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NEW MARINE FUNGI ON *PELVETIA*.

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[WITH FOUR FIGURES IN THE TEXT].

THE small number of known marine fungi stands in striking contrast to the long list of land species, especially when the extent and the variety of possible algal hosts are considered. A natural inference that such forms would be mainly simple Phycomycetes, like the Chytridineæ occurring on fresh-water algæ, has led to a search for these, but, owing to their minute size and to the difficulty of observing them under anything like natural conditions, the investigations have proved distinctly disappointing, both in point of species recorded and the knowledge of their life-histories. Little systematic attention has been paid to other groups, with the result that, while one or two species were known as early as the middle of last century, the additions have been few and accidental. In a survey of marine Pyrenomycetes, published in 1907, Cotton gives a list of nine known species, one of which he describes for the first time. Of these, only two are recorded for this country. Several new and interesting fungi have been collected by the writer in a careful examination of algæ at various points along the coast of Britain. In the present paper it is proposed to deal only with some of those occurring on *Pelvetia*.

The dark olive-green, lichen-like zone of *Pelvetia canaliculata* is one of the most characteristic and distinctive features of our seaboard, forming a narrow belt near the upper reaches of the tidal, and along those outer rocks which project into comparatively shallow water at high tide. The densely crowded much-branched thalli, attached to the rock surface by more or less discoid holdfasts, mark the upper limit of the Fucaceæ. There they lead a dual existence, at one time swaying freely with the advancing and retreating waters, at another exposed to the full influence of sun

and air, without any means of renewing their water supply. They are submerged at most only for a few hours each day, while during neap tides the water may not reach them for two, three, or even four days. This holds only during fine weather; when the sea is rough the plants become drenched with spray or foam, even when the tides are neap. The result is that *Pelvetia* normally spends the greater part, and periodically the whole, of each day under conditions very similar to those enjoyed by many lichens to which its thallus bears a strong external resemblance.

The abundance and wide distribution of *Pelvetia*, coupled with its amphibious habit, tended to mark it out as a possible and likely fungus host, an initial hypothesis which has been fully verified by a systematic examination of this alga throughout the past year. It has been found to possess a rich fungus flora, hitherto undescribed. Some are saprophytic, some parasitic. One symbiotic species is of special interest as its occurrence throws a new light on *Pelvetia*, opening the question whether it should be regarded as a lichen rather than an alga.

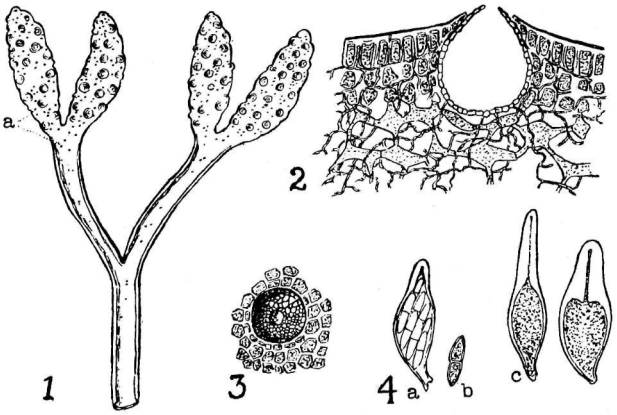

FIG. 1. *Mycosphærella pelvetiæ*. 1, *Pelvetia* thallus and receptacles showing perithecia (*a*); 2, section showing perithecium and intercellular hyphæ magnified out of proportion so as to render more visible; 3, surface view of perithecium; 4, asci (*a*, *c*) and ascopore (*b*).

MYCOSPHÆRELLA PELVETIÆ NOV. SP. (FIG. 1, *1—4*).

Mycelium hyaline, septate, very slender, diffused and entirely intercellular. Perithecia minute, 65–85μ, spherical, with small conical tip, densely aggregated on receptacle. Perithecial wall

thin and membranous except at exposed tip. Asci cylindrical and curved, or tapering, thickened apex almost pierced by narrow canal, 45–55μ × 15–20μ. Ascospores biseriate or bunched, fusiform, 19–25μ × 4·5–5·5μ, hyaline, uniseptate, with slight constriction, cells biguttulate when mature.

Hab. Symbiotic with *Pelvetia*.

The very slender septate mycelium of this fungus permeates every portion of the thallus from holdfast to receptacle, running along the surface of the algal cells, with whose outer walls it is in very close contact. In colour it resembles the latter, and this, added to the extreme tenuity of the hyphæ, may explain why it has been constantly overlooked. The threads, so fine as rarely to exceed 1–1·25μ in diameter, are difficult to detect without the aid of stains; iodine and fuchsin make them readily visible.

The mycelium is entirely intercellular, never breaking through the soft slimy walls unless the cells have been attacked and partially broken down by parasitic fungi or bacteria beforehand. When the material is in this condition the course of the symbiotic mycelium cannot be traced with the same certainty. In the healthy thallus there is no such difficulty. The large intercellular spaces of the medullary tissue and cortex, kept moist continually by the swollen mucilaginous walls, form an ideal home for the hyphæ whose branch tips penetrate even between the cells of the rind or limiting layer. The hyphæ branch freely and are extremely numerous, forming a loose net-like web surrounding the cells. Altogether there is an immense length of fungus to unit-volume of the tissue, but with it all not the slightest trace of injury to the host has been noted in the hundreds of sections examined.

The fungus grows along with the thallus and thus penetrates into the young receptacles, where it commences to fruit just as the reproductive bodies are being formed in the conceptacles. Minute perithecia are produced immediately under the rind, through which the tips burst and barely protrude when mature as in Fig. 1, *2* and *3*. While the immersed portion of the perithecial wall remains thin and membranous, the small projecting tip becomes carbonaceous, and thus readily visible against the olive-green receptacles, so that at this stage the latter become marked by numerous small black specks as in Fig. 1, *1* (*a*). The perithecia are practically confined to the receptacles; a few scattered ones may appear on the thallus immediately below, but they rarely, if ever, occur on the main vegetative body of the host.

These small spherical or pyriform fruiting-bodies contain a small number of asci which are of various ages and formed successively. The diversity in shape of mature asci, found even in the same perithecium, prevents their form being taken as a distinctive feature, notwithstanding the fact that it is often so used by the systematist. While many are broadly oblong and curved as in Fig. 1, *4 a*, others have long, tapering, much thickened apices almost completely pierced by narrow canals with slightly swollen lips as in Fig. 1, *4 c*. Frequently the thickened apical portion forms half of the total length of the ascus when mature. Paraphyses are wanting.

The ascospores, eight in each ascus, are fusiform, hyaline, uniseptate and slightly constricted. Each cell becomes biguttulate when ready to be dispersed (Fig. 1, *4 b*).

Not only is the mycelium of this fungus slimmer than that of *Mycosphærella ascophylli*, first noted by Church in 1893, and described and named by Cotton in 1907; perithecia, asci, and ascospores also differ. These points alone are sufficient to justify its being regarded as a separate and distinct species.

The ascospores are set free from the perithecia about the same time as the oospheres are being liberated. They become entangled in the mucilaginous inner persistent oogonial wall surrounding the latter. There they germinate and their germ-tubes gain entrance to the developing oospore either directly or sometimes a short mycelium is formed which penetrates slightly later. Thus the fungus finds its way into the intercellular spaces at a very early stage in the development of the young alga. Infection is rendered doubly sure by a vegetative process. Mixed with the paraphyses in the conceptacles are loosely coiled, much branched hyphæ. These frequently become entangled in the mucilaginous coating enveloping the oospheres and are torn off as the latter are ejected. This mycelium is also capable of growing and gaining entrance to the developing oospore, when it again lodges between the young cells

Curiously no perithecia have been observed in the thallus of *Pelvetia canaliculata* var. *libera* (Oliver) although abundant mycelium is present. This form, found only at Blakeney, reproduces by vegetative means alone. The fungus has adopted a similar habit being carried into the new shoots before they separate from the parent plant. The absence of perithecia, correlated with the absence of reproductive bodies in the algal host, shows how close the union between the two has become.

Very early in the season smaller and differently shaped perithecia were observed. These appeared to be pycnidia, but unfortunately no pycnidiospores were found in the material collected.

The mycelium of this fungus seems to occur in every thallus. Thousands of plants have been collected and sectioned from stations representative of the North and South, of the East and West coasts of Britain. All showed the fungus; in fact no plant, examined by the writer hitherto, has failed to show it in profusion. In addition, herbarium material at Kew has been gone over with the same result. These specimens were representative of Europe generally, and of collections made from the beginning of last century to the present day. This would seem to confirm the writer's view that this union of alga and fungus is general. It is quite possible, however, that oospores may germinate and plants reach maturity without infection. It will be interesting to determine whether this is so, and also to find out the distribution of such plants if any occur.

The wide, and it may be universal, occurrence of the mycelium along with *Pelvetia* at once raises the question of the relationship of the two. Experiments have been initiated with a view to determine, if possible, the part played by both in this symbiotic union. It may quite well be that the presence of the fungus has something to do with the plant's ability to withstand exposure for so long after submersion. Observations in the laboratory have shown that the loss of water from the thallus is very slight. But here again it is too early to make definite assertions as the experiments and observations are still incomplete.

STIGMATEA PELVETIÆ NOV. SP. (FIG. 2, *1—6*).

Perithecia arranged in almost concentric rings on blackened patch, sunk in tissue, forming hemispherical protuberances with flat or occasionally rounded bases, remaining covered by blackened tissue except at point broken by irregular ostiole, large, 160–200μ in diameter. Peridium only distinctly formed near tip. Asci cylindrical, long with slightly thickened apex, 100–120μ × 12–14μ, 8-spored. Paraphyses present, branched and unbranched, septate. Ascospores egg-shaped, 22–25μ × 9–12μ, hyaline, uniseptate, with distinct constriction. Pycnidia smaller, pyriform, with thick black walls, 70–85μ, at first sunk, but becoming superficial; pycnidiospores 3 × 1·5μ.

Hab. Parasitic on thallus of *Pelvetia* in Scotland.

This fungus is parasitic, attacking the thallus and doing such damage as frequently to sever branches from the basal portions.

The disease is marked externally by the blackened circular patches shown in Fig. 2, *1*, *2*. Usually the margins of these become white owing to the disintegration of, and consequent chemical changes in, the rind of the host. The hyaline, slender, septate mycelium is entirely local, and is easily distinguished from that of *Mycosphærella*, with which it is intermingled, by its greater thickness and irregularity, as well as by its habit. Its average diameter is 2–2·5μ but frequently it reaches as much as 5μ at points where the branching is extensive. The spaces between cells become packed with the hyphæ which penetrate the mucilaginous walls and become closely coiled round the living contents into which haustoria are sent as in Fig. 2, *4*. At other times the tips of branches penetrate directly and act as absorptive organs. In this respect this plant differs markedly from the symbiotic fungus already described.

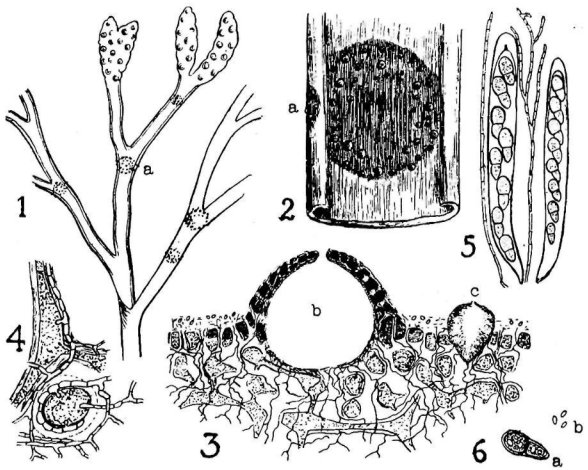

FIG. 2. *Stigmatea pelvetiæ*. 1, thallus with diseased spots (*a*); 2, diseased patch enlarged; 3, section showing perithecium (*b*) and pycnidium (*c*); 4, mycelium with haustoria; 5, asci and paraphyses; 6, ascospore (*a*), pycnidiospores (*b*).

Growth is rapid and shortly after infection fruiting bodies appear on the blackened patches in the form of small pycnidia corresponding to the genus *Phyllosticta*. At first these are immersed but they become superficial by the disintegration of the surrounding tissue. The walls are thick and carbonaceous. Minute elliptical conidia are ejected from the small ostiole. The rapid infection,

following the first appearance of the disease, is doubtless due to the quick dispersal and immediate germination of these small bodies.

The ascigerous fruits appear slightly later on the same patches. They originate in masses of mycelium in the cortex, which increase and give rise to asci before distinct perithecial walls are formed. The place of the latter is taken largely by the superincumbent layers of the host which become blackened to form a kind of false clypeus. This becomes raised to form a hemispherical dome pierced later by the irregular ostiole. A thin perithecial wall is formed under this but it does not extend along the flattened or occasionally curved base, bounded only by the mass of hyphæ from which the reproductive bodies spring. The poor development of the peridium may be accounted for by the protection given by the blackened overlying tissue, represented in Fig. 2, *3*. As this becomes thinner the membranous wall is formed.

This method of perithecium formation has been noted in other fungi examined by the writer in algæ. When placed in the mucilaginous bodies of the latter there is less need for protective walls.

The perithecia are large and readily visible to the naked eye. They contain numerous long cylindrical asci with very slightly thickened tips and mixed with longer septate branched and unbranched paraphyses as in Fig. 2, *5*. The hyaline spores are uniseptate with a distinct constriction marking off the upper broader cell from the lower narrower one. When mature they contain several oil globules.

In the broken-down tissue on the surface of the blackened patch numerous hyaline elliptical conidia are found. As the tips of hyphæ penetrate into this layer it may be that these are cut off from them. They are seen in Fig. 2, *3*.

This species is placed in the genus *Stigmatea* with some reservation. The spore characters, asci, paraphyses and some other features combine to ally it with this genus. It is, however, a moot question whether the origin of the perithecium and its distinctive character do not justify its being placed in a separate and new genus. This can be settled definitely only when the other algal fungi are examined and the whole matter of perithecium formation studied with regard to the new conditions involved in this type of host.

PHARCIDIA PELVETIÆ NOV. SP. (FIG. 3, *1—3*).

Mycelium thick, brown, and parasitic, frequently forming a superficial web in addition. Perithecia minute, 45–55μ, spherical

and with projecting ostiole, at first sunk, finally superficial with brown leathery walls. Asci few, cylindrical with short thickened rounded apices, 30–35μ × 10–14μ, 8-spored. Paraphyses absent. Spores hyaline, two-celled becoming four-celled, surrounded by thin mucilaginous sheath, on the rupture of which the spore breaks at the weak middle into two portions, 12–15μ × 3–4·5μ.

Hab. Parasitic on *Pelvetia* in Britain.

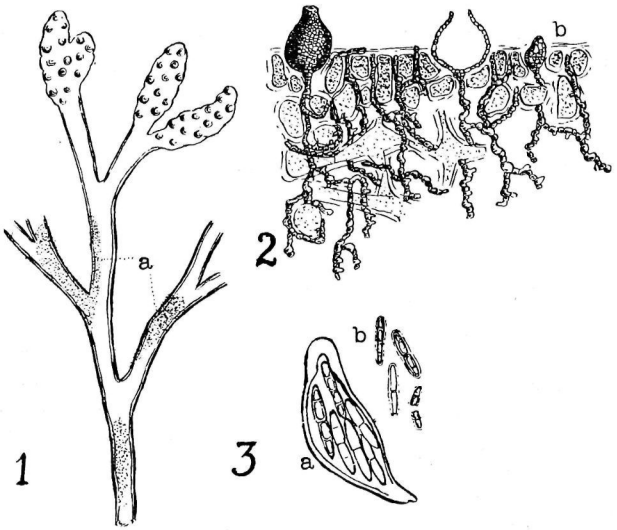

FIG. 3. *Pharcidia pelvetiæ*. 1, thallus with perithecia on diseased patches (*a*); 2, section showing perithecia and dark-coloured parasitic mycelium; 3, ascus (*a*) and ascospores (*b*).

This fungus is likewise parasitic, but is easily distinguishable to the naked eye from *Stigmatea* by the irregular outline of the blackened areas and the immense number of minute perithecia represented in Fig. 3, *1*. Its stout, brown mycelium consists of thick-walled irregular cells with an average diameter of 3–4μ, but frequently of 5μ or even more. It forms close coils around the host cells into which haustoria are sent.

Densely crowded hyphæ riddle the cortical layers and send branches along the surface forming a loose network in the broken-down rind. The rapid extension of diseased patches makes this parasite a powerful factor in preparing the thallus for the subsequent action of saprophytic fungi and bacteria.

Immense numbers of small perithecia are produced shortly after infection. They become superficial and consequently have well developed thick leathery walls. The ascospores likewise are protected by a thin mucilaginous envelope.

PLEOSPORA PELVETIÆ NOV. SP. (FIG. 4, *1—4*).

Mycelium thick, brown with irregular cells. Perithecia at first sunk, becoming superficial, large, black, leathery with short conical opening, 150–200μ in diameter. Asci cylindrical or with free end slightly tapering, thin walled, 110–125μ × 25–30μ, 8-spored. Spores biseriate, elliptic-oblong, yellowish brown, generally seven cross septa and numerous vertical ones, slight constriction, 30–35μ × 12μ.

Conidial form believed to be *Macrosporium pelvetiæ*. Conidiophores branched or unbranched, 3μ, brownish. Conidia clavate, with 5–6 transverse and 1–3 vertical septa, 35–45μ × 11–12μ.

Hab. At first parasitic on *Pelvetia*, then saprophytic.

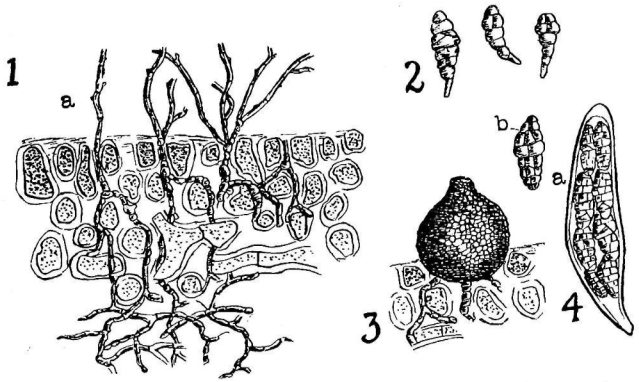

FIG. 4. *Pleospora pelvetiæ* (*1*, *2*) and *Macrosporium pelvetiæ* (*3*, *4*). 1, section of thallus showing mycelium and conidiophores of *Macrosporium* ; 2, conidia of same ; 3, perithecium of *Pleospora* ; 4, ascus (*a*) and ascospore (*b*) of same.

This fungus produces very much the same effect upon plants attacked as does *Botrytis*. The thalli become withered and tend to break down into a brownish slimy mass. The thick brownish yellow mycelium penetrates and destroys the tissue ; the whole medullary portion becomes a mass of parasitic hyphæ as in Fig. 4, *1*. The cortical layers are also riddled and outside arise erect unbranched or slightly branched conidiophores (*a*) on which are borne the clavate muriform conidia shown in Fig. 4, *2*. These vary considerably in shape, but usually show 5–6 transverse and one, two, or three vertical

septa. This undoubtedly parasitic stage would correspond to *Macrosporium pelvetiæ* nov. sp. Later the ascigerous fruits of *Pleospora* appear on the same spots and on identical mycelium. This would seem to make the Imperfect fungus the conidial stage of the Pyrenomycete. But culture experiments are necessary to make this point certain although this type of conidium is common to other species of *Pleospora*.

The perithecia are large and at first sunk in the thallus, but finally become superficial. Paraphyses are present in the immature fruits, but later they break down and deliquesce.

In conclusion I wish to express my thanks and indebtedness to Mr. A. D. Cotton, who noted *Mycosphærella* previously, and kindly handed over to me his material collected at Blakeney and Clare Island, and also to Mr. L. A. Boodle, by whose courtesy this work was carried out in the Jodrell Laboratory.

University College,
Southampton,
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