On Actinococcus and Phyllophora.

BY

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With Plate XV and seven Figures in the Text.

Credidi enim et etiamnunc credo, tubercula illa nihil aliud esse quam parasiticum quid . . .—LYNGBYE, Tentamen Hydrophyt. Dan., 1819, p. 11.

IN 1893 Schmitz published a paper, in which he discussed at some length the *Actinococcus* question. He maintained that all so-called forms of fructification of *Phyllophora Brodiaei* (Turn.) J. Ag. which he had so far been able to examine, belonged in reality to a different Floridea growing parasitically on the former species (5, p. 371). This paper was shortly after reviewed by Gomont, who expressed his full agreement with the views held by Schmitz; so that the true nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag. still remained to be found.

Doubts had long been felt with regard to the true nature of the so-called nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag. The few lines quoted at the head of this paper were written by Lyngbye in 1819 in describing these very same nemathecia. In the beginning of 1894 the author of this paper gave a preliminary account of some observations on the anatomy and development of the Baltic species of *Phyllophora* Grev., in which Schmitz' assertions concerning the parasitic nature of the nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag. were discussed and the accuracy of his conclusions was doubted (1, p. 47). A more detailed account of the author's work on the Baltic Phyllophorae was published about a year later, but unfortunately Schmitz died in 1894. In the second paper just mentioned the author again expressed it as his opinion that the so-called nemathecia of *Phyll. Brodiaei* did really represent the true tetrasporic fructification of this Floridea (2, pp. 2 sq., 23 sq., 36).

The subject has not been worked at by algologists since, and Schmitz' theory has therefore naturally been most generally accepted. Kolderup Rosenvinge alone seems to have adopted an opposite view (4, p. 33).

Since commencing work on the anatomy and development of *Phyllophora* in 1892, the author has devoted much time to the examination of all forms of fructification found on it. Up to 1896 opportunity was however wanting for dredging and examining fresh material during the months of September and October, owing to the author's absence from Kiel during that time. Practically all the material used in these investigations was collected in the Baltic near Kiel. The observations carried out up to this point indicated that the one conclusion to be drawn was that the so-called (*Actinococcus*) nemathecium of *Phyll. Brodiaei* was really the genuine tetrasporic fructification of that plant.

In 1896 for the first time specimens of *Phyll. Brodiaei* were dredged and preserved at all times of the year. This was continued up to September, 1898, when the author left Kiel. The development of the plant in question was followed out, and as a result the author has accepted Schmitz' view of the parasitic nature of *Actinococcus*, the correctness of which view, however, Schmitz unfortunately was not able to establish, as he did not succeed in observing the entrance of the parasite into the host.

The following is an account of the anatomy and development of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv., which forms the 'pseudo-nemathecium' of *Phyll. Brodiaei* (Turn.) J. Ag.

ACTINOCOCCUS SUBCUTANEUS (Lyngb.) K. Rosenv.

Almost at every time of the year small, more or less spherical, dark reddish bodies are found on the flat expansions forming the thallus of *Phyll. Brodiaei.* They are

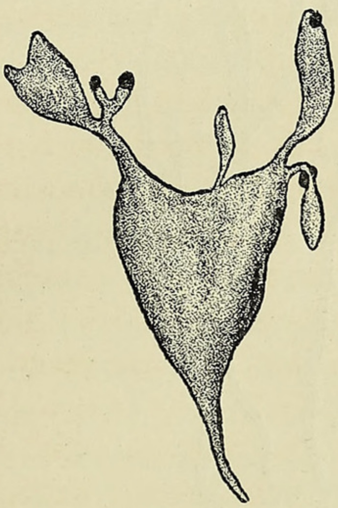


Fig. 1. Nemathecia of *Actinococcus roseus* (Lyngb.). Kold. Rosenv. on *Phyllophora Brodiaei* (Turn.) J. Ag. Nat. size.

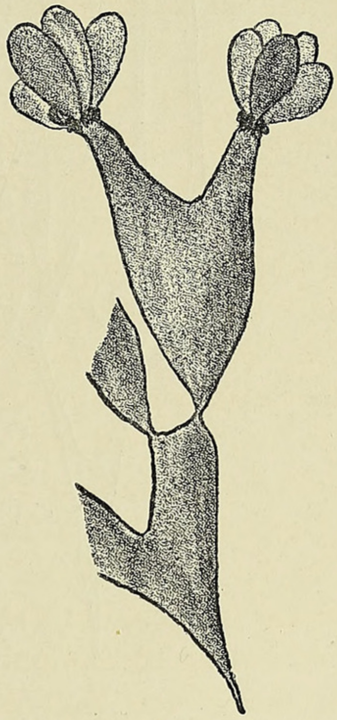


Fig. 2. Nemathecia of *Actinococcus roseus* (Lyngb.). Kold. Rosenv. on *Phyllophora Brodiaei* (Turn.) J. Ag. Nat. size.

sessile on the young shoots at the apex of the thallus, but often appear to be stalked, this appearance being produced by the shoots on which they grow (Figs. 1 and 2) being at first rather narrow.

These red bodies are the nemathecia of *Actinococcus subcutaneus* (Lyngb.) Kolderup Rosenvinge, the Floridea mentioned above as growing parasitically on *Phyll. Brodiaei.*

The young shoots of the latter Alga are not usually much modified by the presence of the parasite, but are on the contrary as a rule well developed.

In the Baltic sea small detached portions of the thallus of *Phyll. Brodiaei* frequently occur lying on the sea-bottom.

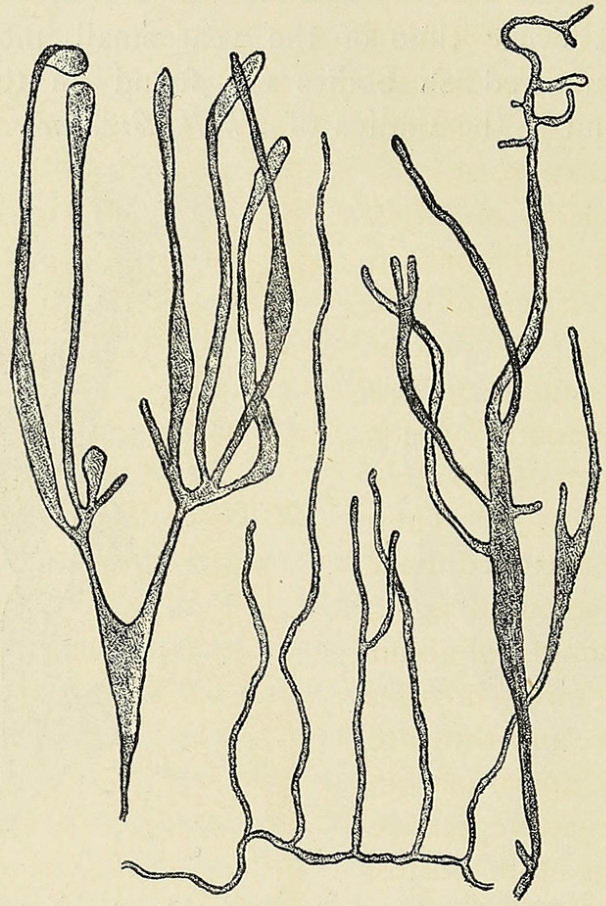


Fig. 3. *Phyllophora Brodiaei* (Turn.) J. Ag.
Sterile, Baltic forms. Nat. size.

They are characterized by being very narrow, elongated and always sterile (Fig. 3, *forma ligulata*, *elongata*, *&c.*, of various authors). They therefore never develop antheridia or procarpia. Furthermore they are never attacked, at any rate not successfully attacked, by the germinating spores of *Actinococcus subcutaneus.* This parasite can only enter the host when the male (or the female ?) organs of the latter are present. It has been known to the author for some time that the antheridial cavities of *Phyll. Brodiaei* often accompanied the presence of *Actinococcus subcutaneus*, but only during the last year has it become possible to explain definitely the significance of this appearance.

As the presence of the antheridial cavities is so intimately associated with the relationship of the two red Algae which form the subject of this paper, it might be useful to recall the structure of the former (2, p. 29).

The antheridia of *Phyll. Brodiaei* are developed in the cortical layer of the spermophores, the latter being shoots more or less modified temporarily for the production of the male organs (Figs. 4, 5). They are slightly flattened near the lighter coloured apex, attaining a length of about 3 mm., being rarely broader than 0·5 mm., and they are borne on the apical margin of the flattened vegetative thallus. In the cortex of such a spermophore, close to its apex and not further down from it than 1·0–1·5 mm., we find the small cavities which contain the antheridia. These cavities are flask-shaped and communicate with the exterior by a small ostiole. Their height is 24–34 μ, their breadth about 20 μ. From the flat bottom of the flask-shaped cavity arise a number of 2, 3 or even 4-celled antheridia, which produce the single male cells or spermatia,

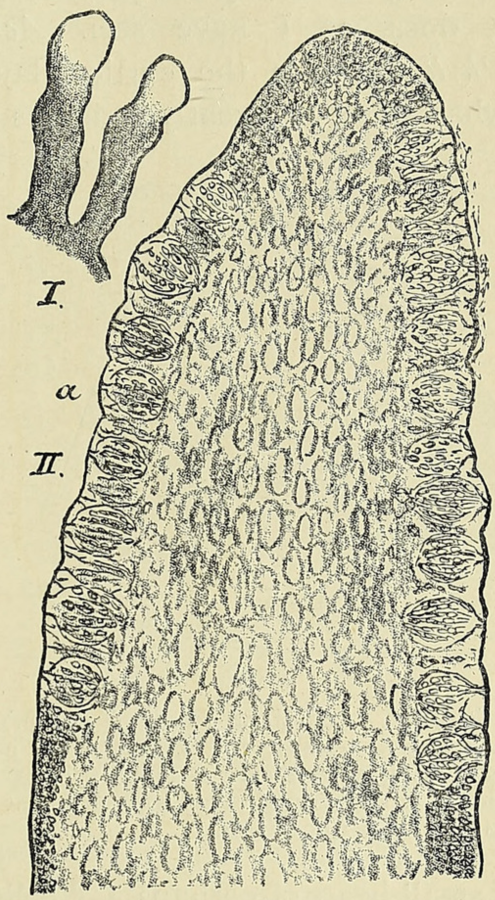


Fig. 4. *Phyllophora Brodiaei* (Turn.) J. Ag. I. Two spermophores. × 10 diam. II. Longitudinal section of spermophore, showing the antheridial cavities in the cortical layer. × 200 diam.

at their apex (Fig. 5). The spermatia pass out of the cavity through the ostioles, which measure about 6–10 μ across.

It is not necessary to describe the carpophores of *Phyll. Brodiaei,* on which the female organs are borne (vid. 2, p. 32, Figs. 46, 47). I have not been able to ascertain definitely whether our *Actinococcus* can enter its host by means of the opening caused by the projecting trichogyne. Very probably it does, as I have seen *Actinococcus*-bearing shoots of *Phyllophora,* in the cortical layers of which could be seen what were apparently remains of undeveloped carpogones. Antheridia and procarpia, moreover, do not occur on the same plant.

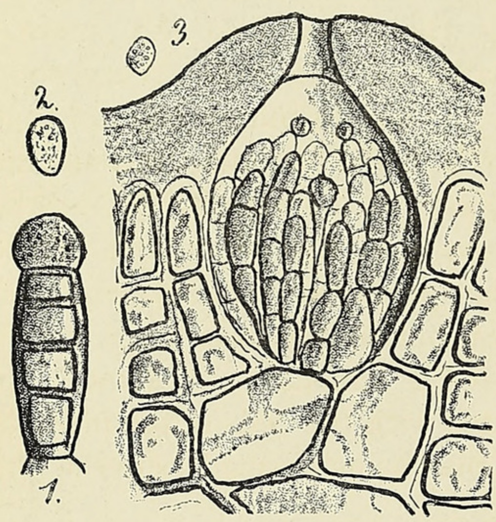


Fig. 5. *Phyllophora Brodiaei* (Turn.) J. Ag. 1. 4-celled antheridium with a spermatium at its apex. 2. Single spermatium. 3. Antheridial cavity with ostiole at its apex. × 1,000 diam.

In the autumn it is possible to observe the entrance of *Actinococcus* into *Phyll. Brodiaei* by the small ostioles of the antheridial cavities. The spores (tetraspores or carpospores) which ultimately give rise to the nemathecia of *Actinococcus subcutaneus* germinate on the surface of the host about this time.

The immediate product of germination seems to be a small heap of perhaps 4–8 cells, one of which always comes to be near an ostiole leading to an antheridial cavity. The antheridial cavities are developed in large numbers and very close together (Fig. 4, II). A filament is then formed, which passes into the host-plant through the antheridial ostiole (Plate XV, Fig. 1).

It is worth while perhaps to draw attention to a former figure which was intended to show the origin of the nemathecium of *Phyll. Brodiaei* (2, Fig. 31). It shows the nemathecium arising from the lower cells of the cortex or the outer cells of the medulla, the filaments in question being drawn darker, to show them up better. The drawing in itself is correct though misleading. It only represents a portion of the initial filament of the nemathecium, which in the other adjoining sections, had they been preserved, would have been seen to originate from cells just outside one of the antheridial cavities. Not unfrequently the spores seem later on to be drawn down into the antheridial cavities, and thus no trace of their external origin is left.

After entering the host by the ostiole of the antheridial cavity, the parasite immediately branches (Plate XV, Fig. 1), the branches at first forcing their way into the internal medullary portion of the thallus of the host. Very soon a differentiation takes place in the primitive thallus thus developed. In between the cells of the medulla the filaments of the parasite branch and some of them grow out in the direction of the cortex. These form the shoot-part of the plant, the former acting as root-portions. The filaments do not enter any cells of the host, but simply force their way in between them along the middle lamella. In some way, however, they become connected with the cells of the medulla of the host-plant, secondary pits being formed between the cells of both organisms.

The normal cells of the medulla of *Phyllophora* as a rule contain large quantities of starch. This store of food-material is the chief source of food for the parasite. The parasite therefore first of all takes possession of this starch, and in older plants we find that the starch of the host has disappeared from the cells of the medulla, the cells of the parasite now being filled with starch. For this reason it is easy to differentiate the two organisms by adding iodine, when the parasite will turn dark blue, the cell-contents of the host remaining unstained.

From the filaments, which are found in the medulla of the host, arise, as already mentioned, the shoot-filaments. They grow in a direction chiefly towards the flat surface of the spermophores, which are infested by the parasite. The filaments soon reach the cells of the cortex, and passing out between the filaments of this layer, they branch outside the host, and gradually the nemathecium of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv. is formed on the external surface of the host-plant. Numerous filaments arise from the root-portion, and by repeated branching the internal vegetative portion (intramatrical filaments) and the external portion (extramatrical filaments) assume large dimensions (Plate XV, Fig. 2). The external filaments form a mass of radiating rows of cells, often branched at the base, which in the end give rise to the tetraspores (Fig. 6).

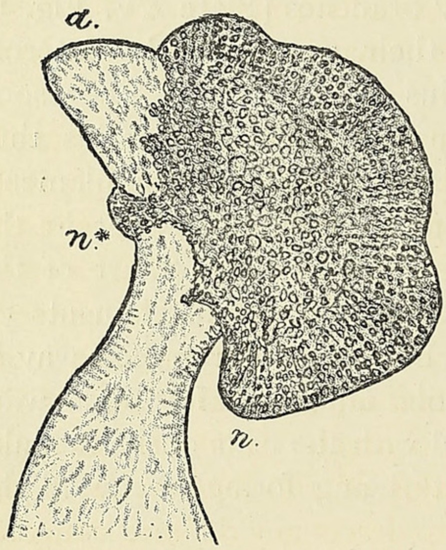


Fig. 6. *Actinococcus roseus* (Lyngb.) Kold. Rosenv. The shaded portion represents the parasitic nemathecium; the lighter portion is the thallus of the host-plant. *n*. large primary nemathecium; *n**. smaller secondary nemathecium just forming. × 40 diam.

The parasite is unable to pierce the outer covering of the host, when entering the latter. It can only attack the latter through the antheridial ostioles. On the other hand this outer coat is easily pierced when the same filaments are passing out to form the nemathecia. In this case they have a firm backing in the solid structure of the medulla of the host-plant. The apex of a filament which is passing through the outer wall of the latter seems to affect the surrounding substance in a peculiar way. This is otherwise quite homogeneous in structure, but in places, where it is being pierced, it seems to become vacuolated. It appears to be corroded in some way by the attacking filament of the parasite (Plate XV, Fig. 3).

A large number of filaments pass out of the tissue of the spermophore within a certain limited space (Plate XV, Fig. 4). On the outside of the spermophore they form dark-reddish bodies, the nemathecia of *Actinococcus subcutaneus* (Fig. 6). They are all joined together in a common gelatinous substance, in which they branch; the branches finally radiating outwards in a direction from the centre of the whole parasitic tissue.

At first as a rule these nemathecial bodies are formed only on one side of the flattened spermophore. Often, however, we see filaments arising from the intramatrical tissue of the parasite, which pass out on the opposite side of the spermophore (Fig. 6). When a second nemathecium is formed, it may be separate, if it is produced exactly opposite to the first: if it is produced close to the first nemathecium and next to it, the two frequently coalesce.

Ultimately, owing to the almost pseudo-parenchymatic development of the intramatrical tissue of the parasite, it is no longer possible in older specimens to distinguish between the filaments of the parasite and the ordinary tissue of the cortex and medulla of the host. As already mentioned, the effect of adding iodine is to show up the cells of the parasite more clearly, as the latter has absorbed all the starch out of the cells with which it has come in contact. It is easy to make out the shape of the starch-grains of *Phyllophora* (2, p. 22, Fig. 26 *n*, 8–10), but impossible to detect any definite structure and shape in the case of the very minute elements composing the starchy mass found in the cells of *Actinococcus subcutaneus*. Those parts of the primitive thallus of the latter, which are presumably most actively growing, as, for example, the apices of the filaments of the shoot-portions, contain no starch or only very little indeed. The root-filaments are usually completely filled with starch.

The internal cells of the whole parasitic cushion vary much in size (2, Fig. 30). The smallest are barely 6 μ in diameter, the largest measure 60 by 30 μ. The latter do not always belong to the parasite, but often form part of the tissue of the host. They are more or less round, the cells of the parasite being slightly elongated as a rule. The latter are connected with one another by fine cytoplasmic strands. The cells of

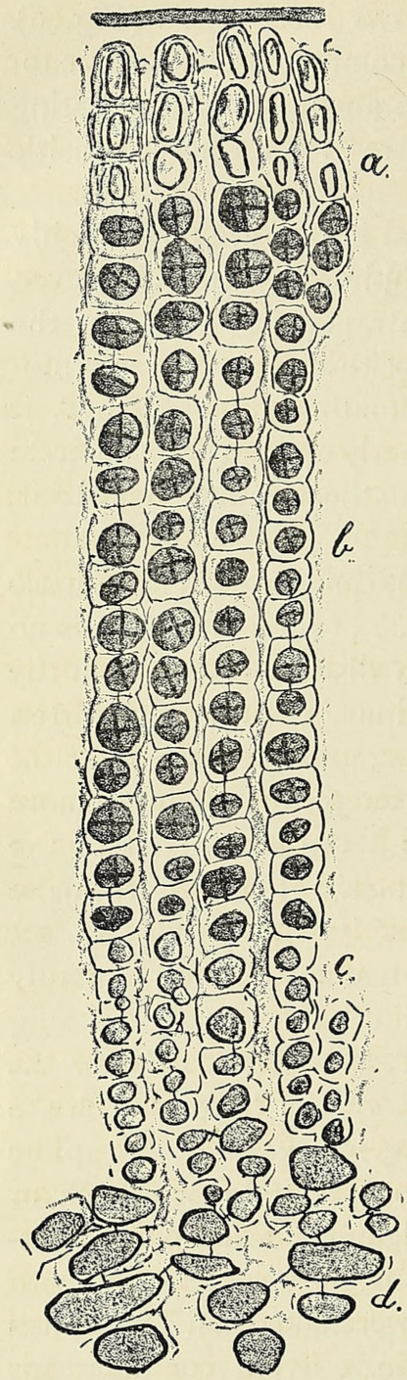


Fig. 7. *Actinococcus roseus* (Lyngb.) Kold. Rosenv. Vertical section of outer layers of nemathecium: *a.* sterile outer cells of nemathecial filaments; *b.* tetrasporangia; *c.* sterile inner cells, and *d.* the cells of the parasitic cushion. × 300 diam.

Phyllophora also possess numerous pits, by means of which their cytoplasm is continuous. Only on rare occasions is it possible to make out any connexion between the cells of the parasite and the host. In cases where the parasite has been growing in the host for some time, the filaments of the former often may grow a considerable distance down into the tissue of the thallus of *Phyllophora* in order to absorb more food-material for the cells of the tetrasporic fructification.

The extramatrical filaments gradually form the nemathecium of the parasite. The outermost, radially disposed filaments gradually develop into tetrasporangia. Each cell of these usually unbranched fertile threads, gives rise to four tetraspores, with the exception of the two to four apical cells (Fig. 7). The inner and lower cells of the fertile filaments also remain sterile. The whole fertile nemathecial layer is in itself about 150–200 μ deep. The sterile apical cells contain some clear, frothy cytoplasm and a distinct, though as a rule very much reduced, rhodoplastid. They are usually larger and longer than the tetrasporangia.

The tetraspores are formed by cruciate division, the first cross-wall formed being placed at right angles to the long axis of the fertile filament. The spore mother-cells measure 12 × 16 μ, the four nuclei being formed before the formation of cell-walls gives rise to the four tetraspores.

The tetraspores themselves are spherical in shape, and when escaping at maturity they measure 10–12 μ in diameter. They are surrounded by a very thin membrane, their cell-contents consisting of a very finely divided rhodoplastid and numerous starch-grains.

On arriving at maturity the nemathecia break up, the tetraspores are set free and presumably germinate. It is impossible as yet to say what actually is the fate of these tetraspores of *Actinococcus subcutaneus*, after they have been set free from the parasitic nemathecium. Do they germinate on another host and there form sexual plants with antheridia and procarpia?

The tetraspores ripen in December and January and shortly after are discharged from the nemathecia, some of the latter apparently continuing to vegetate for some time. With the appearance of the spermophores in the following autumn, we again find the parasite entering the host-plant. What have the spores been doing in the meantime? From what has already been said, it is not unlikely that what we see germinating on *Phyll. Brodiaei* in the autumn is really a carpospore.

It has been possible to follow out the germination of the tetraspores of our species of *Actinococcus*. Briefly the results obtained were the following (2, pp. 25–27, Fig. 32, 33). The tetraspores, derived from the nemathecia of *Actinococcus subcutaneus*, were sown on sterilized and purified parchment-paper. The whole was put in a small glass vessel filled with filtered seawater. Several cultures were started. The spores germinated, and in parts remained in a living condition for nearly two years. The products of germination took the form of small protonema-like organisms. The largest of these consisted of uniserial filaments and larger aggregations of cells, which at the time I took to be the rudimentary basal attachment-disks of *Phyll. Brodiaei*: it consisted altogether of upwards of 250 cells. As yet it still remains to be seen what actually becomes of these products of germination. Possibly they would normally have attacked a new host, be this *Phyll. Brodiaei* or some perfectly different plant, on which ultimately the carpospores would possibly be formed. The carpospores perhaps, one might almost say probably, germinate on *Phyll. Brodiaei*, and eventually give rise to the nemathecia of *Actinococcus subcutaneus*. It is not to be wondered at that the parasite should be able to live separately for nearly two years in an artificial culture, when it is borne in mind that its cells contain rhodoplastids. The long filaments are probably searching for a suitable substratum, whereas the larger aggregations of cells represent portions of the thallus which are chiefly assimilating. On the other hand, the form of the plants resulting from the experimental germination of the tetraspores of *Actinococcus* may be due to the very abnormal conditions under which germination took place.

In discussing the question a short time ago with Professor Reinke, the latter suggested as a possibility, which ought not to be dismissed prima facie, that *Actinococcus* might really be an asexual generation of *Phyll. Brodiaei*, growing parasitically on the sexual generation. Although this is not absolutely impossible, it is not very probable that it represents the true state of affairs. I need only recall to mind the fact that the contents of one antheridial cavity at least are destroyed by the parasite entering the host.

The foregoing paper was written for the purpose of definitely settling the nature of the pseudo-nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag., of the *parasiticum quid* of Lyngbye, of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv. and *Act. roseus* Ktz., all of which represent the nemathecia of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv.

Other species of *Phyllophora* Grev. and of other closely related Gigartinaceae and other species of *Actinococcus* Ktz. and allied genera, are about to be examined by the author. It is a remarkable fact that the nemathecia of *Phyll. Brodiaei* have not yet been found, although this species is as common as *Phyll. membranifolia* (G. and W.) J. Ag., the nemathecia of which are frequently met with, even in the Baltic. It is necessary that this point also should be elucidated.

There are frequently found growing on specimens of *Phyll. Brodiaei* in the Baltic, and more rarely in other seas, certain structures, which have been called 'Traubenkörper' (1, p. 9). Tetraspores, antheridia and procarpia are found on these, but they never apparently attain to maturity. Schmitz also mentions these peculiar bodies, referring them provisionally as a new species to the genus *Actinococcus* Ktz. (5, p. 380). Kolderup Rosenvinge has described a new species of *Ceratocolax*, which apparently embraces the two organisms just mentioned, and to which he has given the name of *Ceratocolax Hartzii* K. Rosenv. (4, p. 34).

The following is a brief description of the genus *Actinococcus* Ktz. and species *Actinococcus subcutaneus*. It is my intention to give a complete list of synonyms, literature and exsiccata in a later paper; as also a discussion of the systematic position of this plant.

ACTINOCOCCUS Ktz.

Literature: (1) Darbish., Beitrag, p. 7, &c.; (2) Darbish., Phyllophora-Arten, p. 36; (3) Gomont, p. 2, &c.; (4) Kolderup Rosenvinge, p. 33; (5) Schmitz, Actinococcus, p. 392.

Thallus parasitic on other Florideae, the vegetative portion consisting of filaments branching in the interior of the host-plant (intramatrical portion), and fertile filaments forming a cushion of parasitic tissue on the external surface of the host (extramatrical portion of the thallus). Antheridia and procarpia unknown. Tetraspores formed by cruciate division in radially disposed rows of tetrasporangia.

Actinococcus subcutaneus (Lyngb.) K. Rosenv.

Literature: (1–4) The same as of the genus; (5) Schmitz, pp. 369–379.
Synonymy: Actinococcus roseus Ktz.

Thallus (asexual plant at least) parasitic on the young spermophores of *Phyll. Brodiaei* (Turn.) J. Ag., which it enters through the ostiole of the small antheridial cavities. The intramatrical portion consists of longish cells in uniserial filaments, which branch in the medullary tissue of the host, forcing their way along the middle lamellae of the cells. The extramatrical portion is formed by filaments arising from the intramatrical tissue which break through the cortex and give rise, on the external surface of *Phyllophora*, to a parasitic cushion of radially disposed rows of cells, which eventually develop into tetrasporangia. The outer and innermost cells of these fertile filaments remain sterile. The tetraspores are formed by cruciate division. Antheridia and procarpia are unknown. It is also not yet known what becomes of the tetraspores, and whether the spores which give rise to the parasitic nemathecia on *Phyll. Brodiaei* (Turn.) J. Ag. are tetraspores or carpospores.

In conclusion I have to express my thanks to the 'Kgl. Ministerial-Kommission z. Untersuchung d. deutsch. Meere in Kiel' for permission to make use of the *clichés* for the illustrations in the text of this paper.

LITERATURE.

1. DARBISHIRE, O. V.: Beitrag zur Anatomie und Entwickelungsgeschichte von Phyllophora: Bot. Centralblatt, Band 57, n. 12, 1894.
2. DARBISHIRE, O. V.: Die Phyllophora-Arten der westlichen Ostsee deutschen Antheils: Wissenschaft. Meeresunters. herausgegeb. v. d. Kom. z. Unters. d. deutsch. Meere in Kiel, u. d. biolog. Anstalt a. Helgoland. Neue Folge, Band 1, Kiel, 1895.
3. GOMONT, M.: Note sur un mémoire récent de M. F. Schmitz intitulé, 'Die Gattung Actinococcus Ktz.': Journal de Botanique, no. du 1er Avril, 1894.
4. KOLDERUP ROSENVINGE, L.: Deuxième mémoire sur les Algues marines du Groenland: Meddelelser om Groenland, XX, Copenhague, 1898.
5. SCHMITZ, FR.: Die Gattung Actinococcus Kütz.: Flora od. Allgem. Botan. Ztg., Heft 5, 1893.

EXPLANATION OF FIGURES IN PLATE XV.

Illustrating Dr. Darbishire's paper on Actinococcus and Phyllophora.

Actinococcus subcutaneus (Lyngb.) K. Rosenv.

Fig. 1. Longitudinal section through the apical portion of a spermophore of *Phyllophora Brodiaei.* The dark cells stained with iodine represent the cells of the parasite, which has entered the host through the ostiole of the antheridial cavity (at *a*). The parasite is branching in between the cells of the host. A differentiation is already visible in the former into a root and shoot portion. x 250 diam.

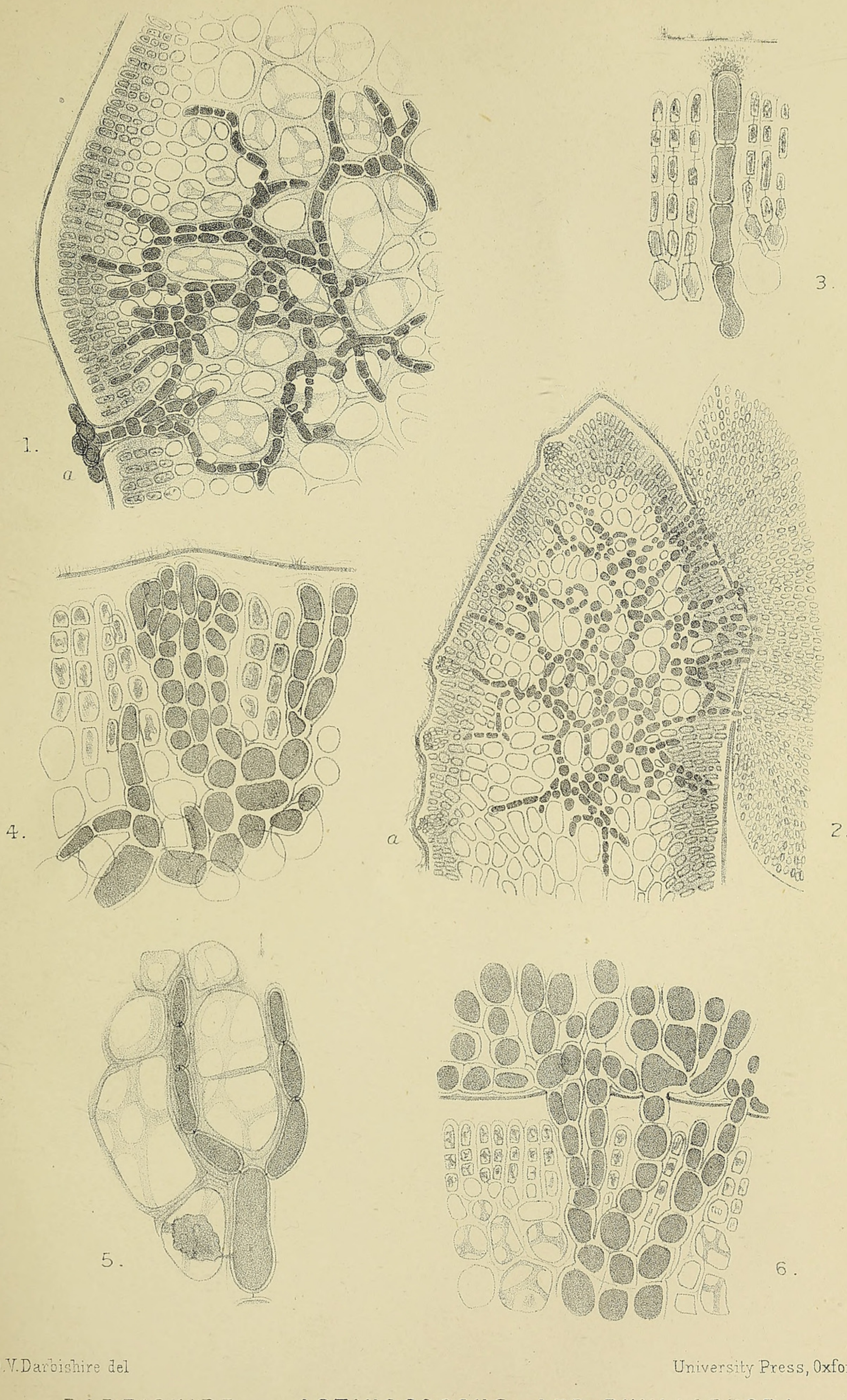
Fig. 2. General view of a longitudinal section through the apex of a spermophore of *Phyllophora Brodiaei,* which has been successfully attacked by the parasite, *Actinococcus subcutaneus.* The intramatrical filaments, stained dark blue with iodine, are clearly seen. From these arise the extramatrical filaments, which have passed out of the tissue of the host, through the cortex. They have formed a nemathecium, only part of which is seen in the drawing. To the left of the section, at *a*, are seen four small antheridia-lined cavities. x 170 diam.

Fig. 3. A single shoot-filament of the parasite breaking through the cortex of the host. The outer covering of the latter is apparently undergoing some change. x 400 diam.

Fig. 4. A bunch of shoot-filaments of the parasite forcing their way through the cortex of the host. They are much branched. x 400 diam.

Fig. 5. A branching filament of the parasite forcing its way between the medullary cells of the host-plant. The arrow points in the direction of the cortex. x 400 diam.

Fig. 6. Section through the basal attachment of an older nemathecium, showing clearly the boundary between the host and the parasite. x 400 diam.



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