " arrangement found in *Saprolegnia* and sometimes in *Leptolegnia* (**2**, pp. 22, 157). The biciliate zoospores on emerging from the sporangia swim actively away as in *Saprolegnia*, *Leptolegnia*, and *Isoachlya* (**2**, pp. 22, 157, 81 ; **6**, p. 83), and it is possible that after encystment a second zoospore emerges as also occurs in those genera. As previously mentioned, the first

zoospores are probably of two sizes, large and small, with intermediate sizes as in *S. anisospora* de Bary.

The *Pinnotheres* fungus is distinguished from species of Saprolegniaceæ growing on freshwater animals and apparently from all other members of the family (**6**, p. 82) by being almost entirely an internal parasite, the discharging papillæ of the sporangia only, with very rare exception, penetrating the chitin of the host and reaching the exterior. It is extremely rare for there to be any growth of the fungus, apart from the discharging papillæ, external to the host, but an exceedingly slight external growth was noticed on three occasions on gills. Such filaments are very different in appearance from the normal internal hyphæ from which they arise, they are very fine and exceedingly vacuolated, the squarish vacuoles stretching across the width of the hyphæ (Fig. 13). Similar very slender, highly vacuolated hyphæ were seen extending from a few well-developed embryos of *P. pisum ;* the hyphal threads radiating from the embryos had the appearance of a halo. These were found on August 21st and 12 days later the female, which was carrying the embryos, was found dead with white opaque patches on the abdomen and with the gills infested with the usual fungus.

Dead infected crabs, left in sea-water, show no external growth of the fungus and it seems probable that it dies soon after the crab. This is in striking contrast to the heavy external growth formed by *S. parasitica* on dead salmon and other fish, dead flies, beetles, etc. (**10**, pp. 321, 331), indeed this latter species is said by Patterson (**13**, p. 1) to show a richer growth on dead than on living salmon, indicating that dead tissue is much more suitable for its growth than the living fish. The apparent inability of the *Pinnotheres* fungus to form a growth on dead crabs may seem to argue in favour of its truly parasitic nature, but putrefaction of *Pinnotheres* is somewhat rapid and it may be that the number of bacteria, etc., present, check the growth of the fungus and kill it (**14**, p. 506 ; **10**, p. 331).

The question as to whether it is a true parasite or only invades tissue which has been broken down by parasitic bacteria, as does *S. parasitica* of the salmon (**13**, pp. 2, 7 ; **4**, p. 200 ; **7**, p. 29) and perhaps *A. Hoferi* of the Bohemian mirror carp (**2**, p. 145), has not been gone into : there is quite a possibility that this may be so, for gills infested with the fungus are almost always swarming with bacteria of several kinds. If it is a true parasite it would seem to be ill adapted to its host, for its penetration into the gills must soon cause the death of the host.

It is very probable that death occurs more rapidly under laboratory than under natural conditions. In the case of the crabs, of which details are given in Table I, they had been taken from mussels, being themselves parasites (**12**), and were isolated free in batches varying in number from

TABLE I.

Mortality of Pea-crabs, Infected with the Fungus, when kept together in Bowls.

Number of crabs in a bowl, all of which died with the infection.	Date of placing in bowl.	Date of 1st and of last death.		Number of days before 1st death.	Number of days between 1st and last death.	Number of days between placing in bowl and last death.
5 (4 adult ♀+1 ♂) from Weir Point, April 10/28	April 10, 1928	April 28	May 18	18	20	38
12 (♀ adult) from Padstow, April 25/28	April 25	May 22	June 6	27	15	42
13 (♀ adult) from Padstow, April 25/28	April 27	May 12	May 25	15	13	28
7 (♀ adult) from Padstow, April 25/28	May 8	May 26	June 8	18	13	31
3 (♀ berried) from Padstow, April 25/28	May 8	June 4	June 13	27	9	36
7 (♀ adult) from Padstow, April 25/28	May 9	May 28	June 15	19	18	37
21 (♀ adult) from Padstow, April 25/28	May 10	May 22	June 11	12	20	32
18 (♀ adult) from Padstow, Sept. 14/28	September 17	October 6	October 20	19	14	33
				Average 19·4	Average 15·2	Average 34·6

5 to 21 in bowls of sea-water and therefore possibly able to obtain little food. When a number of crabs were kept together in a large glass dish, as in the eight instances given in Table I, they gradually died of the disease, one after the other succumbing. Mortality was very much lower among those crabs kept singly in finger bowls, indicating that the disease is extremely infectious. All crabs in the Laboratory had their water renewed every day or every other day.

The death of the host is generally sufficiently rapid, at least in the Laboratory, for the gonad—so far as appears outwardly—not to be affected. This is a condition contrary to that which occurs in infection by a Sarcosporidian identified by Dr. Pixell Goodrich, and in infection by *Pinnotherion vermiforme* Giard and Bonnier (5). In some few cases, mostly crabs which when taken from the mussels were seen to be infected, the crabs were rather orange or milky orange in colour. This possibly indicates that they may resist the fungus longer under natural conditions and there is time for them to draw on the gonad. In one dead crab examined, which had been a milky orange for some time, the fungal threads were found among degenerating ova in the gonad. The main axis of each of the gills was full of a bright yellowish orange substance, which looked like yolk globules, but did not blacken with the osmic acid in Flemming's fluid, and was doubtless derived from the degenerating ova.

SUMMARY.

A fungus, most probably allied to the Saprolegniaceæ, has been found infecting *Pinnotheres*. Pea-crabs so infected always die, but there is not as yet sufficient evidence to determine whether the fungus is pathogenic or only invades tissue which has been destroyed by parasitic bacteria. Although having the general characters of the family it apparently differs from all members so far described in that it occurs in a marine invertebrate and is almost entirely internal in habit, the zoosporangia occurring within the tissue of the host.

ACKNOWLEDGMENTS.

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