# *Phyllocardium complanatum*, a new Polyblepharidacea.

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(With Plates 5—6.)

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As is known, the winter flora of the fresh water basins attracted very little attention of the algologists; partly due to inconvenience of the investigation, partly because this flora seemed to be too poor and uninteresting. It is mainly from the hydrobiological stand point, that some greater basins underwent investigation in the winter time, which in general confirmed the above said. As to the smaller basins, as little ponds, etc., we know almost nothing about them.

There is no doubt however that the winter, while suppressing the summer flora, is the time when a series of other, oligothermic, forms develop, many of them being as yet unknown to the systematisers. Last winter I got convinced in this, when I occasionally turned my attention to an artificial basin in the Botanical Garden in Kharkov, several square meters in size and about half a meter deep, partly overgrown with swamp vegetation. In spite of my perpetual observations on its microflora in warm months of these last years, I found nothing peculiar there. But in december 1927, when the water got covered with ice about 7 cm thick, quite new organisms developed, which I had never seen anywhere. It is true, that the conditions were somewhat peculiar now. Owing to the putrification of the mud at the bottom, water abounded in $H_2S$ and accordingly was poor in oxygen, so that the fishes, which had remained there, perished. The microflora was in fact very poor, as none of the summer inhabitants was found there in a vegetative condition. But there were found in abundance *Chromatium Okeni*, *Chlorochromatium aggregatum* and *Physomonas vestita* Stokes — a very interesting and little investigated Flagellate; further a small species of Ochromonas, one Chlamydomonad, a new colonial Chrysomonad from the group Ochromonadales, and finally *Phyllocardium*. I can naturally say nothing as to whether there is any periodicity in the apparition of these organisms. Later on, in January, both Chrysomonads disappeared and *Phyllocardium* decreased considerably in quantity, apparently owing to the absence of light, as the ice, now about 15 cm thick, was covered yet with a layer of snow.

When brought into the laboratory and kept in a glass vessel, *Phyllocardium* showed a characteristic and very peculiar indifference to light and air, chiefly concentrating at the bottom. This is possibly in connection with the slight intensity of these factors in natural conditions. It is true that other coloured Flagellates, especially both Chrysomonads, gathered to the very surface of the water, being almost absent in the lowest layers. But their want of light and air must have been greater, that in *Phyllocardium*, and this was probably the cause of their disappearance from the basin.

The cells of *Phyllocardium* have a very characteristic form, unknown yet among the Polyblepharidaceae. They are strongly compressed on both sides, showing in this respect a great convergency with *Phyllomonas striata* Korsch. When viewed from the broad side, the body of the mature individuals appears in general triangular, with a more or less bluntly rounded, sometimes almost truncate, fore end. The posterior part is narrowed and pointed at the very end, the sides being usually concave, more rarely straight or even convex (Pl. 5 Figs. 1, 2). Along the margin the body is thinned, almost sharpened, which is well to be seen from the pole (Pl. 5 Fig. 3). The younger cells are narrower, with the posterior end produced into a more or less thin tail, and not so bluntly rounded afore, as in mature cells (Pl. 5 Fig. 6). In general the form of the cells is variable enough, as in all of the Polyblepharidaceae; which on the one hand is dependent from age, and on the other from the medium. This variability is not of the kind of metabolic movements, as in *Euglena*, because I never saw the cells change their form during observation, by normal conditions of course.

It must be noted, however, that *Phyllocardium* is utmost sensible to environmental changes. So, if enclosed in the hanging drop preparation, the cells soon begin to swell, becoming round in a cross section, so that the body takes an ovoid form (Pl. 5 Figs. 4, 5). The posterior end becomes much thinner, and very often transformed into a thread-like appendage, somewhat thickened or even capitate at the free end. What was the cause of such changes, I could not elucidate. But this condition is no doubt abnormal, because the swollen individuals ultimately burst and died. It could be supposed, that they perished due to the rise of temperature and strong illumination during the microscopical observation. But the result was also the same, if preparations were not examined for the first two or three hours. At the same time, the cells in the samples were quite normal.

This sensibility of *Phyllocardium* made its observing very truble-some, and its liefe-history described in the following had to be established mainly by the comparing of different developmental stages, not by perpetual observation of the cells in one and the same preparation.

At the bluntly rounded fore end of the body there is a small portion of the colourless protoplasm, with a minute papilla, from which two cilia project. As is to be seen from Pl. 5 Fig. 3 the cilia are arranged in a plane perpendicular to that of the compression of the body, as is a general rule with biciliate or, sometimes, quadriciliate and compressed Volvocales (*Scherffellia, Phyllomonas, Carteria cordiformis* Dill). The cilia are thinner toward the end and almost invisible at the very tip. In spite of a considerable relative length of the cilia and complanate form of the body, the movements of the organism are very lazy, as compared with those of *Pyramimonas* or *Spermatozopsis.*

The whole protoplast, except at the very fore end, is uniformly green due to a wallsided thin chromatophor without any ribs on its surface, or perforations. At some distance from the fore end there is a small elongated stigma, and at the same level or somewhat lower the nucleus, both placed almost in the mediane of the cell. In the chromatophor there are enclosed numerous and relatively very large starch grains of a lenticular shape, which is to be seen if they are isolated by crushing the cell under the cover glass. Due to the thinness of the chromatophor, the starch grains are disposed uniformly, namely with their broad sides applied to the surface of the protoplast.

There are two contractile vacuoles at the base of the cilia. They are extremely small and almost invisible under usual conditions of observation. In the space between them and the nucleus several spherical bodies of different size are disposed. Methylenblue stains them deeply blue, and this colour is retained after the action of 2 % sulfuric acid; which shows that those bodies are of volutin.

The chromatophor, though closely applied to the periplast, is however an independent structure, separated from the periplast by a very thin and in living cells invisible layer of the protoplasm. But in cells fixed with osmic acid, or any other substance, the chromatophor contracts a little and retreats from the periplast, especially in the posterior half of the body, as is shown in Pl. 5 Fig. 6. In general, the periplast of *Phyllocardium* makes the impression of its being more developed as compared with any other of the Polyblepharidaceae.

In the laboratory *Phyllocardium* felt very well, so that its life-history could be followed up in detail. Division takes place in the evening, and in a motile condition. Successive stages of the process are represented in Pl. 5 Figs. 7—12, Pl. 6 Figs. 13—16. The division is preceded by considerable changes of the form of the body, which begin by the drawing in of the tail and the rounding of the posterior end. Further the body becomes extended in width, which latter exceeds now its length, — and excavated in front, so as to be of a reniform shape. Movements become at this period still slower, than before. The cilia gradually diverge to a considerable distance from one another, and the excavation of the protoplast between them grows deeper, passing over into a furrow all round the protoplast. At this time the chromatophor begins to divide, which is to be seen from the arrangement of the starch granules in it and from its outlines at the posterior end, where it may retreat a little from the surface of the body. The stigma does not divide, but remains unchanged in one of the halves of the protoplast, close near the longitudinal furrow. As to the contractile vacuoles, they also diverge together with the cilia, a second vacuole appearing near each of them.

It is at relatively late stages, that additional cilia begin to grow out, one by each of the old cilia. The time of their apparition is somewhat variable. In some cases they are seen when the furrow round the protoplast is little developed yet (Pl. 5 Fig. 10). In other cases they are absent yet at the stage near to a full separation of the daughter cells (Pl. 6 Fig. 16). Their disposition relative to that of the preexisting mother cilia is to be seen from Pl. II Fig. 14, showing a dividing cell from the pole.

Division proceeds very slowly, so that I never could follow it up completely on one and the same individual before this latter perishes. The very separation of the daughter cells presents nothing peculiar. They are at this time shortly ovoid in shape, and it is only in the following and probably rather long period that they take the form characteristic for mature cells of *Phyllocardium*. Judging from the form of individuals of different age, the young cells first become elongated, and only later a compression takes place.

The daughter cell with the parent stigma develops none such of its own. As to the other cell, it is only at the last stage of division, that the stigma appears in it in form of a small elongated point.

Two or three days after the material had been brought into the laboratory the sexual process was observed. It is apparently preceded by more rapid succession of divisions, because during this period dividing individuals were observed in abundance at any time of the day. The result was that much smaller individuals appeared, which ultimately functioned as gametes. Pl. II Figs. 13—15 show dividing cells of such a kind, to compare with full grown vegetative ones, as in Pl. I Fig. 7.

The gametes in general are similar to vegetative cells, but are not compressed, appearing round in the pole view (Pl. II Figs. 17, 19). Only the larger of them, approaching in size to smaller vegetative cells, may be somewhat flattened. Their body is thus ovoid and prolonged behind into more or less long point. The latter may occasionally be wholly absent, the cells being then rounded behind (Pl. II Fig. 18). As to the structure of the protoplast, it is of course the same as in vegetative individuals, the only difference being that occasionally two stigmata were present, as in Pl. II Figs. 18, 20. One of them is obviously a parent stigma, and the other one that formed anew. Such a case was observed in some of the Volvocales [1]), but it is strange that in *Phyllocardium* this is only a very rare case, not a general rule, as with *Chlamydobotrys*, *Chlorogonium*, and some others.

According to the method of their origin, the gametes cannot be all of the same dimensions, but show some variability in this

[1]) Korshikov, A. A.: Zur Morphologie des geschlechtlichen Prozesses bei den Volvocales. Arch. de la Soc. Russe de Protistol. 1923 Vol. II p. 179.

respect. Of course, there is no possibility directly to compare dimensions of the gametes differing in form. As to the gametes pointed behind, they were from 8 $\mu$ to 12 $\mu$ in length, with a corresponding difference in width. Normally isogamic copulation takes place, but often the gametes of uniqual size are coupled, as in Pl. 6 Fig. 19. They thus show some inclination to the atactogamy, which in a far more pronounced form has been observed be me in some Chlamydomonads.

The gametes going to fuse apply themselves to one another by their fore ends, and for some time perform characteristic oscillating movements on one and the same place. Several other gametes may join them at this period. It is probable, that ultimately two of the gametes becomes connected by a protoplasmatic thread, formed by the fusion of two minute protoplasmic truncs protruding at the tips of the papillae, between the cilia. It was only once that I succeeded in observing such in *Phyllocardium* (Pl. 6 Fig. 21). The connecting thread was exceedingly thin and almost invisible. That in other cases such threads were present, was evident from the fact, that in fixed preparations the paired gametes kept firmly together even when there was some space between their not yet fused ends. *Phyllocardium* presents thus an interesting parallel to some Chlamydomonads, in which I also observed such "copulative truncs" and their fusion before the copulation of the gametes.

The mutual arrangement of the gametes by the copulation is a quite definite one, namely that with the cilia disposed in two planes at right angles to one another, i. e. in alternating directions.

After the gametes have been fused by their fore ends, the copulation passes over into a lateral one, as is shown in Pl. 5 Fig. 20. The fusion of the nuclei takes place somewhat later, when the fusion of the protoplasts has been completed. The chromatophores for a short time remain yet not fused, though closely applied to one another, which is to be seen from the arrangement of the starch granules in the body of the zygozoospore. But soon they all are disposed with their broad sides along the surface of the cell, which shows that the fusion of the chromatophores has taken place. The stigmata lie on one side some distance apart from one anothor. Very seldom the zygotes with three or even four stigmata were observed (Pl. 6 Fig. 26), originating obviously from the gametes with two stigmata.

The not-pointed, ovoid gametes copulate alike with ovoid (Pl. 6 Fig. 28) and pointed (Pl. 6 Fig. 22) partners, without any apparent preference in this respect. Just such copulations showed, that the above described ovoid cells were in fact the gametes of *Phyllocardium*, not the cells of any independent organism.

The zygozoospores do not immediately enter into a resting stage, as in most of other Volvocales. They keep up the motile existance, presenting thus by themselves a diploid motile phase in the life cycle of *Phyllocardium*. They resemble in this respect the zygozoospores of some Chlamydomonadaceae, recently described by me [1]).

The form of these motile holozygotes is quite similar to that of vegetatives cells, with that only exception that the body is generally not compressed, but round in a cross section (Pl. 6 Figs. 23, 25). It is only by the presence of two stigmata that the cells in question can be recognised as the zygotes of *Phyllocardium*, and not the vegetatives cells of any other quadriciliate form. The rounded gametes form zygotes, which are also rounded, as in Pl. 6 Fig. 26. I do not know, however, whether such zygotes become caudate in the following.

As to the duration of the motile period of the zygotes, I can say nothing definite, because it was impossible to keep them in the hanging drop preparations for a time more than some hours. This period must, however, be considerable, judging from the fact that the holozygotes could be obtained from the vessel where the copulation had finished three and more days before. They maintained their former shape, but were considerably larger, attaining up to 20 $\mu$ in length. Some of them were slightly compressed. I did not observe their passing over into a resting stage, but there is no doubt that such took place in my vessels, because after the motile zygotes had disappeared, a large quantity of small green spherical cells were to be found on the bottom of that vessel. These were covere with a thin smooth wall, and provided with two elongated stigmata on one side (Pl. 6 Fig. 27). Any confusion was excluded, because there were none other organisms which could have produced such cells. These resting zygotes grew for some time, up to 13 $\mu$ in diameter. Their wall thickened, the stigmata disappeared, and the chromatophore became overcharged with starch (Pl. 6 Fig. 28).

Later on starch disappeared and the chromatophor became strongly reduced, while in the protoplasm large masses of oil appeared. Haematochrom is absent in ripe zygotes.

[1]) Korshikov, A. A.: Algological Notes II. Arch. Russes de la Protistol. 1926 Vol. 5 p. 137.

Their further development was not followed up.

Any vegetative resting stages, as well as the palmelloid one are unknown.

As is seen from the above description, *Phyllocardium complanatum* represents a quite new, with respect to the form of the cell, type among the Polyblepharidaceae. Therefore it will be reasonable to establish a new genus in this family, with the following characteristic:

*Phyllocardium* n. g.: Cells naked, complanate, produced toward the posterior end, with the chromatophor deprived of the pyrenoid. Two cilia (in the plane perpendicular to the plane of the body). Vegetative multiplication by longitudinal division. Hologamic copulation.

The only species *Phyllocardium complanatum:* Mature cells triangular in their general outlines, rounded or subtruncate in front, produced and pointed behind, not contorted. Length to 19 $\mu$, width to 12 $\mu$. Cilia up to $1^1/_2$ cell length. Two contractile vacuoles at the base of the cilia. Chromatophor entire, wallsided, extending to the very fore end, with a conspicuous irregularly elliptic almost mediane flat stigma above the middle of the cell, and large lentiform starch grains scattered throughout. The nucleus near the middle of the cell. Younger cells narrower and less compressed. Division in motile condition. Hologametes identical with young vegetative cells, but considerably smaller and sometimes rounded behind; occasionally with two stigmata. Holozygote with a long (up to several days?) period of motility, and growth, ultimately approaching to the vegetative mature cells in size and general shape, but only sligthly compressed. Resting zygotes round, with a thick smooth wall, to 13 $\mu$. Vegetative resting stage and palmellisation were not observed.

Found in Kharkov, in a freshwater basin in the Botanical Garden, in winter. Very rare.

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## Explanation of plates.

All the figures, except Fig. 17, were sketched by means of Abbe's camera-lucida from living or fixed by osmic acid individuals, under one and the same magnification 1500.

Plate 5.

Figs. 1, 2. *Phyllocardium complanatum* n. g. et sp. Mature cells somewhat differing in shape. The position of the cilia is typical for fixed cells.

Fig. 3. The cell from the pole.

Figs. 4, 5. The cells swollen after some time having been enclosed in the hanging drop preparation, with the chromatophor contracted in the posterior end.

Fig. 6. The cell some time after the fixation with osmic acid, with the chromatophor retreating from the periplast.

Fig. 7. The cell in preparing to divide; the tail is drawn in.

Fig. 8. A further stage of the balling before division.

Figs. 9, 10. First stages of division; the nucleus is not seen.

Fig. 11. Middle stage of division; additional cilia begin to grow out.

Fig. 12. Later stage of division, with the chromatophor already divided.

## Plate 6.

Fig. 13. The cell in an early stage of division (as in Fig. 10) viewed from the pole.

Fig. 14. Pole view of the dividing cell, as in Fig. 12.

Fig. 15. Daughter cells not long before their separation.

Fig. 16. The same stage, with the additional cilia not yet grown out.

Fig. 17. Isogametes.

Fig. 18. Round isogametes.

Fig. 19. Gametes of somewhat different sizes.

Fig. 20. Gametes joined together by means of fused copulative truncs; one of them with two stigmata.

Fig. 21. Middle stage of copulation of pointed gametes.

Fig. 22. Further stage of copulation.

Fig. 23. Copulation of the unequally shaped gametes.

Fig. 24. Young motile holozygote.

Fig. 25. Full grown motile holozygote.

Fig. 26. Young holozygote formed by two rounded gametes, and bearing four stigmata.

Fig. 27. Young resting zygote; stigmata are yet present.

Fig. 28. Full grown zygote without stigmata and yet with starch in the chromatophor.