CONTRIBUTIONS

TO

THE BIOLOGY OF ZOOTHAMNIUM GENICULATUM AYRTON

BY

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WITH 14 PLATES

D. Kgl. Danske Vidensk. Selsk. Skrifter, naturvidensk. og mathem. Afd., 8. Række, X. 1.

KØBENHAVN
HOVEDKOMMISSIONÆR: ANDR. FRED. HØST & SØN, KGL. HOF-BOGHANDEL
BIANCO LUNOS BOGTRYKKERI
1925

Introduction.

The *Vorticellidae* which are provided with a pedicle comprise a series of Genera of which *Vorticella, Carchesium, Zoothamnium, Epistylis, Opercularia* and *Ophrydium* are the most prominent. Apart from *Ophrydium*, which in many respects differs very much from the other genera, these form a rather well defined unit. They are again divided in two groups, the *Contractilia*, in which the muscle cord is contractile, and the *Acontractilia*, in which this is not the case. To the first named group belong *Vorticella, Carchesium, Zoothamnium*, to the other *Epistylis* and *Opercularia*. *Vorticella* comprises only solitary species, the others live in colonies or stocks. The main difference between *Carchesium* and *Zoothamnium* is that the muscle cord within the pedicle is not continuous throughout but interrupted at each bifurcation so as to permit of the independent extension and contraction of the separate zooids, whereas in *Zoothamnium* it is contractile throughout and not disconnected as in *Carchesium*. The genus *Zoothamnium* is again divided in two groups, the polymorphic and the homomorphic group. As in the other here named genera we find in both groups two sorts of Zooids, the nutritive zooids and the microgonidia; these appear only at special times in the life history of the colony, and most probably all colonies do not pass the microgonidium stage. In the homomorphic group of the genus *Zoothamnium* we only find these two sorts of Zooids. In the polymorphic group, apart from these two sorts of zooids, we find another which is much larger, bulbous, globular, provided with a second ciliary wreath, and capable of detaching itself from the mother colony. This zooid is commonly designated macrogonidium; it is supposed that it is the result of a conjugation between a nutritive zooid and a microgonidium. This is also exhibited by the fact that in most *Vorticellidæ* there is no difference between the common nutritive zooids and the macrogonidia; it is only in the polymorphic group of the genus *Zoothamnium* that such a difference really exists. The polymorphic *Zoothamnia* consist of several species most of them belonging to salt water. Well known are only *Z. arbuscula* Ehrbg. partly lacustrine, partly marine, and *Z. alternans* Clap. & Lachm. only marine. This paper deals only with the anatomy and biology of *Z. geniculatum* Ayrton, found a few years ago by Ayrton and now met with in a little pond in North Seeland. The other species of the polymorphic group are unknown to me; of the homomorphic group, I have found *Z. parasita* Stein upon Entomostraca and *Z. aselli* Clap. & Lachm. upon

Asellius aquaticus. As material for comparison species of the other here mentioned genera of *Vorticellidæ* have often been consulted. In 1898 when occupied with the study of freshwater bryozoa I found the lower surface of the leaves of the *Nymphæaceæ* in the little lake Carlsø near Hillerød covered with numerous beautiful *Zoothamnium* colonies. The colonies were remarkably large and carried all the large so called macrogonidia (bulbi of Trembley) which in the following we will call ciliospores. What specially interested me was that the main rachis or pedicle, when contracted, was not contracted in a regular zigzag line but only bent three times, and further that the three spots where the bending of the pedicle took place presented structures which, as far as I could see, have never hitherto been thoroughly described. Year after year the colony was observed on the leaves; they regularly appeared in the middle of June at a temperature of the water of about 14—16° C. about four weeks after the leaves had reached the surface; they were still found there in autumn when the leaves were brown and perforated by different insects. The colonies disappeared in the last part of October at a temperature of the water of 11° C. some time before the leaves sank downward to the bottom in a decaying stage. Later on the colonies were also found in a lake near Sorø and in some ponds near Hillerød. The substratum was almost always the leaves of *Nymphæacea*, rarely branches hanging down in the water. Whereas many other species of *Vorticellidæ* were found upon the stalks of the leaves — the *Zoothamnium* — during summer, occurred almost only upon the leaves and only a few centimeters downwards along the stalk. On the leaves were now and then found other *Vorticellidæ*; *Carchesium polypinum* especially in spring, different species of the Genus *Vorticella* mainly in autumn. I got the impression that I had to do with a new species, but occupied with now published studies relating to Insects, Plancton and Rotifera I put off a more thorough exploration till another time.

A few years ago I happened to see that Ayrton (1902 p. 407) had described a new species, *Zoothamnium geniculatum*, of which he gives a very good figure (Pl. 21). With regard to the stalk he says as follows: “This can be divided into three regions; the upper part is flexible and contracted by the internal muscular thread into a more or less spiral form, while the two lower parts are perfectly stiff but connected by a highly flexible knee-joint. In contraction this knee-joint is the most remarkable and obvious feature, and has suggested the specific name . . . The muscular fibre is continuous throughout all the branches, and at the summit of the main pedicle the various threads run together to form a short muscular band, which runs down the stem to a point some little distance above the knee; from this point the band becomes very transparent, as if only the sheath of the muscular thread were continued, and just below the knee it is attached to the side of the pedicle. The muscular thread therefore does not run down to the base of the pedicle, as is the case with *Z. arbuscula.* In structure the internal muscular fibrilla consists of a cord with fine transverse striations encased within a delicate hyaline sheath, which itself is very finely striated longitudinally”. Ayrton further says that the nodes are close together and that two or three smaller branchlets usually spring from the underside of each node. Whereas the secondary stalks of the fronds in *Z. arbuscula* are fine, smooth, and elongate, in *Z. geniculatum* they are stout, short, and knotted. AYRTON has seen the two sorts of zooids; the large reproductive ones, being always few in number, are attached to the underside ot the mainribs of the fronds.

The next year I studied the species again and, comparing my specimens with the description and drawings of AYRTON, it was evident to me that I had to do with the new species of AYRTON.

In the long series of years from 1900—1922 the animals were often brought to the laboratory and the ciliospores observed; these were found as well fastened to the colony as in the freeswimming stage; it was further observed that after having fastened themselves they produced a long pedicle and gave rise to a new colony.

Consulting BÜTSCHLI (1889 p. 1629), to my great astonishment I saw that the whole question with regard to the formation and significance of the ciliospores was very little elucidated; in the time from the last days of June to the end of October (1923) I therefore made the study of these charming creatures my chief object of exploration. The animals were studied in the pond almost every week and new material brought to the laboratory. Having finished the drawings and my notices the literature was gone through. I then saw that some points might be more elucidated if the exploration was carried on for another year and the colonies again studied the whole summer halfyear of 1924. —

For my investigation I have mainly used living animals; a colony with its ciliospores was laid in a little vessel containing about 15 ccm. of water from the pond in which the colonies lived. About twenty-four hours later several of the ciliospores were detached from the colony and had fastened themselves to the bottom of the vessel. They were then either detached with a needle and brought under the monocular microscope or studied directly in the vessel by means of the binoculary microscope. Material destined for mounted slides or for cutting was mainly treated in the following way; warm sublimate, Flemming's or de Beauchamp's fluid was poured over the vessels in which the colonies were fastened, the whole process with fixation in sublimate alcohol; staining (borax carmine, Delafield-hæmatoxyline), alcohol and xylol was carried on in the very same vessel, the colonies never coming off from their point of fixation during the operations. Narcotics have always given bad results: chlorale, methylalcohol, cocaine, vapours of alcohol, of formaline, carbonic acid all gave the same results: sooner or later all the heads had dropped off from the colony; fixation without narcotizising always gave some contraction of the stalk, but in the large material some colonies were only slightly contracted. Beautiful preparations of older colonies could be obtained when parts of the leaves with the fastened colonies were suddenly dropped down in 10—15 p. c. formaline.

Loosened from the leaves the large colonies can live in aquaria for about 6—7 days, and in this time throw off their ciliospores. Treated with the above-named fixative fluid they commonly contracted to a ball. If mounted slides were to be made, with these large colonies, I used the following method. Large colonies were laid under cover and slightly pressed. When the moment arrived where owing to asphyxia most of the animals were lying fully expanded but without any motion of the cilia, warm sublimate was poured under the cover and sucked over the colony; in this manner some of the branches were preserved fully stretched out and so also many of the animalcules; later on water was poured over the colony in quite the same manner and lastly 10 p. c. formaline. Then the preparations were closed with the preparation lac of Grübler. —

In 1923 I could never get the colonies beyond a certain point in my vessels; when they had begun to form ciliospores they always died. This was mainly due to the fact that the colonies were devoured by Infusoria and Rotifera. In the autumn of 1924 I had the good fortune to get cultures almost without these organisms, and it was now possible to study the colonies from the very moment when the ciliospore has fastened itself, and when a colony, as far as I could see, died a natural death. The life time of a colony in my vessels was 12—14 days in the autumn at a temperature of the water of about 16—20° C.; the water was the original pondwater filtered through Müllergauze 20; the food was perhaps rather scarce, but copious nourishment caused the water to be blackish and the colonies to be covered with parasites. The water was not renewed, or only if it was absolutely necessary. —

Historical Notes.

In his admirable paper: *Observations upon several species of small waterinsects of the Polypus kind,* 1747, TREMBLEY has studied a *Zoothamnium*-species which may most probably be regarded as *Z. arbuscula.* The paper deals with the anatomy and biology of the animal. Before entering upon a critical survey of the work I wish to make the following remarks. If we take into consideration the extreme simplicity of his microscope and the wonderful observations which TREMBLEY has been able to make with it, it is natural to ask what the eye of a TREMBLEY would have been able to observe if he had been able to use a Zeis microscope for his explorations. Neither CLAPARÈDE and LACHMANN, nor STEIN, nor BÜTSCHLI have been able to contribute to the anatomy and biology of the animal what TREMBLEY did; more than one observation has been correctly carried on by TREMBLEY whereas his above-named epigones, who may all be regarded as the heroes in the protistology of the following century, have interpreted anatomical structures in the opposite way to what TREMBLEY had made out, and often quite incorrectly.

TREMBLEY (1747 p. 649) has correctly seen that the colony has eight or nine branches or leaves. This number is correct but not observed by the subsequent observers. What is much more astonishing is that he adds: "They do not all of them set out from the same point; but the points from whence they do set out are not far asunder". The subsequent observers commonly indicate or draw the branches as proceeding from one single point. Trembley has further correctly seen the physiological result of one of the systematic main characters of the genus *Zoothamnium*, namely that all the branches fold together when only one is touched and that the stem contracts at the same time. Having observed that the heads of other *Vorticellidæ* after detaching themselves are also able to fix themselves, develop their pedicle, and give rise to new clusters, he expected that the animalcules of the *Zoothamnium*-colonies would act in quite the same manner. He found, however, that nothing "was produced by these polypi so detached". He therefore concluded that they did not contain the principles of new clusters, and supposed that they would all perish "without ever producing anything whatsoever". However, searching in his vessels for fixed heads or animalcules, he found other bodies much larger the development of which he studied and traced back to their very source. Upon this point he writes as follows: "Some days after the clusters had begun to form themselves, I saw come out, not from the extremities of the branches, but from the bodies of the branches themselves in different places, small round buds, which grew very fast, and which arrived at their greatest size in two or three days. These bodies much resembled the galls which grow on the leaves of oaks; they were placed upon the branches of the clusters, just as those galls are usually placed upon the fibres of the leaves; and these bulbous substances do really contain the principles of the clusters. Two or three days after these bulbs have begun to form, they detach themselves from the branches out of which they sprung, and go away swimming till they can settle upon some body, which they meet withall in the water, and to which they immediately fix themselves by a short pedicle". It was these round fixed bodies which Trembley found upon the sides of the glass; owing to their large size he did not originally think that they had anything to do with the colony he studied. However, surprised at the enormous speed with which they grew, he studied them more thorougly. On the 2nd of June at five in the evening he took one of these bodies "which was oblong, and had a pedicle three or four times longer than itself. At eight o'clock it began to split from the top towards the bottom, and the two new bodies were nearly of the same shape as the first. "A very little after" he saw the two bodies divide again, and Trembley then understood that this very body which he had but just concluded did not contain the principle from whence "I was to expect the production of one of the clusters I was looking after", might possibly still be the very thing he was seeking for. — At 11.20 Trembley found eight bodies (animalcules) and at 12 sixteen. At 3 o'cl. in the morning the number was twenty-six. At 7.30 o'cl. he counted at least 40 Polypi, in that same part which he could see of the cluster. Trembley repeated the observations upon other individuals and came to quite similar results. In my opinion the most extraordinary of all Trembley's observations is that he has been able to see with his extremely bad instruments that the two "Polypi" which result from the division are not of the same size, the one being "generally larger than the other. The largest remains at the end of the principal branch, whilst the lesser serves to form a lateral branch and is itself the principal of all the Polypi which that lateral branch is to bear". In accordance with this Trembley finds that the Polypi which are at the extremities of the principal branches are always the largest, and those which divide themselves most frequently. Already in the young colonies only containing sixteen animalcules Trembley had observed that they were not of the same size; those which were most distant from the origin of the branches were the largest, and their form was also least like that of a bell. — Trembley (p. 652) further observed that the bulbi, having fastened themselves and having begun the development of the pedicle, continually lengthen it by degrees for about 24 hours; during this time the bulbs change their shape and become nearly oval.

Having detected the bulbi, studied their origin and followed their development, Trembley quite correctly sees their significance for the colony, and the great difference between the other *Vorticellidæ* and the *Zoothamnium*-species which he has studied. The *Vorticellidæ* in general (what Trembley designates as "the first species of Polypi") "come all from Polypi detached from the clusters already formed". In *Zoothamnium* ("Polypi of the second species") they do not arise from Polypi detached from other clusters; but from round bodies or bulbs larger than those Polypi and of a form very different from them". Trembley further points out that the bulbi are not formed like the other animalcules (Polypi), "by division of others like themselves, but they spring from the branches of the cluster, as the flowers and the fruits of a tree spring from the branches of the same". He further remarks that whereas in the *Vorticellidæ* there are considerable intervals of time between their division, in the *Zoothamnium* the first divisions are consecutive and follow hard upon each other. —

Of Trembley's other observations I consider it convenient especially to point out the following: Even if it seems as if the Polypi which we see fixed to the branches of the cluster, do really proceed and spring from those branches in the same manner as the leaves, the flowers, and the fruits of a vegetable spring from the branches of the same, it is nevertheless the reverse of all this that is true. The branches composing the clusters of the Polypi spring from the Polypi which are at their extremities. It is not the branches which draw and give motion to the Polypi; on the contrary the motion in the stem and in the branches is entirely derived from the Polypi which are fixed upon the branches.

In the following years many of Trembley's observations were regarded as doubtful or misinterpreted. This was mainly due to the fact that with regard to the ciliospores (bulbi of Trembley, macrogonids of later authors) Ehrenberg (1838) has in some way misunderstood Trembley. He seems to have supposed that the division of the ciliospores went on upon the old colonies; he has arrived at this supposition because he was unable to corroborate the above-named, fully correct observation of Trembley that the detached individuals of *Carchesium polypinum* and *Epistylis umbellaria* were able to give origin to new colonies. He has, however, correctly seen that the macrogonidia are always placed in the angles of the branches, and supposes that, of the two individuals which are the result of a division, one divides again, giving rise to the two branches, whereas the other is stationary and is not divided more. — Not until a century later were the observations of Trembley fully corroborated by Brightwell (1848). The young colonies were, however, not studied after the stage of nine individuals; he supposed that *Zoothamnium* was subject to an alternation of generations, and that in the way that the larger zooids hibernated in the mud and the following spring gave rise to new colonies; we shall see later on that there is something correct in this supposition.

Claparède and Lachmann (1858 p. 102) have not found *Z. arbuscula*, but on the marine species *Z. alternans* they have found the so-called large individuals (bulbi of Trembley) which however seem to be smaller than those of *Z. arbuscula* (120 μ). They corroborate the observations of Ehrenberg that the large individuals are seated in the angles of the branches, but maintain, as he does, that also the large individuals are capable of division when attached to the mother colony; this has not been observed in any other species, nor has it been corroborated by later observers. Besides, they have not followed the fate of the large individuals but only observed that when fully developed they are almost spherical with the posterior ciliary wreath situated a little behind the æquatorial plan. Greef (1870 p. 366) only remarks that the genus *Zoothamnium* is characterized by the fact that the individuals of the colony show great differences with regard to size, the large individuals being 5 to 6 times larger than the other; their number varies very much; they may be lacking, which is really rare in marine species.

In 1875 Engelmann and later on Forrest in 1879 again saw the large individuals in *Zoothamnium arbuscula*; but none of them have followed their development. Forrest's paper is unknown to me, but Bütschli (1889 p. 1629) says that he supposes that the macrogonidia hibernate.

Kent (1881—82 p. 696) is in many respects the one who, after Trembley, has the best observations upon the development and significance of the macrogonidia. He says that upon the spheroidal zooids devolves the mission of becoming detached and laying the foundation of future colonies. He is the first who clearly maintains that they are at first similar in shape and size to the small individuals and then slowly increase in size, taking no share in the further extension of the branching pedicle. The body gets a circle of cilia, becomes detached and wanders away in search of a suitable site for reattachment. Kent has further set forth a similar idea to what will be found upon the following pages, that the abnormal thickness of the basal portion of the branching pedicle may be explained in the light of these developmental phenomena, its greater caliber being the natural product of a zooid of so much greater a relative size than those which contribute to its further prolongation. Kent further states that Bolton has brought him colonies that bore supplementary zooids five or six times as long as broad and with relatively coarsely annulate surface. KENT is inclined in these bodies to see male units destined to compass genetic union with the large subspheroidal animalcules. As BÜTSCHLI correctly maintains there cannot be any doubt that these bodies belong to organisms which have fastened themselves to the colony and have nothing to do with it.

In many respects STEIN (1867 p. 131) has augmented our knowledge of the propagation of the *Zoothamnium*-colonies; unfortunately the excellent observations of STEIN are very difficult to interpret, especially because they are never supported by illustrations, and many of them are given almost in the form of a diary and not well worked together. Also the fact that all his admirable observations were made to serve his theories (Acinetentheorie, sexual propagation of the Infusoria) has not facilitated the right interpretation and the full appreciation of his work. Most of his observations belong to *Zoothamnium arbuscula* Ehrb. As however he says (1867 p. 131) that he always found the colonies "wiederholt dichotomisch verästelt und zwar meist so, dasz sich der lange gemeinsame Stamm in zwei Hauptäste theilte", it is almost certain that the species has not been *Z. arbuscula*, the branches of which, as far as I know in a number of nine to eleven, almost all proceed from the top of the pedicle. It is more probable that he has had to do with a species similar to *Z. alternans*. If so it also becomes intelligible that he has not observed the macrogonidia. "Auch traf ich auf den Stöcken ausser den gewöhnlichen Individuen nie jene grösseren knollenförmigen Tiere durch welche sich nach Ehrenberg allein die Gattung *Zoothamnium* von der Gattung *Carchesium* unterscheiden sollte". As BÜTSCHLI (1889 p. 1629) however correctly remarks, STEIN most probably observed these animalcules; only they have not been so large as those which other observers have seen, and this again is due to the fact that he has not had *Z. arbuscula* with the great macrogonidia but one of those species where, according to all descriptions and drawings, the macrogonidia do not differ so much from the common zooids as in the above-named species (see e. g. the figure by GREEF Tab. VI fig. 6).

As far as I know STEIN only observed and correctly interpreted the microgonidia of *Zoothamnium*: "knospenförmige Conjugationszustände und Gruppen kleiner Teilungssprosslinge die sich immer zu vieren auf einem Stiele entwickeln". He has also seen the conjugation and quite correctly observed that quite as in *Epistylis crassicollis* and *plicatilis* the microgonidium "senkt sich ganz in dem Körper des Trägers hinein so dasz nur seine Spitze als ein kleiner häufig quer geringeltes erscheinendes Wärzchen nach aussen hervorragt, während sein übriger Teil eine scharf begrenzte im Parenchym des Trägers steckende Kugel bildet" (1867 p. 132). He maintains that the conjugated individuals only occur in a very small number, that they are thicker and more voluminous than the common zooids; they are further contracted in the shape of a ball, cannot develop upon the mother colony, but are loosened from it, whereupon they found new colonies. On the basis of these observations BÜTSCHLI (1889 p. 1630) concludes firstly that STEIN has already observed the macrogonidia, these being only smaller than in the real *Z. arbuscula*, and secondly that STEIN has proved that the macrogonidia are a result of a conjugation ("thatsächlich zur Conjugation bestimmt sind"). This last conclusion I do not think is correct. In accordance with the experience of all other authors relating to other *Vorticellidæ* as well as in accordance with my own, I regard it as quite impossible to distinguish conjugated individuals from not conjugated. What STEIN has seen are only the ciliospores in different developmental stages; then he has supposed that these were the conjugated ones. On the other hand that he really has seen the conjugation is unquestionable. It is well known that after the conjugation the macronucleus is divided in numerous small particles; simultaneously appear some ballshaped light corpuscles (kuglich, lichte Körper) which BÜTSCHLI has shown to be derived from the micronucleus; the number of these bodies is but small, but rather constant for the species. STEIN regarded the fragments of the old MaN as "Keimkugeln" which in *Zoothamnium* are supposed to unite in the so-called Placenta which according to later authors (especially ENGELMANN 1876 p. 620) is only the new MaN; the "Keimkugeln" may be regarded as nucleoli. As far as I can see BÜTSCHLI has not explored *Zoothamnium* and seems never to have seen the macrogonidia. As they are axillary, he (like EHRENBERG) regards them as "Seitensprösslinge". As he regards them as derived from a conjugation it is difficult for him to give credence to the quite correct observation of KENT that they are originally identic in shape and size with the common zooids.

ENTZ (1893 p. 1) has studied the structure of the pedicle and the myonems of the ciliospore. To this work as well as to other literature relating to the pedicle we will return later on.

From this short historical sketch it will be seen that with regard to many points in the anatomy and biology of the *Zoothamnium*-colony we are without any deeper knowledge. The anatomical study of the structure of the zooids is quite insufficient. In the structure and function of the pedicle many questions are unsolved. The rules which govern the admirable regularity of the colony are wholly unknown. The whole question with regard to the ciliospores is quite unsolved. We do not know when they arrive in the life history of the colony, the conditions, outer and inner, upon which their development depends, if they are the result of a conjugation or not; their anatomical structure is very insufficiently known. We are insufficiently informed as to how new colonies originate from the ciliospores, and we have no understanding of the fact why only the genus *Zoothamnium* and only a few species in this genus have ciliospores whilst they are wholly unknown in other genera and in most of the *Zoothamnium* species.

The microgonidia are insufficiently known; the conjugation is only observed a single time. With regard to all questions relating to the nuclei we have only conclusions of analogy from other *Vorticellidæ* to resort to; all questions with regard to hibernation are quite unknown.

In the following pages I have tried to solve some of these questions; especially the ciliospores, their formation, their life, their significance as founders of new colonies, their anatomical structure, their relation to the phenomena of conjugation have been studied. Some questions are still unsolved partly because they demand biochemical or very thorough cytological explorations, domains which I do not master and which could only with difficulty be carried out from a small Freshwaterbiological laboratory to whose main task explorations of this kind also do not belong. In our day in explorations of this kind a division of labour is as justifiable as necessary.

Anatomical Description of the Colony.

The great colonies Tab. I fig. 1 possess a pedicle af almost a length of three to four mm.; the diameter of the umbrella almost equalling in length that of the pedicle. The length of the branch of the first order varies very much, but they may reach a length of almost 2—2½ mm. With regard to *Z. arbuscula* it is commonly maintained that these branches of the first order all proceed from the summit of the main rachis or pedicle; the drawings all show that they all issue from a single point, the apex of the pedicle. That this is not the case with *Z. geniculatum* will be shown later on. The branches of the second order go out either opposite to each other or rarely alternately to the right and to the left side. In the old fullgrown colonies these branches again carry branches of the third order and this is mainly the case with those placed nearest to the centre of the umbrella. Tab. II fig. 5 and Tab. VIII fig. 16 attempt to give a true camera drawing of the ramification of two of the branches of the first order, the one seen fully expanded and en face, the other laterally. The short branches of the third order carry the individuals or zooids in a number of one to four; they proceed mainly from the innerside of the main branches but are turned outwards during growth. Apart from the zooids which have begun the development to ciliospores all the others in the old colonies are almost of the same size, only those placed in the apex of the branches being commonly a little larger. On the spot where the main branches branch off from the apex of the pedicle we further find some clusters of very small zooids often only half the size of the others. These individuals are also characteristic by their darker contents, it seems as if they are loaded with enormous quantities of granules. Always placed at the apex of the pedicle where the main branches go out I have supposed that they have a special significance and that the material with which they are loaded is really of excretory matter. Similar individuals are often found in the angles between the branches of the first and second order.

As far as we know the common zooids of *Zoothamnium* never seem to develop a second ciliary wreath. If this holds good for all species of *Zoothamnium* we do not know, but I have never found this girdle mentioned in literature, and neither Ayrton nor myself have found the girdle developed in the common zooids of

Z. geniculatum. In accordance herewith we also never see the common zooids loosen themselves from the mother. colony, the two zooids developing after fission will both during life be in connection with the mother colony. Upon this point the genus *Zoothamnium* seems to form a contrast to the genera *Vorticella*, *Carchesium* and *Epistylis*; we will later on return to this point. In those *Zoothamnium*-species with which we are dealing here the development of new colonies is confined to quite special individuals differing very much in size and structure from the common zooids. In contrast to the other above-named *Vorticellidæ* we have here a very conspicuous distinction between the common zooids and those which take part in the production of new colonies; only these last-named zooids get a second ciliary wreath and in size and shape as fullgrown are wholly different from the common zooids. These last-named contribute through fissions to the growth of the mother colony; the first-named do not divide as long as they are in connection with the mother colony, the fission does not take place before they are loosened from the mother colony and again fastened upon another locality; their peristome is closed and they are, without themselves taking in food, nourished by the mother colony. We are therefore in these *Zoothamnium*-colonies entitled to speak of two different sorts of zooids: The nutritive zooids and the ciliospores, the bulbi of TREMBLEY, the macrospores or macrogonidia of later authors.

The nutritive zooids (Tab. VII fig. 5—6) in fullgrown colonies are all of almost the same size, very small, only about 36—40 μ long and about 20—30 μ broad; their form is elongated conical. The ciliary disc is flattened; the annular border or peristome only slightly elevated; the adoral spiral describes only $1^1/_4$ winding which is continued in the vestibulum. It is impossible to study the ciliary apparatus upon living animals, and upon preserved specimens it is almost always contracted. If the above-named method of introducing warm sublimate under cover over colonies which are fully stretched out and have suffocated owing to want of oxygen is used, many zooids will remain fully stretched out and may later on be preserved in formaline brought in over the colony under the cover. As the washing out after the sublimate must necessarily be insufficient, a precipitation of crystals of sublimate is inevitable, but this precipitation is never so strong that it prevents the study of the single individuals. In colonies treated in this way it is quite conspicuous that the so-called cilia of the peristome really consists of two undulating membranes which are united basally and have their free border ravelled up in many fibres, the cilia of earlier authors. This picture is in accordance with the sketch which MAIER (1902 p. 114) has given of the ciliary apparatus of *Carchesium* and which already BÜTSCHLI (1889 p. 1339) has assumed.

The two membranes are continued in the vestibulum which goes a little way vertically downwards in the body; then it lies more or less horizontally; it then narrows in, and is continued in the pharyngeal part, the lower end of which lies in the last thirds of the body.

The contractile vesicle seems to be single; it lies very near the disc; when it is in its highest diastyle the disc may be vaulted; it opens into the vestibulum; its greatest diameter is about 14 μ; it commonly contracts about every 40 seconds. A special reservoir supposed to be a part of the vestibulum and in which the contractile vacuole opens I have not been able to detect. As well known this is indicated for many *Vorticellidæ* but not found in others (*V. microstoma, Epistylis plicatilis, E. umbellaria* and in *Opercularia*) (Bütschli 1887—89 p. 1423); Schroeder (1906 p. 97, p. 175)). The horseshoe-shaped macronucleus lies behind the vacuole; the micronucleus is not detectable upon living animals. I have not seen it with certainty but in a few cases I suppose I have seen it in the immediate vicinity of the macronucleus. The number of nutritive vacuoles is commonly but slight. A transversal striation of the cuticula has not been observed. The most characteristic structure of the nutritive zooids is, as mentioned above, that the posterior ciliary wreath ("der Wimperring") in this and most probably in all *Zoothamnium*-species with ciliospores is never developed, nor is any trace of the zone from which it should be developed observable. Of the myonem system only the longitudinal ones have been seen; their number may be about twenty; they proceed from a myonem funnel which seems to be in connection with the muscle of the pedicle only by means of two short strings; in reality the number of strings is greater. The finer structure of the ectoplasma has not been studied. In its lower part the entoplasma is differentiated in a cortical plasma; as far as I have been able to see its structure is in accordance with what Schroeder has found in *Campanella umbellaria* (1906 p. 96).

The ciliospore. With low power it seems, especially at midsummertime, as if every branch of the first order carries only three to five ciliospores (Tab. II fig. 2) almost all of the same size, hut if high power is used, it may be shown that many smaller bodies, some ballshaped, some pearshaped, but all larger than the common zooids, are placed along the branch. Young colonies possess none of the large bodies; old large colonies may carry about 40—50 simultaneously; they do not occur at any special time: they are to be found upon the colonies in June but the number is then but small, commonly not more than 1—5 upon the whole colony, and they only develop slowly. They still appear in October but then the number is again small; it seems then as if, upon each branch, only one or two ciliospores are fully developed. The largest are commonly seated nearest the base of the branches, but fully developed ones may also occur nearest to the apex. Very often in a series of four or five full grown ciliospores we find one or even two which are only half the size in diameter of the large one. The distance between the ciliospores differs very much, and often quite small ones are very near large ones. During the development the ciliospores are turned inwards on the concave side of the crown; when fullgrown they are turned outwards, hanging downwards like fruits upon a fruittree. (Tab. II fig. 5 shows one of these main branches with eight individuals all larger than the common zooids; the leaf derived from an old colony in July). It will be seen that the eight individuals are all seated at that point where branches of the second order issue from the main branch. If now we direct our attention to that point where the stalk which carries a young ciliospore is inserted upon the branch, it will be seen that round this point there are often hanging membrane-like rather irregular flabs, often in a number of two or three (Tab. V fig. 7—8). On closer study it will be seen that these flabs are really stalks which have carried zooids which have disappeared. If we further study these angles in which no ciliospore is formed, we almost always find two to four individuals. It seems therefore that if one of these individuals is destined to be transformed into a ciliospore, this can only take place at the sacrifice of the other two or three individuals which are seated in the same angle; later on when the ciliospore has augmented in size these stalks totally disappear.

I am therefore not quite sure that it is correct to designate the ciliospore only as "Seitensprösslinge", branches of the second order which have not been developed. A supposition which Ehrenberg (1838) has set forth and which Bütschli (1889 p. 1629) has adopted.

As mentioned above, between the nutritive zooid and the ciliospore there are all possible transitions. Tab. VI fig. 13—17 will illustrate this. In the first stages the strongly growing individuals differ from the common zooids only in size; as all the other individuals they possess a very short stalk through which the muscle-cord runs. The body is pearshaped; the peristome is well developed; mouth, contractile vacuole, nucleus and nutritive vacuoles are present. When the breadth has been augmented from above 30 μ to about 80 μ, the peristome is narrowed in, and the cilia are now seen not as a regular *Vorticella*-crown but only as a bunch of cilia stretched forwards on the apex of the body; but now and then the peristome may be almost wholly folded out. The vestibulum and the contractile vacuole are unaltered and in the interior nutritive vacuoles are still present. The outer membrane is smooth and the posterior ciliary wreath is, if present, at all events very inconspicuous. The macronucleus is conspicuous, lying almost in the middle, and is much augmented in size; the micronucleus has disappeared. When the body has reached a length of about 100 μ the breadth is augmented, and it is now almost isodiametric. The peristome is wholly withdrawn, but at the apex cilia may still be seen vibrating in a broad flattened space near the apex; the cilia move rhythmically at small intervals, not uninterruptedly. The vestibulum seems almost to be obliterated. The cytopharynx has totally disappeared. In the lower part of the vestibulum the contractile vacuole opens; this is in connection with the vestibulum by a short canal. All nutritive vacuoles have disappeared, only the contractile vacuole is present; the very large and very broad macronucleus is very conspicuous; behind this a dark mass encircling the point where the ciliospore is fastened to the stalk is every hour more and more obvious. When the ciliospore has reached the size of about 180 μ in length, commonly in the course of the third day, a dark posterior line appears and from this the posterior ciliary wreath is developed. A little before, the pedicle is lowered down in the basal part of the ciliospore at this spot presenting a deep excavation. The ciliospore is fastened to the branch by means of a process which is formed by the wall of the pedicle (Stielscheide) which is here vaulted upwards in the form of a crater; in its interior runs the muscle with its musclesheath which again is in connection with the muscle funnel in the interior of the ciliospore. When the ciliospore has loosened itself a double contoured cicatrice is left upon the branch (Tab. VIII fig. 17), the outer ring indicating the point of attachment for the wall of the pedicle the internal one for the muscle; upon old colonies a long series of such citatrices are seen, placed serially upon all branches of the first order. Whereas the pellicula in the unripe ciliospore is extremely thin and quite smooth, it gets thicker and thicker during the development and at last shows a very conspicuous but very fine transversal striation. Simultaneously the myonem system which has hitherto been very inconspicuous gets more and more conspicuous. We now often see how the ciliospore suddenly contracts its longitudinal axis so that it gets broader than it is long, and for every contraction of the colony this phenomenon is now more and more obvious. For some hours the posterior ciliary wreath moves vigorously; by this motion and the just mentioned contraction the ciliospore is loosened from the colony and darts away. The fullgrown ciliospore is about 230—250 μ broad and about 180 μ long.

The whole metamorphosis from nutritive zooid to ciliospore may be accomplished in the course of 2—3 days, in spring and autumn it commonly takes 4. For more than two days of the development the ciliospore has had no nutriment in solid form. It has only taken in water and pumped it out through the opening; besides it has been fully nourished by the mother colony. We will now study the anatomy of the freeswimming larva a little closer; the pellicula, the endoplasma, the pulsatile vacuole, the myonem system and the macronucleus.

The ripe ciliospore (Tab. II fig. 3—4) shows a remarkably thick pellicula (Tab. XI fig. 7—8); it is 2—3 μ thick and conspicuously stratified with a line in the middle which is more highly stained than the outer and inner layer; in the cuts it is often folded which is most probably a shrivelling phenomenon. A closer examination will however show that below the pellicula which is always stained relatively feebly, lies another extremely thin layer always very strongly tinged and which is never folded together with the outer layer; I am inclined to think that this thin layer is the real pellicula of the ciliospore, and that the outer layer is a secretion originating from it. This would be in accordance with phenomena known from other *Vorticellidæ* (*Campanella* a. o. Schroeder 1906 p. 80). The outer layer seems to be of quite the same structure over the whole surface; near the opening on the apex of the ciliospore the striation disappears, giving place to a collar-like ring quite smooth and surrounding the opening.

The vacuole (Tab. VI fig. 1—5, 8, 9, 10, 11. Tab. VIII fig. 1—14). On the top of the ciliospore is found an opening, always present; below that a fine canal leads downwards into a flattened space; the bottom of this is identic with the discus which is here extremely reduced and in contracted individuals almost invisible. The borders of the discus grow upwards limiting the fine canal; upon their innerside they are coated with cilia and represent the peristome. In the bottom of the space opens a flattened short canal, the rest of the vestibulum. In the interior of this canal an inconspicuous motion of cilia is observed. These cilia, perhaps an undulating membrane, do not move uninterruptedly but at intervals of different length and independent of the grade of filling of the vacuole. A series of horizontal cuts (Tab. VI fig. 8) shows that the opening is constant and that it has jagged borders (fig. 8). In the bottom of this very short vestibulum opens the contractile vacuole. This shows remarkable phenomena which I am not sure I have interpreted in quite the right manner.

Upon the living ciliospore, when fastened to the mother colony or when swimming, we see the vacuole regularly pulsate about every 2.45 to every three minutes. In the first minute the vacuole reaches a diameter of 50 μ, in the second of 67 μ, and in the third of 84 μ. In full systole the spot where it will appear again shows a peculiar foamlike structure (Tab. VIII fig. 13) owing to numerous small vacuoles which disappear slowly during the diastyle, giving place to a single one which slowly augments at the expense of all the other smaller ones. It has first jagged borders but the lines are slowly smoothed out and in the last phase of the diastyle the contours are circular and the space ballshaped. Simultaneously a bright zone (Tab. VIII fig. 14) appears round the vacuole and immediately before the systole the whole vacuole is moved up and down. The systole goes on rather slowly during five to six seconds. The vacuole is in connection with the vestibulum by a short canal almost invisible during diastylis; it opens in a little excavation of the vestibulum bordered by a little hill on one side. Immediately before the systole we see the skin in the excavation burst, and the lumen of the canal becomes visible. Then the canal almost quite disappears again. Simultaneously with the opening of the canal a violent sudden movement of the cilia near the opening of the canal in the vestibulum sets in, whereupon it is again discontinued. Very often, but not always, we find behind the great vacuole one or two of the so-called "Bildungsvacuolen" which are connected with the great vacuole by short canals. In some cases it may be seen that the smaller vacuoles are first filled, whereupon they pour their contents of fluid into the greater one and then disappear. The cuts often show one or two of these reserve vacuoles. Tab. VIII fig. 1—10 shows a longitudinal series of ten cuts, each 7.5 μ thick, through the vacuolar room; the second to the fourth show part of the vestibular room in which the vacuole opens, the others the vacuole which is in the last part of the diastyle. As well known, different authors (Wrzerniowski 1877, Bütschli 1889 p. 1419) have also found three vacuoles, of which one has been interpreted as a reservoir, a part of the vestibulum into which again the true vacuole with its reserve vacuole was supposed to open. Now and then I have supposed that the vacuole was really the reservoir in the bottom of which the two vacuoles opened. As however it is always maintained that the reservoir is mainly obvious during the systole and I have seen the slow filling of the room during the diastyle upon the living animal, I am inclined to suppose that the great pulsating hollow room is really the true contractile vacuole, and that the two smaller ones can only be designated as so-called “Bildungsvacuolen”. The great problem now is whence comes the water in the vacuole? In the nutritive zooids it may be supposed that the water goes in through the vestibulum and cytopharynx and comes into the entoplasma with the food as nutritive vacuoles. In the ripe ciliospore the vestibulum is rudimentary, there is no cytopharynx; nutritive vacuoles are not found, and no nourishment of any kind is brought into the organisms. The water can only come in through the small opening at the apex or through the whole surface of the body; as just the skin of the ciliospore is very thick, covered with a thick cuticula, this last named supposition is improbable. It is most probable that the water comes in through the opening at the apex, the opening for the rudimentary peristome. In the cuts of the vacuole two small canals lying immediately under the insertion of the cilia (Tab. VIII fig. 6, Tab. VI fig. 11) are always obvious in the walls between the vacuole and the rudimentary peristome room. I suppose that the water in some way goes in here, but with regard to the necessary driving forces I have no supposition at all. I have been unable to see any regular motion in the cilia; commonly they do not beat only now and then, they suddenly beat some few strikes and then the motion ceases again; the motion takes place during the whole diastyle, but just during the systole the cilia do not beat. How the cilia alone should be able to conduct water into the interior of the ciliospore, I do not know. It is possible that more thorough explorations will show a system of ring myonems, but hitherto I have not been able to see them. As well known, we commonly assume that the contractile vacuole in the Infusoria gathers the water from the whole organism during the diastylis; in this case we are almost forced to suppose that the water is pumped out and in directly from the surrounding medium. I have tried to solve the question by means of vital staining methods, but I could get no exact results. We will return to the question when the formation of the pedicle is mentioned.

Myonems: In all cases where more thorough explorations have been carried on, the myonem system of the *Vorticellidæ* may be referred to five different systems. 1. The ring myonems of the basal part of the zooid, 2. the longitudinal myonems, 3. the ring myonems of the peristome border, 4. the spiral myonem of the discus and 5. the retractors of the disc (Schroeder 1906 p. 85; Fauré-Fremiet 1905 p. 207). These five systems are by no means found everywhere, but it seems that all myonems found in a zooid may be referred to one of these five systems. Of these five systems, as far as I can see, only one, No. 2, the longitudinal myonems, exists in the ripe ciliospore. As the peristome and almost the whole ciliary apparatus has disappeared, it is easily intelligible that the three last systems have been obliterated; also the ring myonems of the basal part of the zooid seem to be wanting. Of the longitudinal myonems of the ciliospore Entz (1893 Tab. II fig. 3) has given a rather good figure. Their number may be about 40; they do not reach the apex of the ciliospore; these longitudinal myonems are, as well known, in connection with the muscular fibrillæ of the pedicle which are dissolved in a number of longitudinal myonems, the number of which differs in the different species. In the lower part, before reaching the posterior ciliary wreath, the fibrillæ of the muscle-cord are separated from each other and from the so-called muscle funnel (Muskeltrichter of the authors); the bottom of this funnel is seen in Tab. VI fig. 7 where the longitudinal myonems are seen proceeding from the borders of it. Each of these longitudinal myonems carries numerous branches which again are divided in very fine fibrillæ (Tab. XII—XIII fig. 5); these form a regular myonem layer between the endoplasma and the pellicula; the branches anastomose with each other; before the secondary ciliary wreath they lie far away from the surface, but above it they come nearer to it; they do not reach the apex of the ciliospore. In longitudinal (Tab. VI fig. 10) but not median sections the longitudinal myonems are seen as long lines, the number of which are augmented in the direction of the apex owing to the branches proceeding from them. In a median longitudinal section (Tab. VI fig. 11) is seen the bottom of the muscle funnel, one pair of longitudinal muscles, and a number of cut ones. In *Vorticella putrinum* (1906 p. 404) SCHROEDER has seen the longitudinal myonems giving off fibrillæ for the posterior ciliary wreath. I suppose that this is also the case with those of the ciliospore.

Cortical plasma and Entoplasma. The whole interior of the ciliospore is of an extremely homogeneous structure. In the basal part of the ciliospore to the ciliary wreath lies the cortical plasma (Tab. VI fig. 10—12), above it the entoplasma; I have been unable to point out with certainty a cortical plasma between the myonem layer and the entoplasma, at all events it is extremely thin. The cortical plasma as well as the entoplasma shows an inconspicuous alveolar structure, but that of the cortical plasma is much finer than that of the entoplasma. Both are overfilled with enormous masses of granules which lie closer to each other in the cortical plasma than in the entoplasma. The single granules are also much finer in the cortical plasma than in the entoplasma. In the middle of the entoplasma is a hollow space into which the entoplasma projects columella-like; in this hollow space round the columella lies the nucleus. In the ripe ciliospore the entoplasma does not contain nutritive vacuoles; it is of an extraordinary homogeneous structure; only round the pulsatile vacuole it is not so coarse. In the living ciliospore a remarkable dark mass, arranged in the form of a circle round the point of attachment, is observed; this mass I cannot find in the cut material; it disappears when the ciliospore is prepared for cutting.

The macronucleus (Tab. VI fig. 6, 11. 12) is very conspicuous even without the use of any reagentia, and of a really extraordinary size; its position differs a little; it commonly lies in the middle of the sphere; it is a more or less spirally twined band; its length is about 230 μ, its breadth 30—35 μ; under the binoculary microscope it may without difficulty be prepared out by means of two needles. It is commonly as Tab. VI fig. 11 shows twined round the columellar projection of the entoplasma; it always lies in a nuclear space, being not in connection with the entoplasma on its convex sides, but only on the concave side which is pressed round the columella. It possesses a conspicuous nuclear membrane. In a homogeneous mass lie numerous bodies which stain very strongly with hæmatoxyline; they are of different size, the largest ones are surrounded by well-defined clear spaces; most probably these bodies are identic with the protomicrosomes and protomacrosomes found by GREENWOOD in the nucleus of *Carchesium*.

The micronucleus: During the whole development of the ciliospore and till the first fissions take place I have been unable to see any trace of the micronucleus either in the living organism or in stained ones or in cuts.

The stalk or pedicle presents a very peculiar aspect, as far as I know, very different from the stalk of other *Zoothamnium* species. Tab. XII—XIII fig. 1—2 attempt to give sketches of the pedicle in erect and in contracted conditions. In cros-section the pedicle is a flattened band in its upper part nearest to the points from which the branches issue; in the middle the cros-section is more elliptical, and only in the part nearest to the leaf which carries the colony, the cros-section is almost circular. It will further be seen that it is only nearest its base, where the pedicle is fastened to the substratum, that the sides of the pedicle are parallel, whereas, nearest to the summit and almost to the middle, they are conspicuously undulated, but only and always with two broad undulations. A closer examination will therefore show that the pedicle may be divided in three divisions (Tab. XII—XIII fig. 1—2); an upper basal part which is thin, rounded in diameter, and with perfectly parallel sides, a middle part which is elliptical in diameter and has almost straight sides, and a lower part which is flattened, bandlike, and undulated. Between the two last parts the pedicle shows a more or less conspicuous narrowing, between the two first parts we find a rather conspicuous knee in which peculiar chitinous structures are seen; they will be described later. The interior of the pedicle is filled with a hyaline glassy mass already mentioned by earlier authors. Through the interior of the pedicle runs the wellknown muscle with its muscle sheath (Fadenscheide BÜTSCHLI). The muscle itself is, as far as I can see, quite straight without windings; the muscle sheath and the walls of the pedicle wind with one winding round the muscle. In accordance with this, in the lower part of the pedicle the muscle lies laterally in two spots and so that if nearest the ramification it lies to the right side, a little below it lies to the left. In other words, the whole pedicle (Stielscheide BÜTSCHLI) in its lower part winds round the muscle in one single revolution. The muscle is therefore not axial in the pedicle. Where the pedicle is broadest, it will, when bent, show a system of fine lines, commonly two systems of different length, and both split up in lines which diverge almost under equally large angles. When the pedicle is straight, not bent, these lines totally disappear (Tab. XII—XIII fig. 1). Round the muscle, following the same revolution as the pedicle itself, winds the muscle sheath. Where the muscle sheath is broadest, the pedicle will when the colony is alarmed be deeply bent and in the way indicated in Tab. XII—XIII fig. 2, 3. The figure further shows that from the interior of the wall of the pedicle where the muscle sheath is broadest there runs a system of parallel fine threads which are fastened to the muscle sheath, showing jagged borders. Whereas the muscle itself in the whole upper part is almost of the same thickness, the muscle sheath has two broad extensions; if the one nearest the point of ramification lies to the left, the second lies to the right. Whereas the muscle itself presents the wellknown longitudinal striation best observable (Tab. XII—XIII fig. 3—4) when the mucle is strongly bent, the muscle sheath is of a granular structure.

From the tip of the muscle proceeds a very fine yellow band which runs through the knee and at last is divided in two strings (Tab. X fig. 5; Tab. XI fig. 6). These two strings are expanded into two discs, the contours of which run out in many fine threads (Tab. XI fig. 4—5). The disc is well figured by Ayrton (Tab. XXI fig. 7) but in the description we find no allusion to this highly remarkable structure. It is obvious that this fine band with its two strings may be interpreted as a tendon which is fastened with its two expansions just behind the peculiar cuticular structure (Tab. XI fig. 4—5) which allows the pedicle to be bent kneelike just at this spot. If high power is used, it will be seen that the muscle sheath (Tab. XI fig. 6), even in the upper part of the muscle nearest to the knee and where the muscle seems to be nude, is really present as a very thin coating to it. Further, that it really seems as if it was the muscle sheath from which the tendon proceeds. This seems more evident later on when we have seen how the muscle and muscle sheath are developed. In the knee itself the pedicle shows a highly remarkable structure. When the pedicle is straight and not bent in the knee, hardly anything can be observed (Tab. XI fig. 4—5); half bent we see in the knee a system of fine striations which runs transversally and almost reaches the other side of the pedicle; when fully bent the striations radiate in the shape of a fan from the angle between the two parts of the pedicle. It will further be observed that the pedicle here is of a darker colour and strongly longitudinally striated, these striations being perpendicular upon the first named. It will be clearly understood that this spot, which is found almost in all pedicles of *Z. geniculatum* at the same distance from the substratum, is predestined to a very sharp bending. This bending is thus partly due to this structure, and partly to the highly remarkable phenomenon that the muscle is furnished with a well defined tendon which is fastened with its expansion on the other side of the peculiar cuticular structure allowing the stiff pedicle to bend just at that point.

The pedicle of *Zoothamnium* has often been studied e. g. by Claparède & Lachmann (1858 p. 86), Wrzesniowski (1877 p. 267), Kent (1881—82 p. 693), Bütschli (1887—89 p. 1306 and 1555), Entz (1893 p. 23). The object has hitherto always been *Z. arbuscula, alternans, nutans* and *mucedo*. Between these earlier descriptions and mine of *Z. geniculatum* there are obvious differences; the last named agrees fairly well with that of Ayrton. Most of the differences between the earlier authors and the two last named are due to the fact that the species *arbuscula* and *alternans* bend the pedicle in many zigzag lines, whereas *Z. geniculatum* only bends it in three points; in the two lower of these points the walls of the pedicle are somewhat altered, and the bending is not total; in the upper point the walls are of a special structure which allows a total bending so that, when the bending is total, the basal part and the middle part touch each other. With regard to the descriptions of the authors before Bütschli I refer to his work (1889 p. 1306). I only wish to call attention to a single point. Bütschli writes "Nach diesem Erfahrungen scheint es doch möglich, dasz die Schlängelungen welche Claparède & Lachmann bei *Z. nutans* beobachteten, nicht wirklich in einer Ebene lagern sondern lose Schraubenwindungen waren . . . Kaum dürfte sich in dieser Weise jedenfalls der Ehrenberg'sche Fall erledigen lassen, wo die zickzackförmigen Biegungen des Stiels so stark sind dasz sich die einzelnen Knickungen berühren" (p. 1314). In *Z. geniculatum* the facts are as follows: During the contraction the whole crown is swept round almost a whole revolution; during the unfolding of the crown, which goes on much more slowly, it is often possible to see, especially in the last phase, how the crown is swung back again. This sweeping round of the crown is due to a spiral motion once round, and takes place in the two lower parts of the pedicle. It is in accordance with the single revolution of the muscle sheath round the muscle. Simultaneously with this spiral motion, owing to the peculiar structure of the walls of the pedicle in the two above-named spots where the muscle is fastened to the inner wall of the pedicle, the pedicle is bent in two conspicuous elbows. But at the same moment when the crown is swept round, and the two lower parts of the pedicle are bent, the last part is bent in the knee. The two lower bendings are a combination of a spiral and a zigzag bending; the more basal one only a zigzag bending. The result of it all is that the basal part of the pedicle nearest to the crown is bent so deeply downwards that it touches the basal, stiff, totally inactive part. The latter is however fastened to the leaf in such a way that it may be laid down horizontally, often so that it touches the leaf in its whole length. Therefore the colony which originally hangs down from the leaf with fully expanded crown, often about four mm. removed from the leaf, when contracted is suddenly flung down as an almost invisible greyish little body touching the leaf with its contracted crown- When the colony is getting old, the spiral motion of the upper part of the pedicle ceases, and a little later the bending of the pedicle in the knee is also discontinued. This is due to gradual destruction of the muscle. It was first attempted to study the movements of the pedicle when the colonies were fastened to the substratum; this was almost an impossibility, especially when high power was to be used. Later on the colonies were loosened and laid under cover; the movements could then easily be observed. The method has unfortunately the drawback that when the pedicle was not fastened to a substratum, the contractile cord could not attempt the strong motion. In the course of some days the muscle falls to pieces and the contractions are now slow. This is most probably due to the fact that, when the pedicle is fastened to a substratum, it is the crown which is carried downwards to the point of fixation when the colony is contracted, whereas, when the colony is loosened, it is the basal part which is now drawn upwards, and often deep into the crown itself.

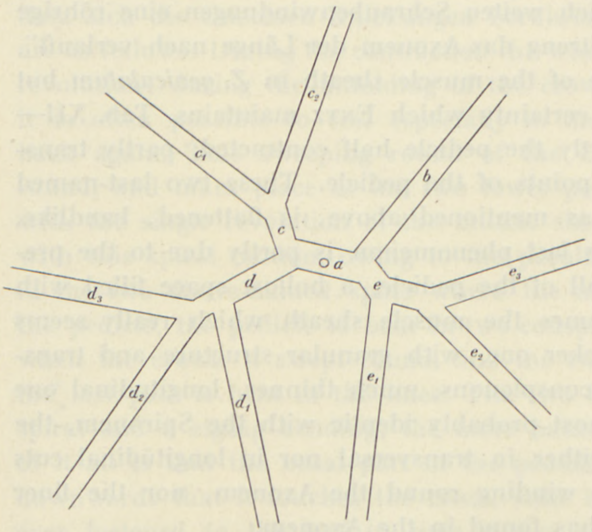
Entz (1893 p. 27) has explored the pedicle of *Z. arbuscula* very thoroughly and comes to results which differ highly from those of earlier authors. In the pedicle he finds three different threads: the Spasmonem, the Spironem and the Axonem; the Spasmonem is identic with the muscle; the Spironem and Axonem with the muscle sheath. He says correctly that the Spasmonem is bandlike "der Querschnitt entspricht einer Sichel, dessen eine Ende gedunsen, das andere zugespitzt ist" further that it "in einer homogenen Grundsubstanz aus parallel verlaufenden Längefibrillen zusammengesetzt ist". The muscle sheath is "zusammengesetzt aus dem Spironem, welches mit ziemlich weiten Schraubenwindungen eine röhrige Rolle vorstellt, in welcher der andere Streng das Axonem der Länge nach verlauft".

I have tried to find this structure of the muscle sheath in *Z. geniculatum* but have not been able to see it with the certainty which Entz maintains. Tab. XII—XIII fig. 3, Tab. XI fig. 1—2 show partly the pedicle half contracted, partly transversal cuts through the two bending points of the pedicle. These two last-named cuts show firstly, that the pedicle, as mentioned above, is flattened, bandlike, further, that it is strongly curved; this last phenomenon is partly due to the preparation. The cuts show the thick wall of the pedicle, a hollow space filled with the above-named glassy fluid; then comes the muscle sheath which really seems to consist of two strings, an outer thicker one, with granular structure and transversal furrows, and an inner, more inconspicuons, much thinner, longitudinal one striped like the muscle. The first is most probably identic with the Spironem, the second with the Axonem of Entz. Neither in transversal nor in longitudinal cuts have I been able to see the Spironem winding round the Axonem, nor the finer structure which Entz asserts that he has found in the Axonem.

Near the point where the crown branches off from the pedicle this is flattened with the muscle lying as a flattened band in the middle; at both ends of the muscle lies the muscle sheath. Tab. XI fig. 3 shows four cuts at the point where the two main branches go out. It will be seen that the muscle is narrowed in in the middle, and that the connection between them has burst. In the thinner branches it may often be observed that the muscle is composed of muscle fibrillæ. It seems as if they here only lie pressed together, and may be torn from each other either by pressure or by pulling. This has been illustrated in Tab. X fig. 6—7. On entering the zooid the muscle is spread out in a funnel-shaped body from which the longitudinal myonems proceed; it may also be described by saying that the muscle dissolves in a number of longitudinal muscles which are held together in their lower part by means of a common membrane.

The Laws of the Ramification of the Main Branches.

Much interest is connected with the questions relating to the laws of the ramification. If the colonies are loosened from their support, many of them will lay themselves upon one side; some of them however will place themselves vertically with the pedicle stretched out laterally. Tab. II fig. 2 gives a rather schematical camera drawing of such a colony. It will then be seen that the number of main branches is nine, and that they branch off at almost the same distance from each other. As all branches are almost of the same length, the whole colony gets an aspect of extreme regularity and beauty. The colony resembles an umbrella where the pedicle is the handle and the nine main branches the ribs. The apices of these ribs divide the periphery in nine points, almost all at the same distance from each other. Between the many hundreds of colonies I have seen, I have never found a greater number than nine; if a smaller one has been observed, it has almost always been possible to show that the variation was due to attacks from organisms which preyed upon the organism. If a branch has been amputated, no new branch appears, but the cut off branch has a remarkable longevity; if it carries almost ripe ciliospores, they will be fully developed and regularly loosened from their stalks; the branches have no power of regeneration. That the number of main branches is invariably nine is very peculiar; these nine branches seem to proceed from one single point and with angles of almost the same distance. A more thorough exploration will however show that the nine branches do not proceed from a single point, but from a line. The figure above is drawn with a camera and as I have sketched twenty five colonies with the camera and always found only very small differences, the figure may be regarded as a paradigm of the ramification of the colony. The pedicle carries on its top a horizontal middle piece *a*, from the corner of which four branches proceed. One of these branches, *b*, does not divide any more; one, *c*, divides in two branches c_1 c_2; the remaining two, *d* and *e*, in three; of these three branches d_1 and e_1 are branched off before the other branches e_2 e_3 and d_2 d_3, in such a way that they do not go out from a single point. It is only by drawing the corners of the middle piece out to a different extent, by branching off the branches d_1 d_2 and e_1 a little sooner or later from the joining points of d_2 d_3 and e_2 e_3, and by altering the angles a little between the branches, that the great regularity in the ramification of the colony is reached.



Still it may be added that it is the relations to space and light which in the last instance governs the mode in which these nine branches are branched off from each other. We will return to this point when the development of the colony is discussed.

It seems as if the number of nine main branches is a specific species character for the species.

How the ramification is in the other species of the genus, we do not know. These nine main branches carry the branches of the second order, and these again those of the third.

Life History of the Colony.

Sooner or later the ciliospore will loosen itself from the stalk, and the same moment it darts away with considerable speed through the water. It swims remarkably well, rotating during swimming, and is able to live in this freeswimming stage for about 36 hours. If the body is not fastened by that time, it has invariably died off in my vessels, resting upon the bottom where it soon flows out and disappears. In the swimming stage the body invariably alters its form, it may be ballshaped and then quite resemble the body in the fixed stage, but it may also, and this is the most common case, be coneshaped, either as a very flattened or as a very high cone. In Tab. II fig. 4 it has been drawn in the very flattened stage. In this stage the myonems are not so conspicuous as in the ballshaped, not detached stage. When swimming the contractile vacuole most probably contracts once a minute, perhaps a little faster, at all events faster than when the ciliospore is connected with the mother colony, and in the later fixed stage.

If colonies have been laid in a small vessel in the morning hours, they will almost invariably in the course of the day give off numerous freeswimming stages which swim along the edges of the vessel with great speed. In diffuse daylight they are commonly gathered on the side of the vessel turned away from the window, during the night mostly on the opposite side. Next morning many new ciliospores will be thrown off, but it will also be seen that a good deal are now fastened to the bottom and sides of the vessel.

When the time arrives when the ciliospore is to fasten itself, we see it for some time rotating in the same spot. The base is turned downwards, and we look down upon the apex with the closed peristome. In the centre is the vacuole, and more peripherically, the nucleus; the posterior ciliary wreath is in strong motion; suddenly it is stretched out, the motion ceases, and the animal now stands still.

I have now followed one and the same individual from 9 o'clock in the morning till 8.50 in the evening (Tab. III fig. 1—13). In the course of the day 13 stages have been drawn with the camera. After 15 minutes the cilia are thrown off. In this position it is commonly not possible to see what further happens; but in those individuals which have fastened themselves upon the sloping sides of the vessel it is possible to see the process which is going on during these fifteen minutes. In the course of two minutes the ball has risen about 2 μ. It now seems as if the ball stands floating above the substratum; a little later we are able to observe a very broad gelatinous stalk in which two layers, an outer and an inner one, may be seen (Tab. III fig. 2—3). Simultaneously, the ball diminishes a little in size; from c. 250 μ in diameter it has gone down to about 235 μ. The ciliary wreath has disappeared, but the ring from where the cilia have been developed is still visible. At 10.30 the stage is almost unaltered, the stalk has, however, become much narrower, being about half the breadth of the former stage; it is now well defined from the body and rests upon a little disc with a thicker central plate surrounded by a more outflowing jelly mass with irregular contours. It is impossible to observe directly from where the jelly comes; most probably it is formed as a secretion from the base of the ciliospore. The above-named ring has now disappeared. At 11.05 the pedicle has reached a length of 230 μ. Simultaneously it has become still narrower and the dark mass in the ciliospore mentioned in pag. 19, and which has been drawn in the two preceding figures, has now altered its form showing in its central part a little bud. At 12.50 the stalk has reached a length of 340 μ; the breadth is almost the same, and it is still of almost the same breadth at apex and base; the little bud is a little more conspicuous. At 2.05 we find that the pedicle (fig. 6) has reached 460 μ. The pedicle now shows the great peculiarity that a lower, broader, part is conspicuously set off from an upper, much narrower, and longer part. If now we throw a glance at fig. 6—11, we see that in the course of the day this upper piece gets narrower, but in all figures is almost of the same length. This piece is unaltered during the whole life, its walls get thicker and thicker; they are often of a yellow tinge; it looks as if this part may soon be regarded as a dead hollow tube with relatively stiffened walls. Simultaneously the large, broad, gelatinous plate on the substratum has disappeared, giving way to a small, slightly expanded disc which is unaltered during the following development, and to which the colony is fastened for the rest of its life. The bud from the middle part of the dark mass in the ciliospore has augmented in size and simultaneously the dark mass has got smaller. At 2.30 (fig. 7), only twenty-five minutes later, the pedicle has augmented very considerably, the whole augmentation falling only upon the lower part. From 460 μ the whole pedicle has grown up to 805 μ. It is further seen that the bud from the dark mass now presents itself as a long dark rod which is slowly pushed downwards in the pedicle. It will already now be understood that this rodlike body is really the muscle or contractile cord. As it is further evident that the longer the rod gets, the more does the dark mass in the posterior part of the round body disappear, it is rather probable that this dark mass is a material originating from the mother colony, stored up in the ciliospore before detachment, and destined for the perfection of the contractile cord. To this point we will return later on. It will further be seen that the round body through all figures to fig. 12 is of almost the same size. The figures seem to show that the differences in size are relatively large. It must, however, be kept in mind that the body is able to contract and to change its form to rather a great extent; most probably the dimensions of the ball are to some extent also dependent upon the amount of water which the pulsatile vacuole possesses at the moment when the ball is drawn. Also the form of the various fixed larvæ differs considerably; it is by no means always globular; often it is rather oblong, and this form may be present during the whole development, but it may also happen that in the middle of the development it will be altered from oblong to isodiametric or vice versa.

At 3.30 (fig. 8), an hour later, the pedicle has reached a length of 880 μ; the upper part is now very conspicuously cut off from the lower part; two or three rings between the two parts are now often visible. Hitherto the whole pedicle has been quite stiff. If the head were moved by a needle, no movements of the pedicle would have been observed. From 4.30, an hour later, this is altered. The pedicle is now 920 μ. The contractile cord has a length of 340 μ. The dark mass in the posterior part of the head, still at 2.30 visible as a shadow, has now disappeared. If now we touch the head, the pedicle immediately bends in a zigzag and always where the three transversal lines are indicated in the figure. Simultaneously a slight bending is also visible a little below the head. — At 5.55 the pedicle has reached 1.10 mm. and the contractile cord more than half of this length. At 7 o'cl. the pedicle has reached 1.3 mm. and the contractile cord 800 μ. At 8.15 (fig. 12) the pedicle is 1.7 mm. long and the contractile cord 1.1 mm. In this stage it will further be seen that, whereas the pedicle has hitherto been almost straight and the sides parallel, now the pedicle begins to be curved and is not of the same breadth. Simultaneously the muscle sheath gets more and more conspicuous, but to the formation of that we will return later on. At 8.50 the stalk has reached a little over 2 mm, but the augmentation is greatest for the piece lying between the end of the contractile cord and the part nearest the crown. Curiously enough, the contractile cord has only augmented slightly. At 9.40 the division of the head begins, the stalk now being 2.5 mm. We further see that the pedicle shows a conspicuous narrowing, and that from this point also the contractile cord diminishes in size.

Just after the attachment the contractile vacuole functions very slowly, contracting only every fifth minute, the size of the vacuole during greatest extension being c. 80 μ. In the first minute it reaches the size of c. 50 μ, in the second c. 67 (observed without cover). During the formation of the stalk it functions faster and faster, at last about once a minute, the diameter being only about 50—60 μ.

The macronucleus is almost unaltered in size during the whole process till the fission takes place; the form is the same, and in the position there is but slight difference. In the last stages the fine striation of the pellicula is more and more inconspicuous. In the first stages peculiar sudden contractions of the whole head are often observed, later on they diminish in number.

At the moment when the first sign of the first fission was observed, the observations were broken off. Before describing the fissions there are some points with regard to the pedicle which must still be considered.

If we compare the pedicle immediately before the fission with that of an old fullgrown colony (Tab. III fig. 13, Tab. XII—XIII fig. 1), we shall find that it has here reached a length of about 3 mm. It will however be seen that the whole pedicle, from the head to the knee, has only been augmented with about 350 μ, the last part with about 700 μ or about two times its own length. At page 26 we have regarded this very part as a dead hollow tube, a supposition which at the first glance seems to be without any connection with the fact that in the old colonies it has grown more than two times its own length. — This apparent contradiction is due to the fact that the whole series in Plate III fig. 1—13, has been studied in an individual which was not fastened, but was lying horizontally under a cover. By comparing the specimen just mentioned, as well as many others which were either freeswimming in the vessel or fastened to the bottom and grew upwards, with specimens which were growing downwards from above, it could be shown that the last, dead piece of the pedicle suspended its growth much sooner in the first cases than in the last. It seems that, not only adhesion to a substratum, but also growth from above downwards, is a necessity, at all events an advantage, for the full development of the last piece of the pedicle.

The whole hitherto described development, from the moment when the freeswimming ciliospore has thrown off its cilia and fastened itself, and till the first fissions take place, is completed in the course of only about twelve hours. More than forty times during the summer 1923 and 1924 fresh material has been brought into the laboratory; in the course of 12—14 hours my vessels have always had colonies with two to four animalcules. The temperature in the room has been 14—20° C. The development went on by day as well as by night. During this very period the larva, without taking in solid food, has produced a stalk which is more than eight times its own length, the larva having a diameter of about 250 μ, the pedicle of more than 2 mm.

Moreover, we know from the above that the pedicle is a highly specialised part of the whole organism. It is composed of an outer wall, a glassy fluid, a muscle sheath, which may perhaps be divided in two strings: the Axonem and the Spironem, and finally the muscle itself with its tendon; the wall of the pedicle is of an extremely complicated structure in two spots, especially in the knee. All these different parts originate from one single cell which, in spite of its enormous efforts, seems to be almost unaltered with regard to size and form. In my opinion it seems quite unintelligible how this is possible, and moreover that this very cell, when this production has been finished, should still be able to begin the fissions from which the new colony is to develop.

It will be understood that in this connection it is of the greatest interest to be sure that there has not really been any considerable diminution in size and form of the cell and of the macronucleus. Three newly fixed larvæ were therefore followed regularly again and drawn every second hour; this time however with higher power. The result for one of these larvæ has been given in plate X fig. 8—15. The same individual has been drawn immediately after fixation at 9 o'cl. in the morning when the pedicle was only 146 μ, and at 9 o'cl. in the evening when the first fission was just about to begin and the pedicle was a little over 2 mm; between morning and evening the individual has further been drawn at 10, at 11, at 12, at 1.40, at 3, at 5, and at 7 o'cl. The length of the head only varied from 256 to 280 μ, the breadth only from 220 to 230. At 5 o'cl. it is almost isodiametric; but this is accidental; at 7 ocl. it has again its normal aspect. The whole development has been carried out under the microscope; no cover has been used. The contours of the macronucleus have been rather conspicuous, they have been drawn with the camera; as they are of course not quite exact, they have been given with dotted lines.

Still the contours have been sufficiently clear to show that, during the whole development of the pedicle, no important diminution in the size of the macronucleus has taken place; there are some variations with regard to form and position; but an important diminution in size does not take place before the first fission. As it is always said that it is the vegetative process which belongs to the domain of the macronucleus, I confess that the result rather troubled me and that it was really the opposite of what I had expected. Neither at the moment when the contractile cord begins to glide downwards in the pedicle, nor when the muscle sheath is produced and the pedicle begins to be bent in the knee, do we find the slightest variation in form or size of the cell. The question is therefore: From what part of the cell comes the material to build up the different parts of the pedicle?

As mentioned above, the ciliospore is built up by an ectoplasma with its pellicula enclosing an entoplasma containing the myonem system, the vacuole, and the nucleus. At the bottom of the cell lies the cortical plasma. If an alveolar layer is present, is questionable. The question therefore is whether there is any real connection between the different plasma structures of the ciliospore and the pedicle. With regard to this very difficult point of the exploration I can only remark as follows:

During the development of the ciliospore on the mother colony we saw a dark mass appear in the lower part of the ciliospore, a material which has in some way been accumulated in the cortical plasma in the larva, and which disappeared during the formation of the stalk. This dark mass has already been observed by Engelmann, who describes it as strong light refracting granules. He supposed that he had here to do with the material for muscle and muscle sheath, especially because he "von einer Fortsetzung oder einen almähligen Ubergang des neugebildeten Stielfadens in die Leibeshöhle durchaus nicht zu erkennen vermochte, namentlich die konische Ausbreitung der Fibrillen nicht beobachtete". Bütschli (1889 p. 1312) denies the whole supposition, presuming that these granules are "Excretkörnchen" and that it is the very presence of these granules that is a hindrance to the understanding of the real fact. The funnel-shaped expansion of the fibrillæ which Engelmann has not seen was observed by Entz.

In Tab. VI fig. 12 is shown a longitudinal cut of the ciliospore; it will here be seen that the cortical plasma in the middle carries a little projection which is unquestionably the beginning of the muscle cord, and which so to speak flows out and downwards in the central part of the pedicle. I am therefore inclined to suppose that Engelmann was really right, and that the granulated mass behind in the ciliospore is really in some way the store material for the formation of the muscular parts of the stalk; that they should be produced only from the myonemsystem of the ciliospore, I regard as highly improbable. Already during the development the muscle shows a longitudinal fibrilla structure.

If we study the muscle with high power at the hour 3.30 (fig. 8), about five hours after fixation of the larva, (Tab. X fig. 5) we shall see that along the muscle there is now appearing a hyaline fluid which, when it has reached the apex of the muscle, continues in a very thin hyaline cord a little broader at the free end. This hyaline coating of the muscle is the beginning of the muscle sheath; it comes from the interior of the cell, but more I cannot say. The thin cord in a very short time reaches the point of the pedicle where the knee is to be produced. It is then divided in two strings, and each of these strings is expanded and fastened to the inner walls of the pedicle. In this manner the muscle is fastened to this point, and it is owing to this fixation that bending of the pedicle can take place. Till 8 o'clock, about 10 hours after the fixation of the larva, the muscle sheath is about the same breadth everywhere. The sheath is first hyaline, later on it gets a granular structure and, when bent, shows a transversal striation. Then it begins to be broader at two points; simultaneously the walls of the pedicle are no longer straight. The sheath is now twined in one revolution round the muscle, and it seems as if the muscle in the two following hours is first pressed to the inner wall of the pedicle at these two points, and later on fastened to it; its position is no longer axial. With regard to the formation of the wall of the pedicle (Stielscheide Bütschli) I cannot see better than that it is originally formed as an exudation from the posterior part of the larva which is agglutinated to the substratum by means of this exudation.

On the basis of these insufficient observations it is most reasonable, as already Bütschli has done, to regard the whole pedicle as an exudation and not, as some authors have supposed, as a continuation of the pellicula. It originates from the hind part of the ciliospore; in the cortical plasma has been accumulated material which is used for its development.

As mentioned above, during the whole time which has been spent in the formation of the pedicle, the peristome has been closed; it has further been closed for 2½ to 3 days during which the ciliospore has been fastened to the mother colony, and also during the freeswimming stage of the larva, all in all about four to five days. It must however be kept in mind that, even if the organism has had no solid food during this period, the contractile vacuole has worked incessantly. In this connection it is of smaller interest whence the water comes; the main point is that during this very period the organism has been run through by an unintermitting stream of water. When I began the exploration, I had supposed that the vacuole would work faster during the development of the pedicle than before and after, and faster in the ciliospore than in the nutritive zooids. As mentioned above, this is not the case; on the contrary it works about five times slower and further, in the peculiar way that it reaches its greatest extension in the first part of the diastyle, and already a diameter of 50—60 μ in the first minute, whereupon it stands with the water for two to three minutes without getting much larger during this time. This peculiar behaviour seems to indicate that the vacuole has really other important tasks than commonly in the Infusoria.

The problem was now to determine whether the water taken in by the animal had any significance for the formation of the pedicle. As it may be regarded as a matter of fact that the freeswimming larva, and the larva after attachment and till the first nutritive zooids are produced, gets no food in solid form, it should be permissible to suppose that, if the water had no significance at all, the whole development of the pedicle could take place in distilled water. — With this supposition in view twenty larvæ were isolated, ten of them were put into common pond water, ten others in distilled water. Time 7 o'cl. evening. The next morning the ten first-named larvæ were all fastened and had produced pedicells of different length; the last named were all swimming round; they were still living in the freeswimming stage after 24 hours, all in all 36 hours, but after that they lay lifeless at the bottom and died in the course of a few hours; not one of them had fastened; the experiment was repeated; the result was always the same; no larva fastened in distilled water, but the length of time during which they lived here was very different; some died in the course of only a few hours. Then 10 new larvæ were taken at 7 o'cl. in the morning and put in a vessel with pond water. At 7 o'cl. in the evening five of them had formed a pedicle of almost full length; the water was poured out and distilled water poured in; in the course of two hours all the pedicles had thrown off their heads, and these were lying dead at the bottom of the vessel. At 7 o'cl. in the morning 10 new larvæ were put into pond water and often observed; at 9.30 four of them were fastened and produced pedicles, the length of which was only once or twice the diameter of the head; not one of them could bend the pedicle, and the muscle itself was only detectable as a very small rod. In the course of the day the pedicle was to grow to the normal length. If now again the pond water was replaced by distilled water, it could be shown that the further development of the pedicle immediately stopped. The larvæ were under observation every 15 minutes; they died about 7 o'clock but without any augmentation of the pedicle and its muscle.

As mentioned above, the contractile vacuole on the animals living in pond water in the first period during the development of the pedicle pulsates about every fourth to every fifth minute. When the animals got distilled water, this caused an enormous acceleration; it now pulsated about every minute (more correctly every 60—70 seconds). When the animals had been for some hours in the fluid, the form of the vacuole got irregular; very many small vacuoles appeared round the large vacuole; the systole was not full, and now and then there appeared two vacuoles, almost equally large, and pulsating alternately. At last the whole vacuolar system was disorganised; the contents of the animal were opaque; for a short time the nucleus was very conspicuous, and then the whole ciliospore flowed out.

From these observations we are presumably most entitled to maintain that pond water is a conditio sine qua non for the attachment of the larva and for the development of the pedicle.

I for my part cannot suggest any other solution of the problems which have for a rather long time occupied me than the following: During the development of the ciliospore, material from the mother colony has in some way been conducted into its interior; it has here been accumulated in a very condensed form. By means of water this material is again made more fluid and is now used for the development of the pedicle; how this development takes place, I have tried to show. Scientists with a more extensive chemico-physiological training could unquestionably have brought the exploration a step nearer to its solution.

The fact that the development of the pedicle is stopped when it is to be accomplished in distilled water, seems to indicate that the water is really taken in directly through the vacuole, and not endosmotically through the cell walls, the water taken in in this way being more diluted than that which flows directly into the vacuole.

We will now return to the fixed larva which we left at the moment when the head was broadest and the first fission was about to begin.

Six freeswimming larvæ were caught on the ^{28}/VIII and at 7 o'clock in the evening brought into six vessels. Twelve to sixten hours later all the larvæ were fixed and fissions had begun. One of the larvæ which showed the first sign of fission was loosened and laid under the microscope. It was studied from 7 in the morning till 7 in the evening. It was drawn at seven different times of the day; the drawings are reproduced in Tab. IV fig. 1—10.

At 7.30 we see the first fission begin. The breadth of the head is 280 μ, the length 160. The nucleus is bandlike and lies in the middle of the cell. At 8 o'cl. the fission is completed, and the two animalcules are sitting each upon their stalk. The two animalcules are not quite of the same size, but the difference is only very small; the diameter being about 160 μ. The vacuole in the two zooids pulsates every 50 seconds. In full diastyle the size is only 33 μ. They do not pulsate simultaneously; in the fully contracted peristome the cilia beat very irregularly and independently of the pulsation of the vacuole.

Already two hours later, at 10 o'cl., we find 4 animalcules, but it is now evident that the result of the fissions has been that two of the animalcules are much larger than the others, in other words, that the mother animal is cleft in one larger and one smaller animal. Especially the third fission is commonly very unequal, one of the animals being much smaller than the other. The smallest one is at a very early stage bent downwards, hanging down like a bell from the attachment of the branch to the pedicle; already in colonies with only four heads the peristome of this little zooid is opened, and in this way nutriment is conveyed to the young colony; this little zooid, when divided, gives origin to a cluster of very small zooids later on hanging down below the main branches of the colony.

As far as I have been able to see, inequal fissions are common in the first days of the life of the colony; later on this is not the case; they are most common, at all events most conspicuous, in young and small colonies; in older colonies the result of the fissions, especially for all those animalcules belonging to the branches of the third order, is that these animalcules are almost of the same size, at all events the difference is but little pronounced; inequal fissions only occur in those animalcules which are sitting in the apices of the main branches. At 10.40 the colony possesses 6 heads, at 11.20 7 heads, at 3.30 12 heads, and at 6.40 16 heads or zooids. It will further be seen that at 10.40 the peristome is open in one of the other zooids and that at 6.40 all individuals have opened the peristomes. The opening of the peristomes manifests itself in the enormous speed with which the fission takes place. Already the next day on the $^{30}/_{\text{VIII}}$, the number of heads is 40—50, on the $^{1}/_{\text{IX}}$, it is 60—70; on the $^{2}/_{\text{IX}}$ about one hundred, and on the $^{3}/_{\text{IX}}$ about 200. On studying the figures Tab. IV and V it will be seen that the size of the zooids invariably diminishes until the colony of about 200 zooids is reached; then the size of the head does not diminish considerably; it is then about 45—50 μ; in old and very large colonies with many thousand zooids it goes down to 30—40 μ. It will then also be observed that the great differences in size of these animalcules which are the results of fissions in the apices of the main branches disappear more and more. On page 24 I have tried to give a sketch of the ramification of a full-grown old colony; in the following it will be shown that this admirable regularity, which prevails in the ramification of the colony during its development, is the result of quite special laws which rule the mode of ramification. It is rather difficult to clear up these matters in colonies which are seen laterally; it is necessary to observe the colonies from above. Tab. IV fig. 11—14 and Tab. V fig. 1—4 are all drawn with the camera from living animals and illustrate the following. Fig. 11 shows the first fission from above. In Fig. 12 it will be seen, as indicated in pag. 24, that the branches proceed not from a single point but from a rectangular piece originating from the first bifurcation of the pedicle. Already in this very young colony, only consisting of seven animalcules, we see the pedicle implanted in the middle of a rectangular piece deriving from the pedicles of the two first zooids; near the point of attachment are seen two small animalcules originating from the above-named small zooid, appearing already in the third fission. In each of the four corners of the piece there is a zooid; in one of them fission has taken place. In fig. 13 the piece has been more stretched; we shall be able to find in it the same piece as in the textfigure on pag. 24, and the same branches proceeding from the four corners. Apart from the two animalcules near the attaching point of the pedicle we now find eight animalcules; on comparison with the schema on pag. 24 it will be easily understood that these eight animalcules represent eight of the nine branches of the first order; this is much more conspicuous in fig. 14, where we see c_1 c_2 branched off upon c; d_1 branching off upon d, and d_2 d_3 a little above d_1. Tab. V fig. 1—4 show a further development of what has been pictured in Tab. IV.

As soon as the nine main branches are sharply set off, a stage which answers to Tab. IV fig. 10 or Tab. V fig. 3, no more branches of the first order are formed; the nine branches adjust themselves at almost equal distances from each other, and it is easy to see that the colony is already now umbellate. On the sides of these main branches new zooids have been set off, developed from fissions of the large zooids on the apex of the branches; from them are developed branches of the second order; these zooids divide again, and the results are clusters of two to four individuals situated upon small dwarf branches, the branches of the third order.

If now, in these young colonies, we study the large animalcules at the apex of the branches a little more closely (Tab. V fig. 5—6), we shall see that they differ from the other animals not only with regard to size but also with regard to form; they are much thicker, and especially much darker; they are further characterized by an enormous nucleus very conspicuous and spiral shaped. In the other small zooids the nucleus is very inconspicuous, only faintly detectable without staining. On looking over a colony of about 40—70 animalcules it will be seen that such a colony is provided with about 10—20 such large individuals all seated in the apices of the branches and always most pronounced in the apices of the branches of the first order. On laying such a living colony under the microscope and pressing it a little under cover one gets the impression that the whole nuclear material which the colony possesses is only distributed over these c. 20 dark individuals, the nuclei in the bright small individuals not being detectable in living animals. It is as if the colony possessed a fairly fixed number of nucleus carriers. As mentioned above, as soon as the colony has reached a number of about 150—200 individuals, the size of these large individuals diminishes; the largest individuals are also in old large colonies always seated in the apices of the branches, and the result of a fission is also here that the individuals are not quite of the same size, but the great difference in size which characterizes the fissions of the young colonies in the apices of the main branches, we do not find later on. — All fissions are now almost equal.

The microgonidia. When the colony has developed about 150—200 animalcules, a quite new phenomenon is observed.

It will often be seen that, upon the third or fourth day after the freeswimming stage has attached itself, the vessels in which these young colonies are growing up teem with numerous bright corpuscles, all extremely small and all only detectable when the vessels are studied with rather high power. The corpuscles swim with incredible force, and it will soon be observed that they mostly gather round the colonies. On closer study it will further be observed that these microorganisms crawl round upon the colonies glide, over the animalcules. and often attach themselves to the sides of them. Moreover it is observed that all these microorganisms are produced by the colonies themselves. They are the microgonidia of the colonies, and are produced upon special short branches, as far as I can see, always near their apices. Tab. XIV fig. 1 shows part of a system of branches carrying six normal individuals, clusters of microgonidia in different development. In this species always 4 are produced upon the same branch; an animalcule which does not seem to differ from the others is first divided in two smaller individuals, and most probably each of them divides again in two others without getting larger; the result is 4 small individuals which always arrange themselves in such a way that the apices with the contractile vacuole are turned inwards. The second cilia ring is developed, and with the cilia of this ring in constant motion, and with the peristome half opened, they may sit for many hours in the same position always with the apices turned inward. The vacuole pulsates three to four times a minute. Now and then one or two detach themselves and dart away, but often come back to the colony and, when here, find another microgonidium arranging itself in the same manner as before.

The stage of microgonidia formation only lasts about twenty-four hours in the history of the colony; it commonly sets in when the colony has about 150—200 zooids; in old colonies I have never observed it. Very often I have seen a microgonidium attached to the sides of nutritive zooids. The microgonidium has been remarkably flattened and the wall of the other animal has been vaulted. It is as if the whole microgonidium sinks down into the other zooid, at most a little projection is visible (Tab. VII fig. 3). Quite the same observation has been made by Stein with regard to *Epistylis crassicollis*, *E. plicatilis* and *Zoothamnium arbusculum*. In these species we do not find the microgonidia after copulation hanging down as an empty sack on the sides of the macrogonidium, as mentioned or figured in the papers by Greef (1870 Tab. V fig. 6) and Wallengreen (1899 p. 157).

It seems as if the microgonidia are formed during the night; they always occurred in the vessels in the early morning hours. A vessel with many young colonies without microgonidia was placed under the microscope, and a little box was placed over the vessel in such a way that all light was shut out. I wished to see if the colonies should not just in this stage be in a labile period in which I should be able to force them to build microgonidia. After six hours the box was removed, and it could then be observed that the colonies were quite contracted. It took some minutes before they were again expanded, but no trace of microgonidia formation could be observed.

A more thorough study of the nuclei during and after the copulation has not been carried on. The microgonidia seem to conjugate with the other individuals quite independently of where they are sitting; at all events they try to do so. Engelmann (1876 p. 625) maintains "dass die Kleinsprösslinge, die durchschnittlich nur kurze Zeit (etwa $^{1}/_{4}$—$^{1}/_{2}$ h.) zu schwärmen schienen, sich nur auf solchen grösseren Individuen fixirten, unterhalb welcher — auf tieferen Zweigen derselben Äste — sich bereits Mikrogonidien gebildet hatten oder noch bildeten. Sie verschmähten, so schien es, alle Individuen, sowohl der nämlichen, als anderer Stöcke, welche nicht bereits zu Rosetten sich entwickelnde Theilsprösslinge geliefert hatten". In *Z. geniculatum* I have seen the microgonidia creeping over and attached for conjugation upon all the common nutritive zooids, and I have seen total fusion even in colonies which possessed only two to eight individuals. After the conjugation the zooid seems quite unaltered; that the conjugation should produce giant growth of the cell so that the result should be formation of a ciliospore (macrogonidium) I have never had any proof of. Also in other *Vorticellidæ* giant growth is never the result of a conjugation.

With regard to the influence of the conjugation upon the nuclei I only wish to call attention to a single observation. Commonly the nucleus lies as a long curved band either laterally or in the middle of the cell (Tab. VII fig. 1—2); now and then, especially in young colonies which only possess a few (three to six) individuals, we find a nucleus of a very peculiar structure; it is here almost altered to an often extremely irregular clue and in that case filling up a great deal of the whole cell (Tab. VII fig. 4); colonies of three to four individuals in which all the zooids have carried nuclei of this kind have often been observed. For a long time it was quite incomprehensible to me why the nuclei in some cases, and often only in some vessels, had this structure, in others not. In some of my vessels I had simultaneously colonies which threw great quantities of microgonidia, and numerous larvæ attached with long pedicles; a few of them had just begun the first fission. Placing them under the microscope, I found numerous microgonidia running over the two zooids, and some of them in different stages of fusion. Of these stages slides have been made, and one of them drawn (Tab. VII fig. 3). As well known, different authors have observed conjugation with more than one microgonidium, and Bütschli (1889 p. 1609) has collected these cases. The further fate of these remarkable phenomena is quite unknown. As I found the peculiar nuclei just in those individuals which were derived from the vessels with enormous quantities of microgonidia, and upon those which carried a number of microgonidia, I have been inclined to suppose that these two phenomena are in some way connected with each other; further and more thorough explorations may elucidate this.

When a colony has developed microgonidia in the morning hours, it will begin the development of the ciliospores in the evening of the same day.

I have now tried to solve the questions: how long a time the ciliospores use for their development, how many a colony is able to produce, and lastly, the lifetime of the colony.

The method employed to solve these questions was the following. Pieces of Nymphæa-leaves were brought into high aquaria vessels containing about $^{3}/_{4}$ liters. The vessels were made of cut-glass. The water was invariably the original pond water in which the colonies lived; it was always filtered through Müllergauze No. 20. The vessels stood in the shade; temp. 15—18° C. If leaves with colonies were brought into the aquaria, many larvæ were thrown off or fastened the next day; most of them were sitting upon the leaves, but some of them were fastened to the side ot the vessel. When about 5 to 6 were fastened in this way, the leaves were thrown away and new water given. Later on the water was not renewed. The spots where the larvæ were fastened to the vessel were marked with indian ink on the outer side of the glass. Then the vessel was placed in a permanent place and not later removed; behind the vessel was placed a strong electric lamp, before it the aquarium microscope of Zeisz. It was now possible by means of the system Objective 55 and orthoscopic oculars to follow the development of the colony quite plainly from hour to hour without disturbing the animals. Every colony got its own paper in the protocol, and every morning and evening at 7 o'cl. rough freehand drawings were sketched. The renewal of the water is always dangerous; on the other hand the sides of the vessels are, in the course of about a forthnight, always covered with coatings of algæ which cause much trouble.

The method employed has the fault that with the feeble powers it is impossible to observe the ciliospores at their first beginning; it is therefore necessary to add about eight to ten hours to the time during which the ciliospores have been observed. On the other hand the method has the great advantage that owing to the horizontal position of the microscope the whole process can be followed without injury of any kind to the animal.

In Tab. IX fig. 1—20 I have tried to sketch the life history of two colonies. They were in the same vessel; the distance between them was only 3 cm. They were both attached by $^4/_9$ in the morning; the first fission began during the night between the $^4/_9$ and the $^5/_9$. By the $^8/_9$ they both counted about 200 zooids. A microsporidium stage was overlooked. By the $^9/_9$ in the morning both had developed ciliospores which in both colonies appeared in the angles between e_2 e_3 and d_2 d_3. In one colony, which is called *A* (fig. 1—12) these two ciliospores were ripe with a fully developed ciliary wreath by $^{14}/_9$ in the morning; they were loosened in the course of the day. In colony *B* (fig. 13—20) they were not fully developed by the $^{11}/_9$, and thrown off $^{12}/_9$. In the *A* colony the development had taken five days, in the *B* colony only three days. Already by the $^{12}/_9$ in the evening the *A* colony had new ciliospores developed upon several branches of the first order, by the $^{13}/_9$ almost on them all.

On the *B* colony they occur at $^{11}/_9$ in the morning. Most of these ciliospores in the *B* colony are thrown off in the course of the night between the $^{13}/_9$ in the evening and the $^{14}/_9$ in the morning; simultaneously a new, third, set appears. In the *A* colony the second set is not thrown off before the $^{17}/_9$, but of them all only a few are ripe (four); the rest have perished. By the $^{17}/_9$ the *A* colony begins to lose the zooids, and this is also the case with the *B* colony on the $^{16}/_{9}$ in the morning. By the $^{18}/_{9}$ there is only an insignificant remnant of *A*, and by the $^{17}/_{9}$ morning the same is the case with *B*.

The two colonies are developed under quite the same outer conditions; nevertheless the one lives two days longer than the other (relatively 12 and 14 days); the one colony produces 11 ripe freeswimming ciliospores, the other only four. They are both destroyed by Rotifera and Infusoria, and both marked by the life under abnormal conditions. I suppose that life in nature is about one week longer, and that the colonies are here able to develop two or three sets of ciliospores more than is the case in captivity. For colonies taken in in the days $^{7}/_{9}$—$^{14}/_{9}$ showed two to three almost ripe ciliospores upon most of the branches, and between them three to five in different stages of development. In Nature too the colonies succumb under the attack of Infusoria and Rotifera.

More than fifty colonies have been studied in the manner mentioned above; the exploration was carried on in September. The first ciliospores almost always arrived on the fifth day after attachment; these first ciliospores always appear in the angles between two of the nine branches of the first order, and almost always between the angles of $e_2\ e_3$ and $d_2\ d_3$; they are ripe in the course of two to four days; the next set always appear along the branches of the first order and where those of the second order branch off.

Their lifetime is also about 2—4 days.

The colonies produce ciliospores to their last days, but in September they have never produced more than about 20 in my aquaria.

The lifetime in my aquaria in September is about 14—16 days. In July and August at a tp. of 20—25° C. the colonies have, also in the aquaria, produced 40—50 ciliospores; they are fully developed in the course of only two days, but the lifetime of the colony is only about 10 to 12 days.

In the period when the ciliospores appear along the branches of the first order, the colony is in its prime. Each of the branches may carry a long series of ciliospores in July—August, and the weight is so great that the colony, if fastened horizontally, is now pendent.

In the last days of their life the nutritive zooids are devoured or sucked out by Rotifera etc. There remain only the branches carrying the ciliospores in very different stages of development. These unripe ciliospores seem not to be attacked and are unable to loosen themselves. The result is therefore colonies carrying upon the top of the pedicle the nine main branches without any other animalcules than the ciliospores in different developmental stages. At last the whole is a shapeless floccous body which dissolves and disappears.

The great question is now: Are these ciliospores the result of a conjugation with a microgonidium, or may they arise quite independently of this process. It must be remembered that in a colony with about two to three hundred individuals, even if a conjugation is observed, it is a matter of extreme difficulty to follow this very same individual and show that after a conjugation just this owing to excessive growth should be transformed into a ciliospore.

The microgonidia in the *Vorticellidæ* having been discovered according to common usage all the other individuals of the colonies have commonly been designated macrogonidia. In the other colony-forming *Vorticellidæ*, *Carchesium*, *Epistylis* and *Opercularia*, the macrogonidia cannot be distinguished from the common individuals; this is only the case with a few *Zoothamnium* species where the macrogonidia owing to excessive growth are distinguished from the other nutritive zooids. Without any more thorough exploration it has been regarded as a fairly established fact that the macrogonidia of *Zoothamnium* were really a result of a conjugation, and that a conjugation invariably resulted in the formation of the large macrogonidia. The difference between these *Zoothamnium*-species and all other colony forming *Vorticellidæ* should then be, that conjugation should only in the first named case produce excessive growth, in all the others not.

I for my part regard this supposition as rather hazardous and am of opinion that the macrogonidia are formed independently of any conjugation between a microgonidium and the individual which begins excessive growth. For that very reason I have, in contrast to all previous authors, not used the name macrogonidium, but proposed the name ciliospore, which only says that we have here to do with a means of spreading which is furnished with a wreath of cilia.

I base my supposition upon the following facts and considerations.

1. I have seen the microgonidia appear epidemically in numerous colonies but always only in young colonies with two to three hundred zooids, never in old ones; the microgonidia only occur in my vessels with young colonies, never in those with old ones.

Nevertheless the old colonies produce numerous ciliospores six to eight days after the microgonidium stage of the colony has been passed.

2. I have often seen young colonies begin the formation of ciliospores in spite of the fact that I have never been able to see the swarms of microgonidia round the colony; of course it is possible that the stage has been overlooked. At all events we are entitled to suppose that the microgonidium stage is time-fixed in the life history of the colony, beginning commonly on the fourth day after attachment; further that this stage is perhaps not passed through by all colonies.

3. The formation of the ciliospores is however not only time-fixed in the life history of the colony; it is also bound to quite definite places in the colony; the two first ciliospores almost always appear in the angles between the branches of the first order and almost always in the angles between e_2 e_3 and d_2 d_3, the others always along these branches, and where the branches of the second order branch off from those of the first order. Keeping in mind the fact that in the other *Vorticellidæ* conjugation, as far as we know, takes place with all the common zooids of the colony, it is rather improbable that, in *Zoothamnium*, it should either take place only with

some very few zooids with a fixed position, or should only predestine just these zooids to excessive growth.

4. I have fairly often seen microgonidia in contact with young ciliospores (Tab. XIV fig. 2—5), especially in the stage where the peristome was not quite closed and the fine striation of the pellicula not fully developed. The microgonidium hollowed the skin of the ciliospore; there was formed a curve in the skin in which it was sitting; the posterior wreath moved very vigorously, but the skin of the microgonidium did not burst, and after 15—20 minutes the microgonidium fell off, and the curve in the pellicula of the ciliospore disappeared again.

5. I have seen conjugation in colonies which only consisted of two individuals only an hour after the first fission had taken place, and about four days before the first ciliospore would appear.

I am therefore of opinion that the transformation of a nutritive zooid in a ciliospore is quite independent of a conjugation between it and a microgonidium.

The question is now: What is the real significance of the ciliospore for the colony and for the formation of new colonies.

It is worth noting that the ciliospores are unique in their sort in the kingdom of Infusoria; they are only found in a few species of the genus *Zoothamnium*, and only in those in which there is a rather great number of branches of the first order almost all proceeding from almost a single point. They are never found in all those *Zoothamnium* which are fastened to living freemoving organisms, all those species being, as far as I know, very small. The question therefore arises: Why are the zooids divided in two sorts just in these few species: common nutritive zooids which under favourable conditions are unable to detach themselves from the mother colony, and which never develop a posterior ciliary wreath, and a rather inconspicuous number of individuals characterized by excessive growth, a second ciliary wreath, and destined voluntarily to be loosened from the mother colony. To solve this question we may take the following points into consideration.

As the so called nutritive zooids in the genus *Zoothamnium* are tied to the mother colony by means of the contractile cord, they are unable to loosen themselves, swim away and produce new colonies. As far as I can see, we lack more thorough explorations with regard to the formation of new colonies of the other colony-building *Vorticellidæ*; from our present knowledge there is, however, no doubt that some of the common zooids here now and then produce a posterior ciliary wreath, detach themselves, and develop new colonies in another spot. In the development of this secondary wreath in these genera there is, however, something casual, and more thorough explorations are necessary. I have often observed that colonies of *Epistylis* and *Carchesium*, when their hosts which carried them (*Copepoda, Cladocera*) are killed, developed the secondary wreath in the course of one to two hours, whereupon they dart away. They were then, while swimming, transformed into ciliospores with withdrawn peristome. The common zooids of *Zoothamnium* are unable to do anything of the kind. In spite of the many hundred colonies I have had in my vessels, I have never seen nutritive zooids producing a secondary wreath. They do not detach themselves, and if they fall off, they die immediately or are dead before they drop off. This, however, means that these *Zoothamnium* colonies are unable to propagate vegetatively by means of the common zooids, and in this way produce new colonies. In the ciliospores of the *Zoothamnium* species we are therefore inclined to see the aids by means of which it was made possible for the species to produce new colonies. If there were not in these species some zooids which were able to loosen themselves from the connection with the mother colony, new colonies could not be founded, at all events not in the way which was the natural one for the family. Whereas in other colony-forming *Vorticellidæ* a great number, and perhaps all individuals, are able to loosen themselves from the mother colonies, of the thousands which constitute a *Zoothamnium* colony only very few, most probably only a few hundred, are destined to be founders of new colonies.

Even if we are now able to understand the significance of the ciliospore, we have not solved the question of what has conditioned the excessive, gigantic growth. In my opinion these gigantic ciliospores are in the first place carriers of material which is to be used for the development of the pedicle, and it is just upon this main task that their excessive growth depends. It must be remembered that the pedicle just in these species is of an excessive length and strength and of an extremely complicated structure, treats which are all necessary if the pedicle is to be strong enough to carry a number of branches proceeding from almost one single point. It must further be remembered that the whole pedicle is to be fully developed before the first fission takes place ɔ: before the organism has any solid food; the ciliospore must in itself carry all the material which is necessary to build the pedicle. In the other colony building *Vorticellidæ* the pedicle is by no means so strong and of such a complicated nature, and fission takes place long before the stalk is fully developed. If Copepods covered with *Carchesium* and *Epistylis* colonies are killed, in the course of a few hours the colonies have thrown off their zooids which now swim away with great speed. If then in such a vessel I put in some few *Cyclops* or *Diaptomus*, which are quite free from *Vorticellidæ*, they will the next day be covered with heads of *Carchesium* and *Epistylis* which in the following days grow up into large colonies. Only one or two hours after attachment the peristome is fully open but there is still no pedicle. The pedicle is therefore here built up by an organism which takes in food; in the *Zoothamnium* species by organisms which have not had food for 4—5 days.

It is justifiable to suppose that the very small nutritive zooids of *Zoothamnium*, being only about 40 μ long, could not carry with them all the material which was necessary to develop the pedicle. In the peculiar fact that all zooids of the genus *Zoothamnium*, apart from the ciliospores, are tied by means of the contractile cord to the mother colony and therefore unable to loosen themselves, and in the strong and complicated structure of the pedicle, I for my part am inclined to see the main factors which just in these species of *Zoothamnium* have produced the gigantic ciliospores. Loaded with this store material, to which in my opinion the enormous volume of the ciliospore is due, it is detached from the mother colony. During the reception of water this material is brought in circulation and used for the formation of the pedicle.

In accordance with all this, the ciliospores are developed in those localities of the colony where it may be supposed that the circulation of constructive and destructive material is greatest i. e. in the angles between two branches. On this view it is also intelligible that of these zooids, which are seated in these angles, those which are not transformed into a ciliospore are destroyed and perish.

The resting-organs (Tab. X fig. 1—4). When autumn comes the whole carpet of Nymphæaceæ leaves sinks to the bottom; the half decaying leaves, before they sink down, are covered with a brown coating partly consisting of Diatoms and other Algæ. The *Zoothamnia* have disappeared. Unquestionably resting stages have been developed; they were searched for in the autumn of 1923, but without success. The resting stages are quite unknown, at all events in the polymorphic group of *Zoothamnium*; that the pedicle in these species should play any rôle in the formation of them was highly improbable; hypothetically it might be admitted that they would originate either from the nutritive zooids or from the ciliospores. With this supposition in mind large quantities of material were explored but without success. Concurrent causes to that result were that the forestry suddenly removed almost all the *Nuphar*-leaves from the surface of the lake; owing to lack of material I was forced to put off the exploration till the next year. — The result of my studies in these two years in the months of September—October are the following.

When the temperature of the water has sunk to about 15—17° C. and heavy showers have thrown great quantities of water to the surface of the lake and diluted its watermasses, the number of *Zoothamnia* decreases. In our climate we commonly get some warm days in September with bright sunshine. This was the case as well in 1923 as in 1924. The result is that the temperature of the water again rises to about 20° C. This causes an enormous production of new *Zoothamnium* colonies. Before the warm period sets in, a certain number of old colonies have endured the cold rainy period and now, owing to the increasing temperature and better life conditions, begin a new production of ciliospores, the result is that in the course of a week the leaves are covered with great quantities of quite young colonies; these may be present in such a considerable number that the leaves, when taken out of the water, shine whitish grey. Simultaneously also the stalks of the leaves are covered with colonies, and that to the very bottom of the lake. Also dead branches from the bottom, floating leaves on the surface of the lake, get their contingent. It now depends on the climatic conditions if these colonies will live to produce ciliospores; most of them die before they are fullgrown, but a rather considerable number will grow up into fullgrown colonies.

If these large colonies from the last days of September are examined, it will be seen that they often carry a great number of ciliospores, often about 13—14 fullgrown and a lot of smaller ones in all stages of development. Taken into the aquaria they will throw off many ciliospores and new colonies originate from them. On further study it will, however, be seen that some of the fullgrown ciliospores are not thrown off; they may remain for six or seven days upon the colonies; the cause is that these ciliospores do not develop any ciliary wreath. Otherwise they are of quite the same structure as the regular ciliospores; the pulsatile vacuole pulsates regularly about every third to fourth minute; and in the posterior part we find the same accumulation of dark material mentioned above. On further study it will then be seen that the connection with the muscle of the mother colony is suddenly broken off; but the ciliospore is not therefore thrown off but still remains in connection with the walls of the pedicle. Shortly after, the body presents itself as quite globular, the diameter being exactly 200 μ. The dark mass in the posterior part of the regular ciliospore disappears, but simultaneously the whole interior of the globule gets more and more opaque; the macronucleus cannot be seen any more, and the whole body gets darker and darker. Simultaneously with all this it will be seen that the wall of the body gets thicker and thicker, it is first quite smooth but later on of a faintly granular surface; where the ball is in connection with the pedicle it carries a little, flattened protuberance and this persists on all balls, at all events during the whole autumn; in this spot the wall is hollow, and it is perhaps possible that the animal leaves the cyst through this spot in spring. When these bodies have remained for some days upon the colony, they drop off and always during the strong contractions of the colony; when the colonies have been disturbed, I have seen under the microscope the bodies being flung away during the contractions and later on lying upon the bottom of the vessel. It seems as if the surface of the ripe cyst is of a rather gelatinous matter; at all events detritus of different kinds is adherent to it. If mashed it will be seen that below the outer wall there is another, extremely thin, hyaline membrane, and now and then I have got the impression that there exist two such membranes. During the pressure the contents of the ciliospore flow slowly out, and the macronucleus lies conspicuous and unhurt in the outflowing mass which contains many smaller and larger oilglobules. The contractile vacuole is at all events present during the whole autumn and acts regularly, under the conditions which my vessel offers, about every fifth minute.

That we have to do with the cysts in these bodies is unquestionable; cysts of a similar aspect have been found by Bresslau upon *Systylis Hoffi* (1914 p. 41).

It may be added that the colonies may simultaneously carry normal ciliospores, which are thrown off and give rise to new colonies, and such as are transformed into cysts (Tab. X fig. 2); I have the impression that it is in rather a late stage of the development that it is decided whether a globular body shall be transformed into a ciliospore or into a cyst. I have had colonies under the microscope which have thrown off ciliospores and carried six cysts, further many young globular bodies, all of the same aspect, and of which some were transformed into cysts, some into ciliospores.

The normal further fate of the resting organs is unknown to me. That they drop off the colonies and sink to the bottom may be regarded as certain. In June we find the colonies again on the leaves. A freeswimming stage may have developed from the cysts in May—June and arrived at the surface. Whether this stage has had the form and aspect of the regular summer ciliospore we do not know, but this may be regarded as highly probable.

Theoretical Remarks.

The question now arises: How shall we interpret the organisms which have now been studied. At a first glance they seem to belong to those which Doflein has designated as "Zellstöcke" or "Kolonialindividuen". This may be true for *Epistylis* and *Opercularia*, hardly for *Carchesium*; but the designation is very unfortunate for those *Zoothamnium* species which have been treated here. If these species are designated as colonies consisting of as many individuals as we find heads or zooids upon the branches, the question arises: what then are these branches and especially, what is the pedicle? Each of the zooids consists of protoplasma, and nucleus; and the pedicle has no nucleus at all; nevertheless it is a living part of the whole organism. The pedicle is so to speak a part of every one of the zooids of the whole colony. If we irritate only a single zooid no matter where it is sitting, the whole organism will immediately answer to the irritation by contraction of the muscle of the pedicle, and this again will cause the whole pedicle to be curved and zigzag-bent. In other words, that means that an irritation of one of the individuals will immediately be conducted through the muscle cord downwards to the base of the colony. How was this to be understood if we had only to do with simply "Kolonialstöcke", aggregates of individuals? As a matter of course the thought is directed to the colonies of Hydrozoa and Bryozoa, in the organisation of which it is a well known fact that we find structures which at a first glance to a very high degree are comparable with the two main parts of the *Zoothamnium* colonies, the zooids, and the branches with their protoplasma strings. Most probably it was just this apparent comformity which caused an Agassiz to refer the *Vorticellidæ* to the Bryozoa, especially to *Loxosoma*.

Just because we have to do with unicellular organisms, it is so difficult to find the justifiable term. If we will content ourselves with the designation "Zellstock", we are forced to remember that in these "Zellstöcke" composed of as many organisms as there are zooids there exists a special part of the Entoplasma, the contractile substance which is common to the whole "Zellstock", in which all the zooids participate, by means of which they are all in connection with each other and which, when hurt, causes the death of the whole colony.

The difficulty of assuming that the colony is a simple "Zellstock" is augmented when we remember that the contractile elements of the protoplasma, common to the whole stock, at distinct times and in distinct localities of the whole body, in a form and manner of which we have no knowledge at all, are deposited in special individuals, the ciliospores, which, after being detached from the mother colony are used again during the formation of new colonies. How the polymorphism of the whole stock, with its four sorts of individuals, the two sorts of nutritive zooids, the common ones, and those sitting where the branches of the first order issue from the apex of the pedicle, further the ciliospores and the microsporidia, is carried out is difficult to understand if the organism is to be interpreted as a "Zellstock".

In my opinion it is impossible to interpret the *Zoothamnium* in this way. The question is if it was not permissible to regard the whole colony as one single giant cell, or perhaps better as an acellular organism, with the nuclear material peripherically distributed in as many portions as there are zooids, and with the protoplasma to a very high degree differentiated in the different parts of the cell. If we take one of these ways, two great difficulties in the apprehension of the organisms will disappear. The peculiar fact that the irritation of a zooid is conducted through the pedicle to the attachment of the colony presents no difficulty at all. If we regard the whole organism as a single cell or as an acellular organism, the ciliospore may in some way really be regarded as the result of a conjugation. It would then be more intelligible that the ciliospores appear about 12—14 hours after the conjugation stage of the whole organism, that conjugation is necessary for the development of the ciliospore, and that this giant cell once fertilized preserves the ability to produce ciliospores the rest of its life.

Furthermore, if we regard the whole organism as a cell, we are better able to understand that, during the growth of the ciliospore, store material for the pedicle is deposited in it, a process which cannot probably be denied, and which almost seems impossible to understand if the organism is only to be regarded as cell congregations, where the single individuals are not in living connection with each other.

The Parasites of the *Zoothamnium*-Colonies.

It is a well-known fact that different organisms are to be found on or in the colonies of *Vorticellidæ.* It seems as if some species only occur on quite distinct species of *Vorticellidæ*; thus an *Amphileptus* species is almost only to be found in other colonies of *Vorticellidæ. Zoothamnium geniculatum* is as already mentioned by Ayrton characterized by being the host of a very peculiar Rotifer which, during the summer halfyear, seems to spend almost its whole life on the colony. Already Ehrenberg (1838 p. 427) states that he has found a Rotifer upon *Epistylis* and *Carchesium* colonies, which he designated as *Notommata petromyzon* Ehrbg.; it is also said to be found in *Volvox*. *Proales petromyzon* (Ehrbg.) has later on often been found by Gosse (Hudson Gosse 1889, p. 38; Weber 1898 p. 469; Voigt 1912 p. 90) but almost always in the freeswimming stage. My animal (Tab. XIV fig. 6—8) very much resembles that of Ehrenberg. If it is the same which later authors have always found freeswimming, I am not quite sure. The enormous, distended stomach hides almost all the other organs; I have been unable to study the musculature and see any sign of lateral canals and vibratile tags. According to Weber's description and figures all these organs are here very conspicuous.

The animals are to be found on the colonies from the last part of June to October; they occur two or three weeks after the first colonies are to be found, and were sitting upon the last colonies I have seen. They do not appear in the plancton of the lake; but if the plancton-net is drawn through the *Nymphæa*- and *Nuphar* leaves, they occur in great number in the sample.

Now and then most of them sit upon the pedicle; if so, commonly horizontally extended from it. A close examination will then show that every time the pedicle is contracted in zigzag, the Rotifer is stretched out in its whole length, and when the crown touches the pedicle, the animal snaps one of the heads and devours it. Commonly most of the Rotifers sit in the crown itself slowly creeping round on the branches, and often to be found extended from the apices of the branches. They devour the animalcules one after one, being able to strip off one branch after the other; at last only the large bulbs are left. A colony may simultaneously harbour about 30 Rotifers; if so, the large eggs are found in great numbers as well upon the pedicle as on the crown. No males have been observed, and only parthenogenetic female eggs, neither resting eggs nor male eggs.

In the crown itself I have found no other Rotifers, but on the pedicle representatives of the genera *Oecistes*, *Melicerta* and *Lascinularia* are very often found. Species of *Vorticella* (Tab. XII—XIII fig. 9) are also very often fastened partly to the pedicle partly to the crown; very often the pedicle is almost covered with *Cothurnia* (Tab. XII—XIII fig. 6), most probably *C. crystallina* Ehrbg., and especially upon the lower part of the pedicle, with some round bodies with short stiff hairs. As the pedicle always carries numerous diatoms and different other algæ, it will be understood that it may be wholly covered with epizoits of very different kind. A very common form is *Cladothrix dichotoma*, kindly determined by Prof. Kolderup Rosenvinge. Most probably they differ from lake to lake.

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Both begin the formation of ciliospores on the same day $^{9}/_{9}$ and the ciliospores as always appear in the angles between the branches $e_2\,e_3$, $d_2\,d_3$. In *A* these two ciliospores are thrown off on the $^{14}/_{9}$, using five days for their development, in *B* they are thrown off on the $^{12}/_{9}$, using only three days. On the twelfth the development of a second set of ciliospores begins in *A*, they always appear on the branches of the first order; of these only four get ripe and are all thrown off before the $^{17}/_{9}$; they have used five days for their development. On the $^{17}/_{9}$ the branches still carry small ciliospores which do not reach full development. On the $^{18}/_{9}$ the colony dies off, having only produced six ripe ciliospores.

The other colony, *B*, begins the formation of the second set of ciliospores on the $^{11}/_{9}$, one day before *A*; all these ciliospores are developed and thrown off by $^{14}/_{9}$. On the $^{14}/_{9}$ begins the development of the third set of ciliospores which are almost ripe by the $^{15}/_{9}$. On the $^{16}/_{9}$ an attack of *Proales* begins, and the colony dies between $^{16}/_{9}$ and $^{17}/_{9}$; it has produced 11 ciliospores and would most probably have produced a new set if it had not been devoured.

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Fig. 3. The two bendings of the pedicle; the wall of the pedicle with the two peculiar systems of bending lines. The muscle sheath which, when bent, shows transversal stripes, and in its upper part seems to be attached to the wall of the pedicle by means of extremely fine threads. The muscle is conspicuously longitudinally striped, marked off from the muscle sheath by means of a darker part (the Axonem of ENTZ). On the concave side of the two slings the wall, when bent, is split up in a series of short pieces. Zeisz Obj. 4 Oc. 6.

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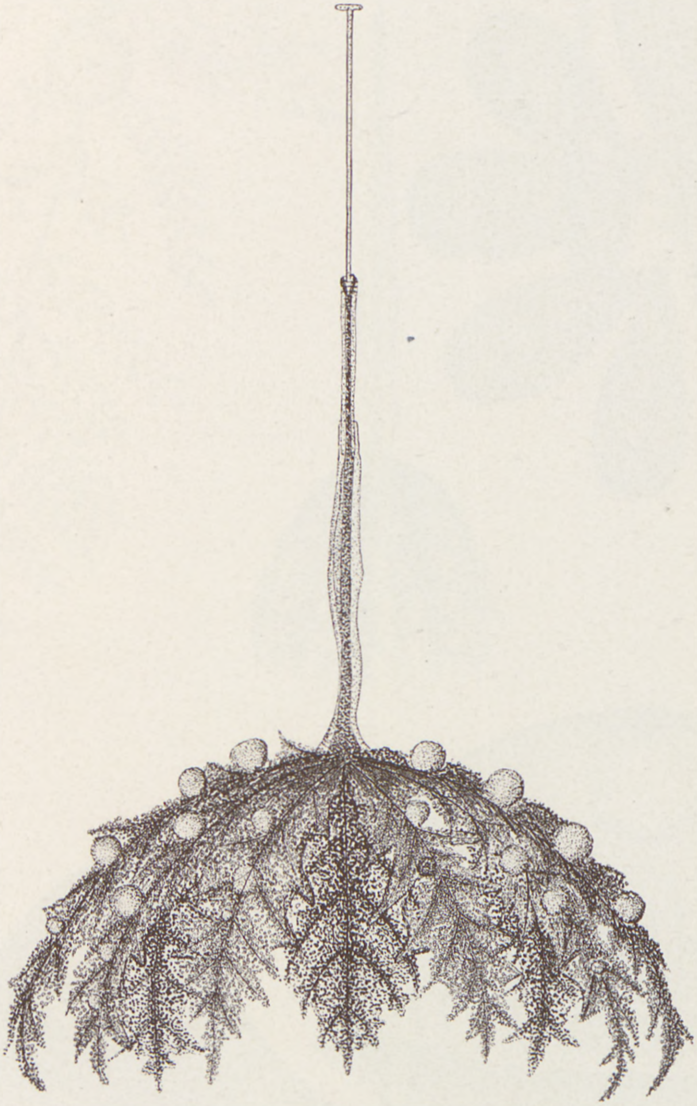

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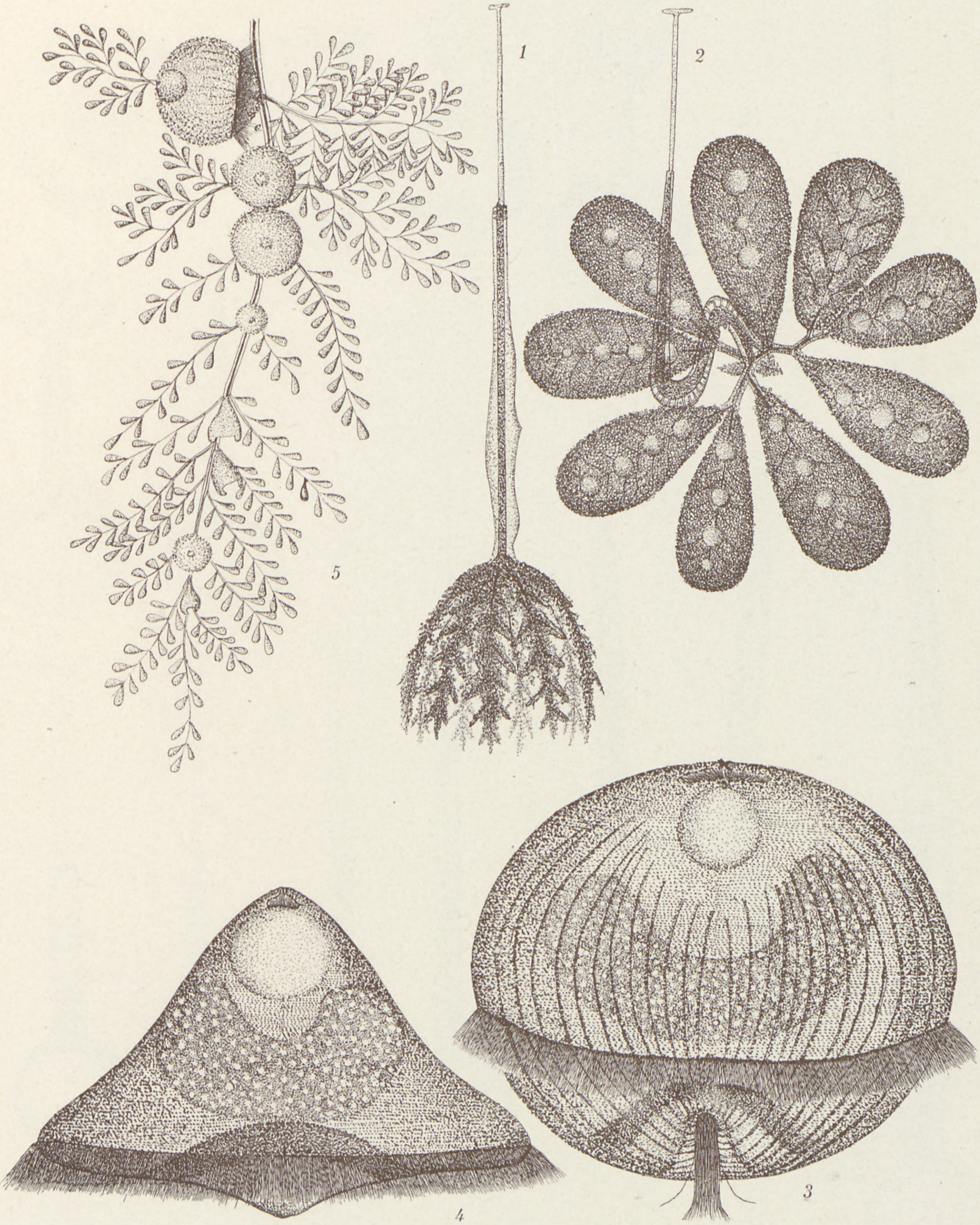

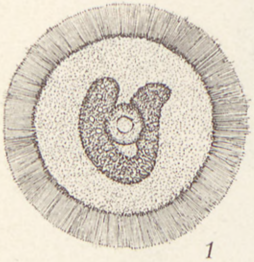
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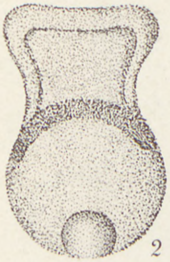
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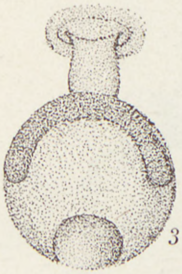
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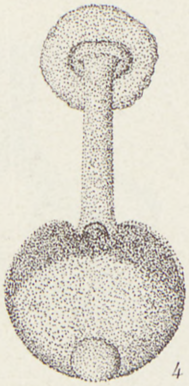
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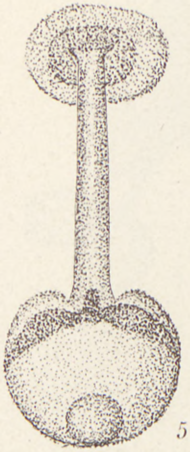
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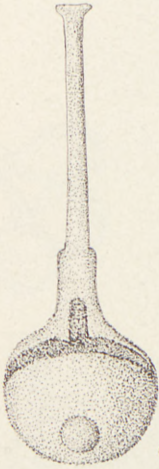
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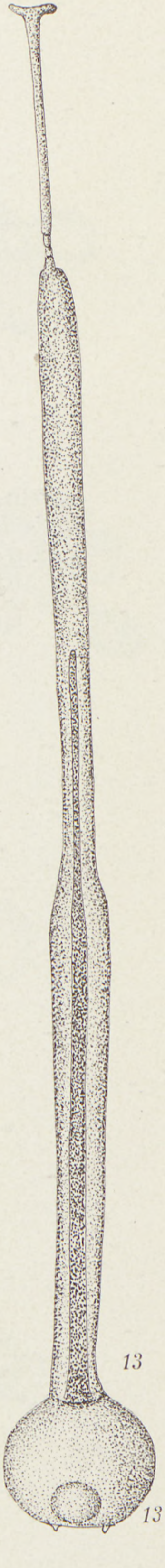
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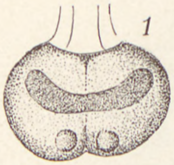
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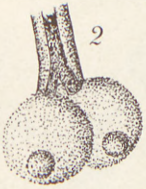
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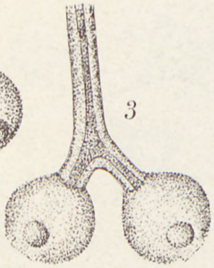
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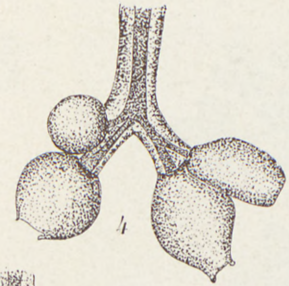
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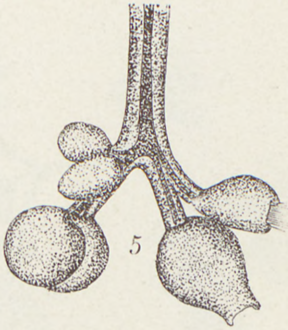
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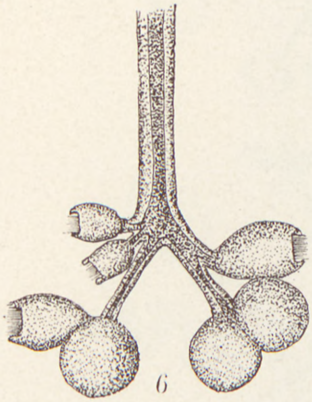
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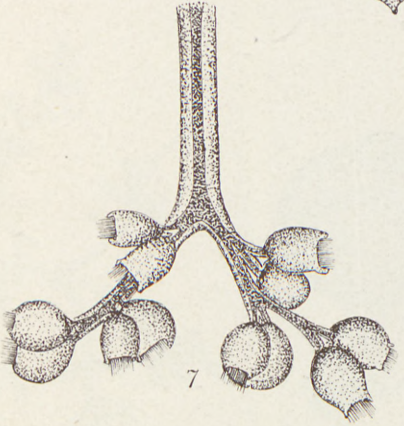
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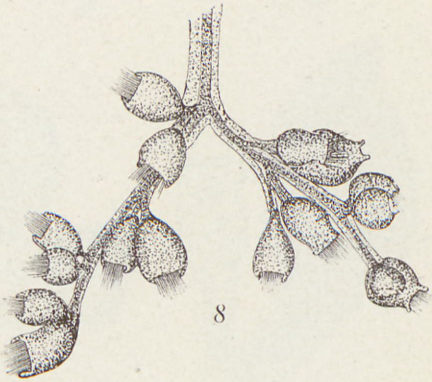
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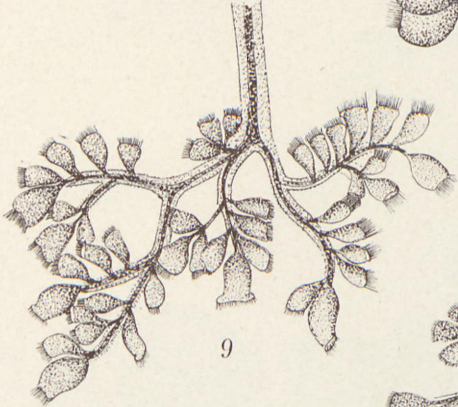
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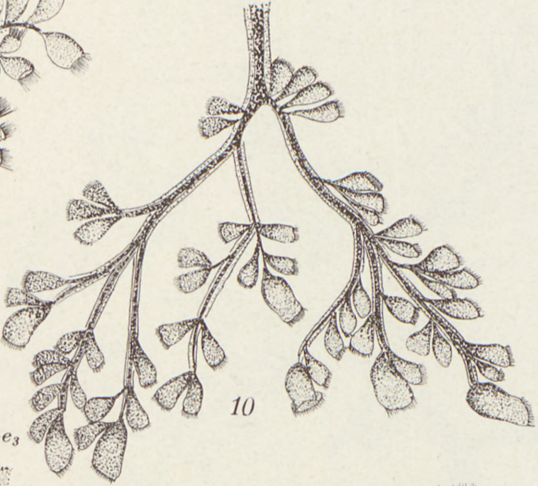
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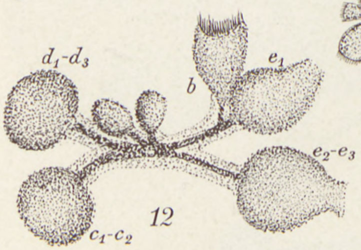

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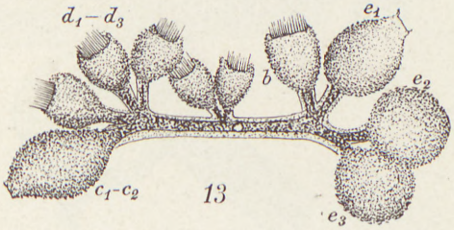

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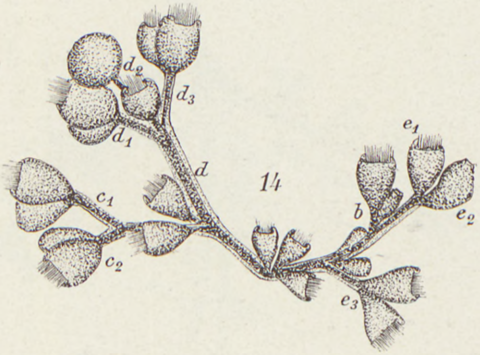

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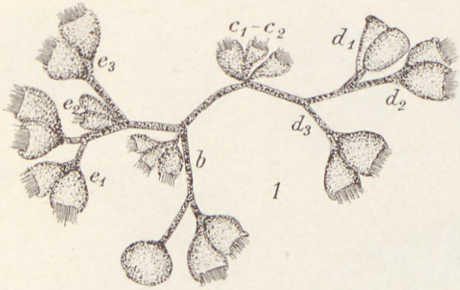
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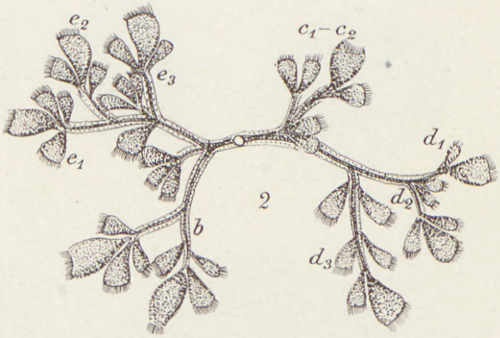
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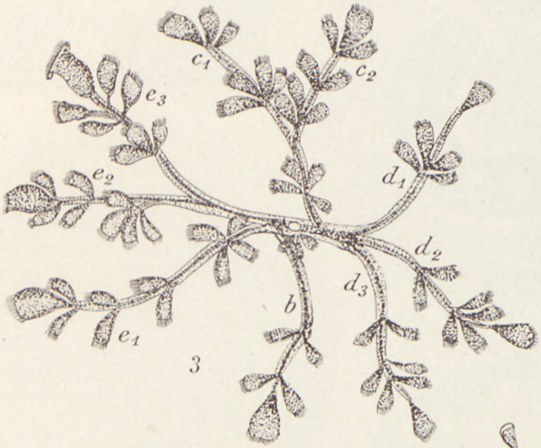
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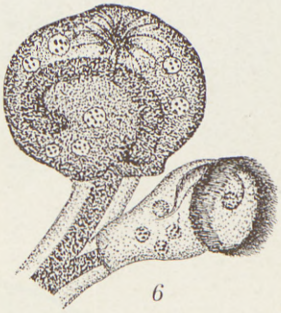

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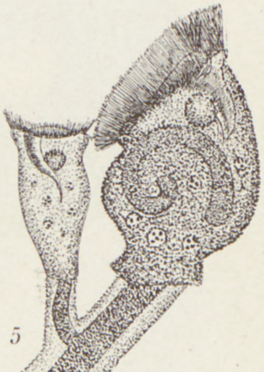

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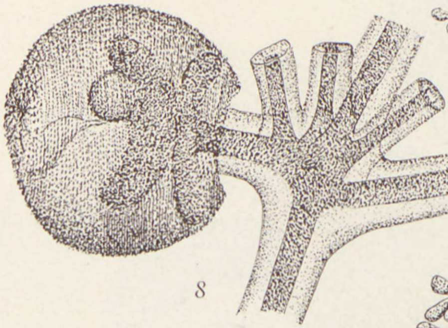

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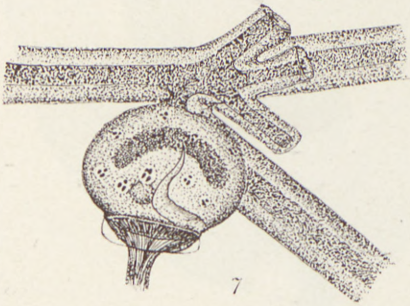

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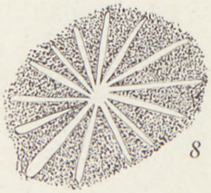
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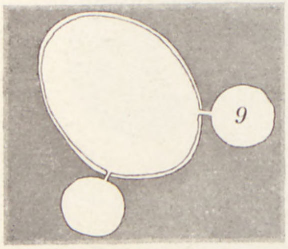

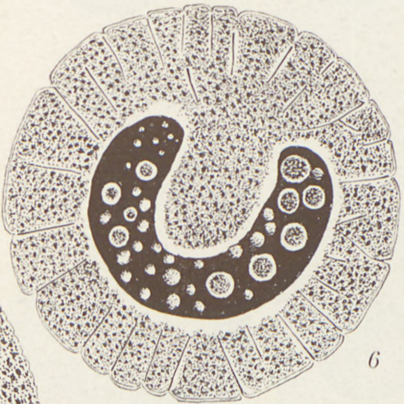
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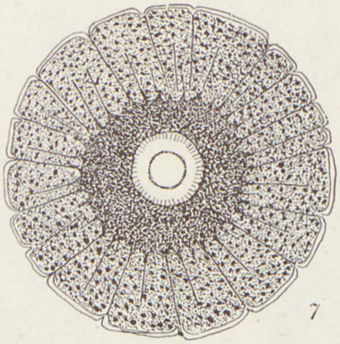
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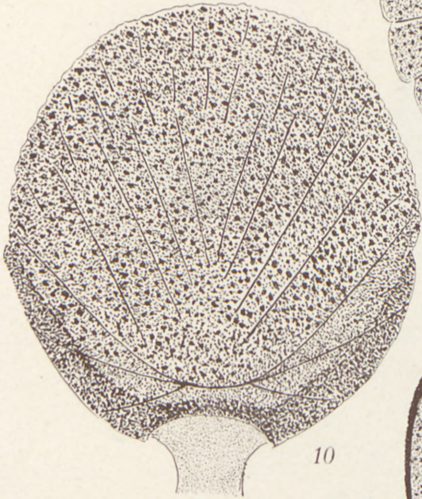
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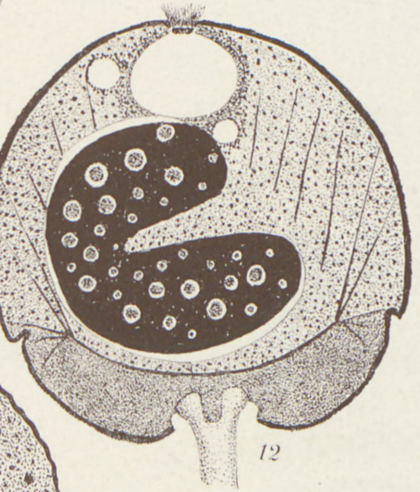
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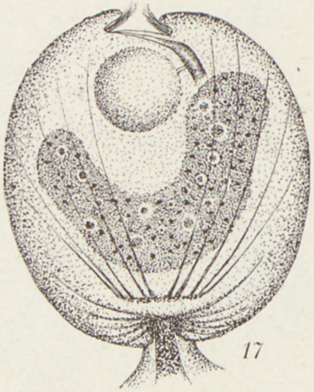
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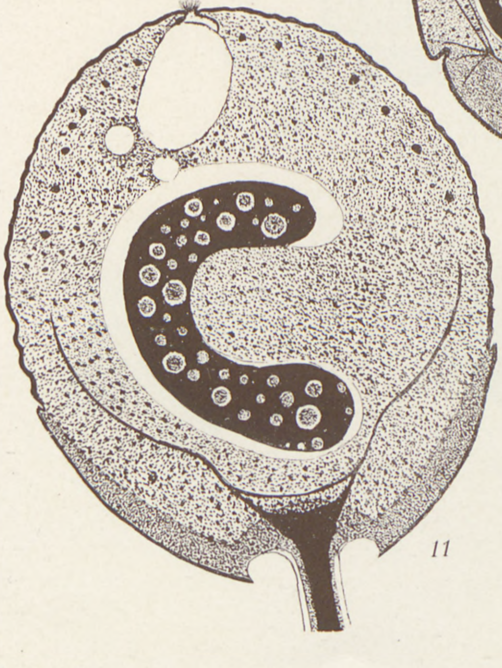
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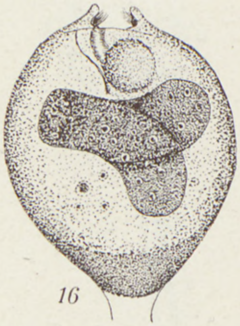
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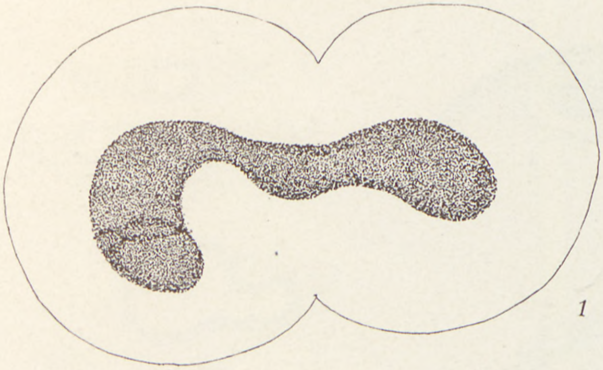
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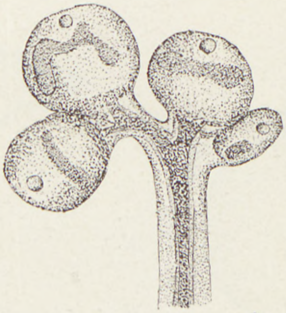
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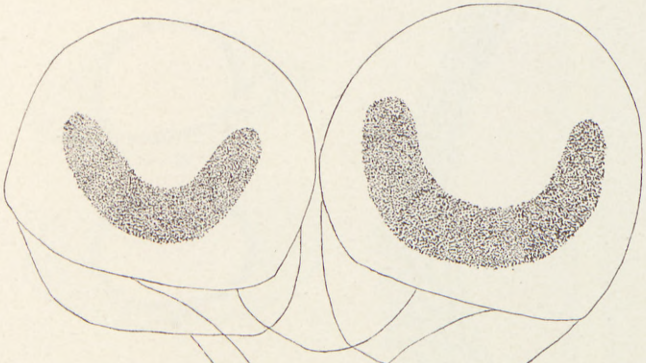
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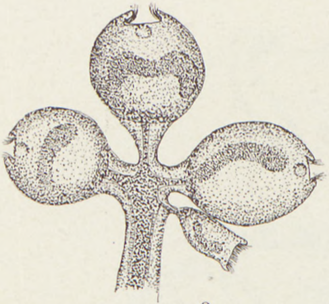
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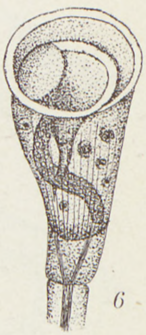
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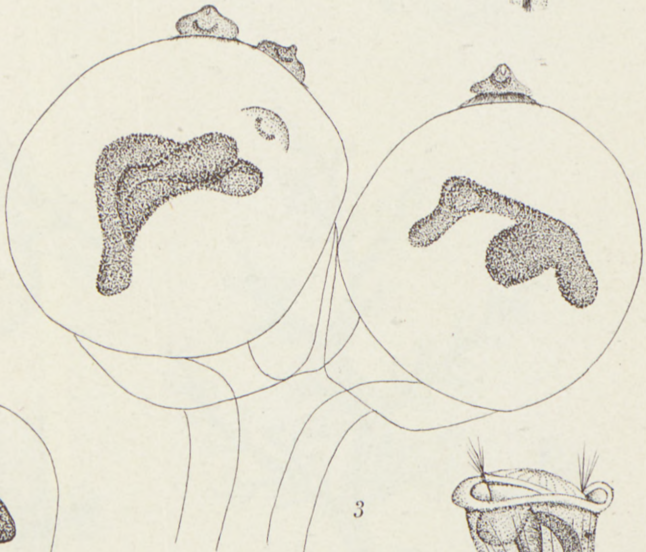
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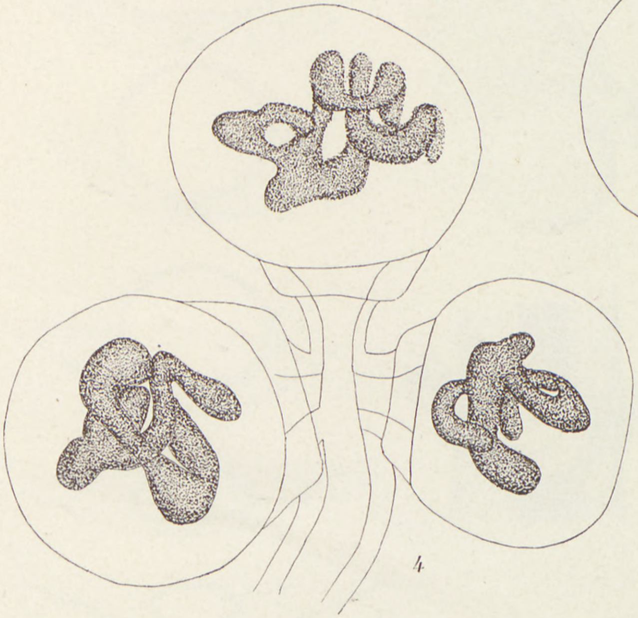
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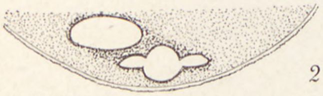

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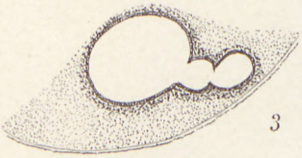

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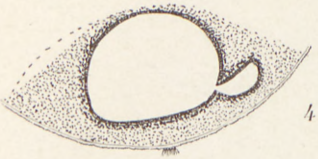

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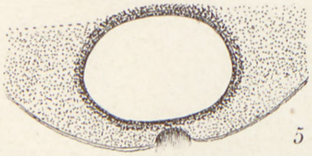

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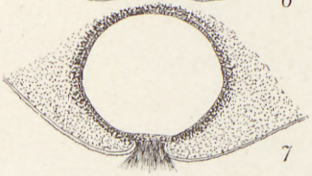

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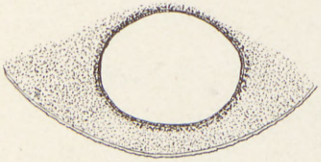

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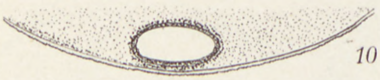

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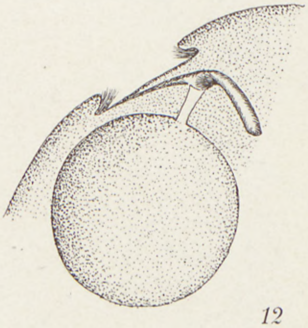

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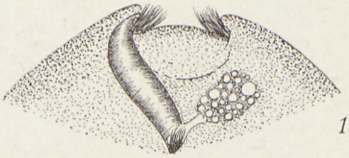

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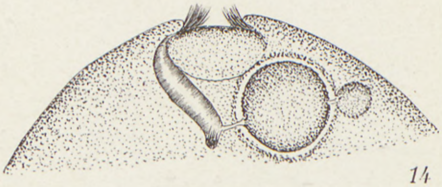

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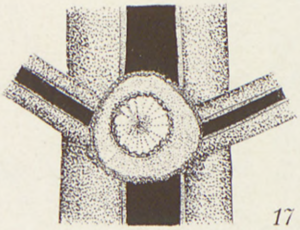

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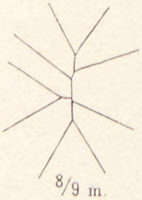
TAB. IX



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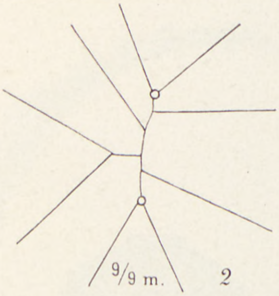

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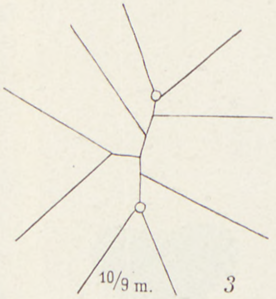

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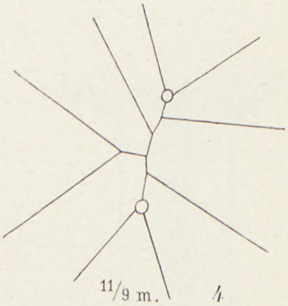

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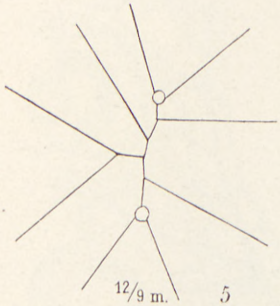

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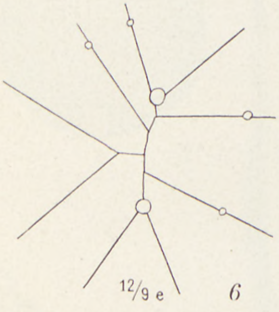

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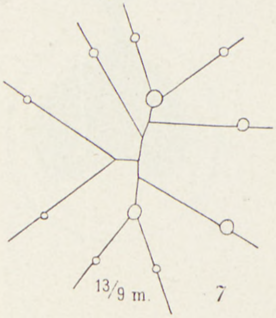

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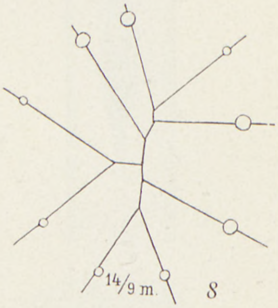

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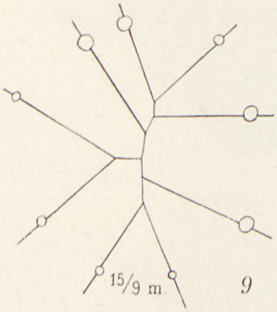

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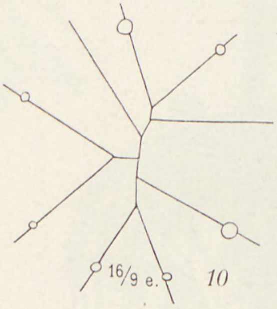

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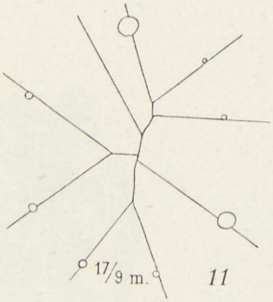

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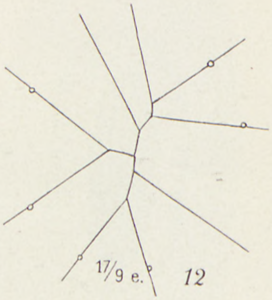

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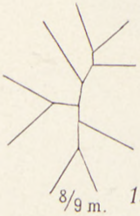

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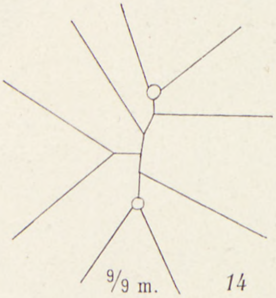

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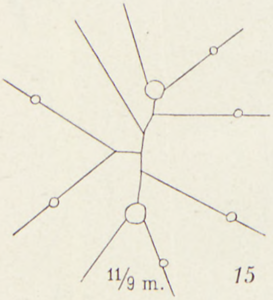

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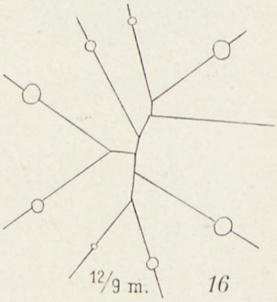

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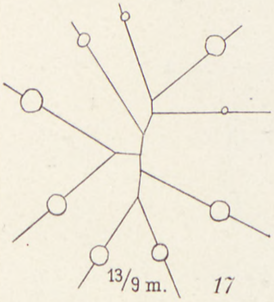

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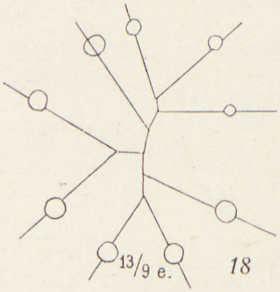

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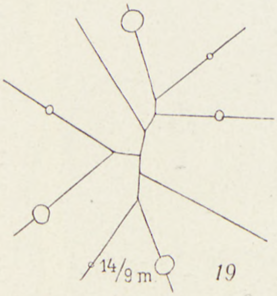

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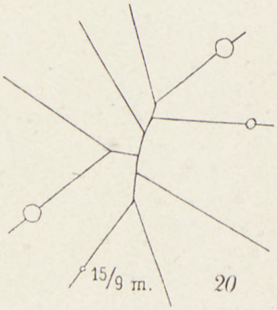

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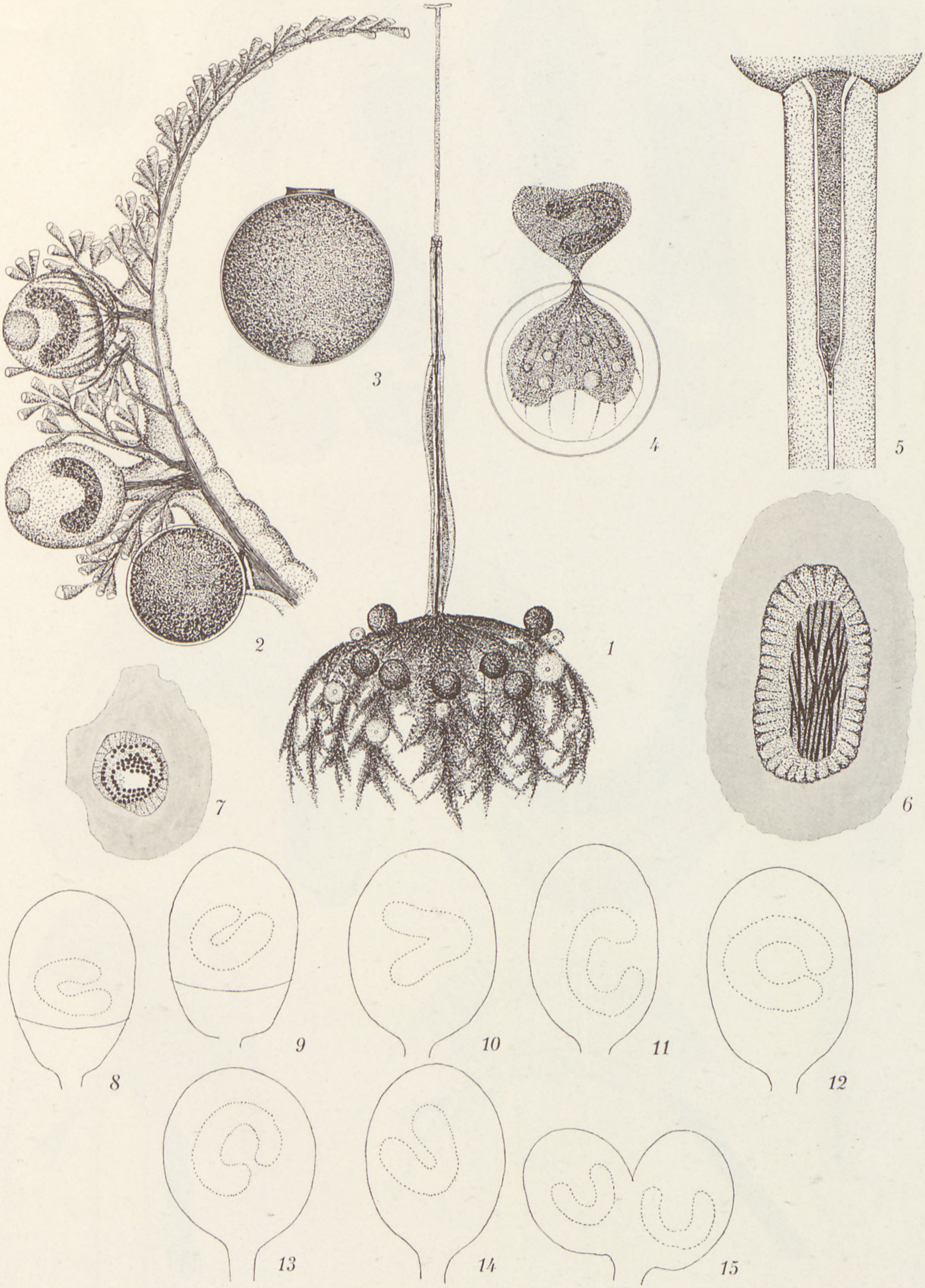
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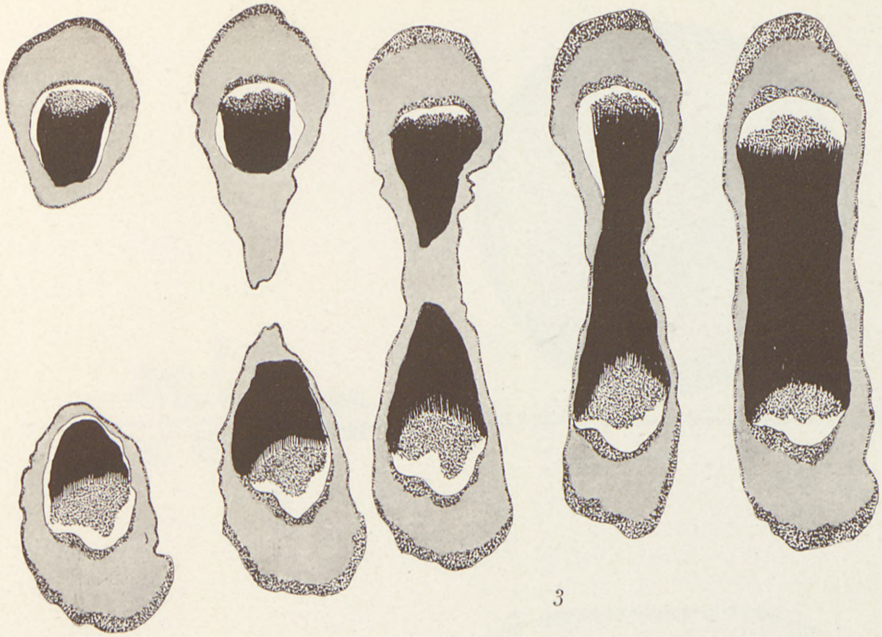

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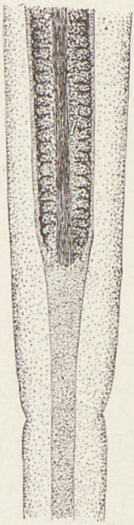

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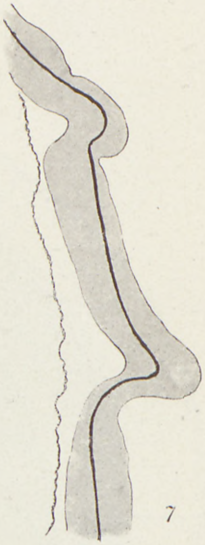

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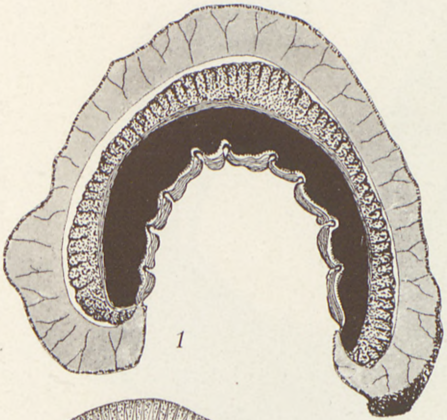

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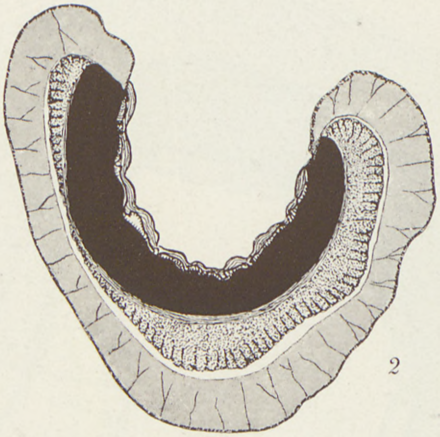

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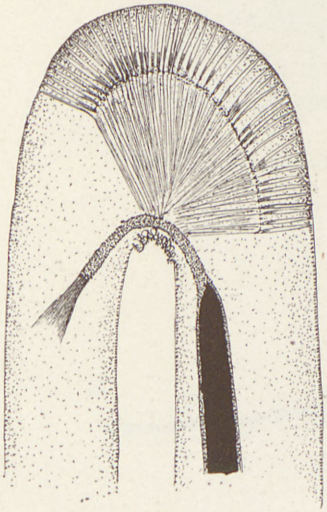

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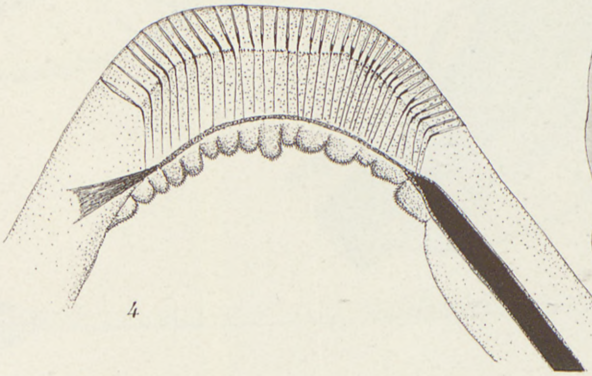

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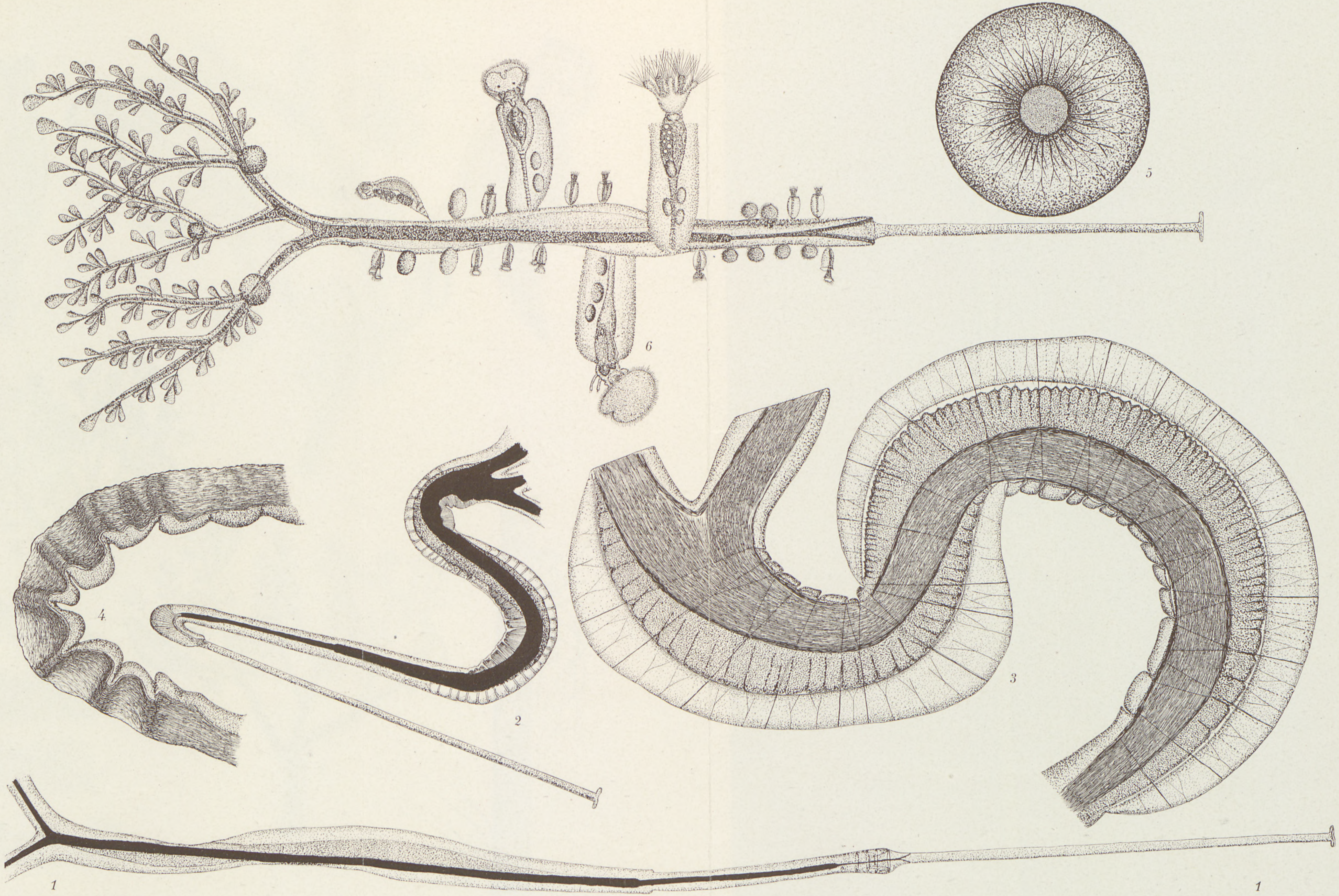
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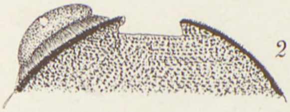
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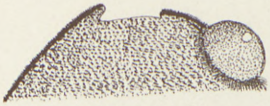
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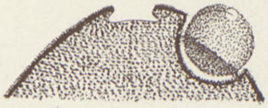
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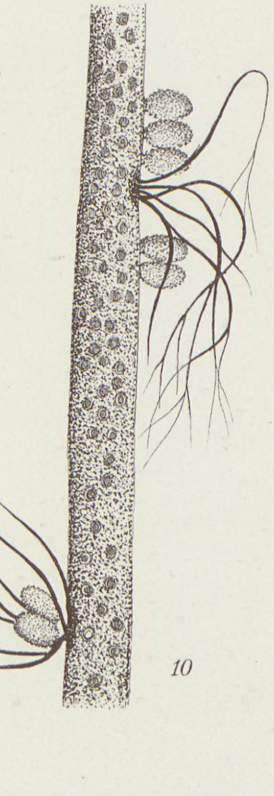
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