Herrn Prof. Dr. H. Joseph, Wien, spreche ich für die Anfertigung der Photographien meinen besten Dank aus.

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2. Notes upon Opalina.

By Maynard M. Metcalf.

(With 21 figures.)

eingeg. 9. April 1914.

I. Chomosomes in *Opalina*.

Thru the kindness of Professor E. A. Andrews, I received some time ago half a dozen live toads, *Bufo agua*, collected in Jamaica in connection with the summer laboratory of the Johns Hopkins University. In the recta of these animals were large numbers of a good-sized *Opalina*, a new species which has proven remarkably favorable for study of nuclear phenomena. *Opalina antilliensis*, as I am naming it, is a large flat species, as flat as *O. ranarum.* It is one of the binucleated forms and its nuclei are huge, tho not so large in comparison with the body as are those of *O. macronucleata* described by Bezzenberger. Raff's *O. binucleata*, according to published measurements, tho not from the figures, has nuclei of about the same size as those of *O. antilliensis.* Figures 12 and 13 show two nuclei of *O. antilliensis* magnified 1780 times. One realizes their large size upon comparing with figs. 14 to 21 which represent nuclei of an undescribed *Opalina* (*O. intermedia*) magnified to the same degree as those shown in figs. 12 and 13. The latter nuclei are of the size of those seen in the well-known *O. ranarum.* The nuclei of *O. antilliensis* are three times as wide, as those of the multinucleated Opalinas, in corresponding condition.

The diameter of the nucleus is but very little less than the thickness of the whole ectosarc (fig. 3). It is unnecessary, therefore, to make sections for the study of nuclear phenomena, for in total preparations there is only the ectosarc tissue above and below the nuclei, and this does not at all obscure vision. The chromatin in each nucleus is not abundant for the size of the nucleus and, as in all binucleated Opalinas studied, is loosely arranged at the surface of the nucleus, just beneath the membrane. These conditions make the nucleus of *Opalina antilliensis* by far the clearest finest nucleus for study I have seen among the Opalinas, or in the whole group of the Protozoa. Doubtless the nuclei of *O. binu-*

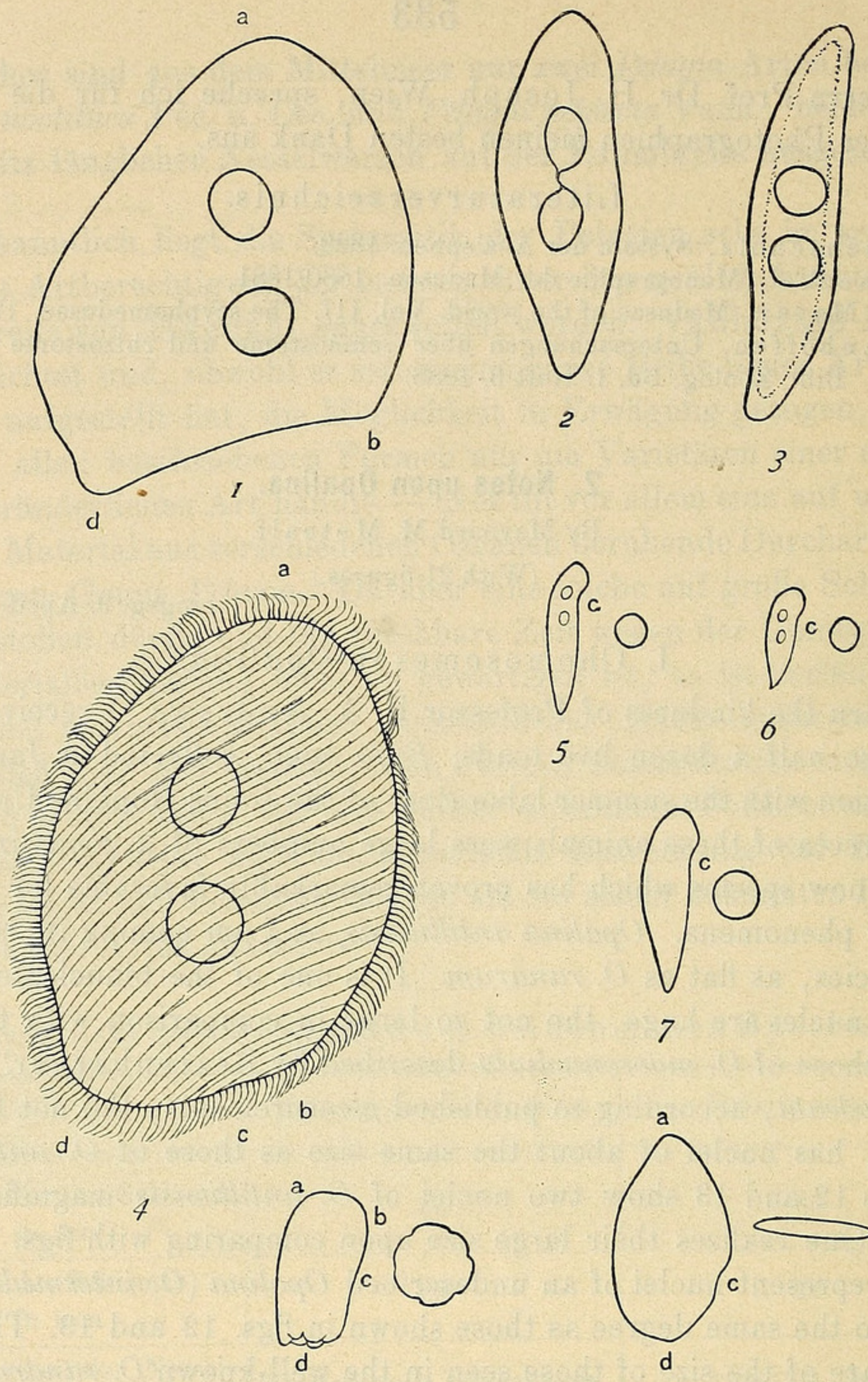


Fig. 1. An individual of *Opalina antilliensis* which has recently come from longitudinal division i. e. division parallel to the lines of cilia, *c-d* being the line along which the division occurred, and *a-b* the broad anterior end. $\times$ 280 diameters.

Fig. 2. An individual of *O. antilliensis*, which has just come from transverse division, i. e. division transverse to the lines of cilia. The nucleus has not yet completed the division by which the binucleated condition is to be reestablished. $\times$ 280 diameters.

Fig. 3. An optical section of *O. antilliensis*, showing that the diameter of each nucleus is practically the same as the thickness of the whole endoplasm. $\times$ 280 diameters.

Fig. 4. An ordinary full sized individual of *O. antilliensis*, showing the lines of cilia insertion, some of the cilia at the edge in profile, and the two nuclei in outline. $\times$ 280 diameters.

Fig. 5. *Opalina intestinalis*, side view and cross section.

Fig. 6. *Opalina caudata*, side view and cross section.

Fig. 7. *Opalina dimidiata*, side view and cross section. The very numerous nuclei are not drawn.

Fig. 8. *O. zelleri*, side view and cross section. The very numerous nuclei are omitted.

Fig. 9. *O. ranarum*, side view and cross section. The very numerous nuclei are omitted.

cleata are as fine. The greater thickness of the body in *O. macronucleata* must make its nuclei a little more difficult to study.

Observation of the nuclei of *O. antilliensis* has shown a very interesting condition as to the chromosomes, which my studies of *O. intestinalis* and *O. caudata* did not reveal. There are two distinct sets of chromosomes, one massive and the other granular, both sets lying just beneath the the nuclear membrane (cf. figs. 12 and 13). Leger and Duboscq have described similar granular linear chromosomes and massive "parachromosomes" in *O. saturnalis*. I can confirm their description of these conditions in *O. saturnalis* from my own study of this species. Comparisons between *O. saturnalis* and *O. antilliensis* must be left for the more complete paper now in preparation.

The massive chromosomes in *O. antilliensis* resemble those of *O. intestinalis* and *O. caudata*. Some are large, some small, some intermediate in size. As in *O. intestinalis* and *O. caudata* there are several characteristic sizes and shapes, and chromosomes of these sizes and shapes are seen in every nucleus during the anaphases of mitosis. During the anaphase stages the several diverse chromosomes at one end of the nucleus correspond each to a chromosome of the same size and form in the other end of the same nucleus, and the pattern thus shown in one nucleus is closely reproduced in any other nucleus in a corresponding stage of mitosis. The massive, and also the granular daughter chromosomes, are connected in pairs across the equator of the nucleus by easily staining threads that aid in determining which two chromosomes belong to each pair.

The granular chromosomes consist of lines of granules which are hardly larger, but stain more deeply, than the achromatic granules. The achromatic granules are found thru the whole nucleus, not being confined, as are the chromatin elements, to the superficial layer. The granular chromosomes differ considerably in the number of granules they contain. One cannot have entire confidence in the accuracy of his counting of these granules, but it can be said that in some of the longer of these chromosomes there are more than twice as many granules as in the shortest. The chromosomes of any one pair, one at each end of the spindle, are alike.

I have not yet reexamined the nuclei of *Opalina intestinalis* and *O. caudata* with sufficient care to be sure of the condition of the granular chromosomes in these species, tho it is evident they are present. Fresh material of these species is not obtainable in America.

Interesting as is this remarkable double series of chromosomes in *Opalina antilliensis*, its interest in enhanced when we realize how different are the conditions in the multinucleate Opalinas. The accompanying series of figures of mitotic phenomena (figs. 14 to 21) are from the nuclei of one of two multinucleate species of *Opalina* abundant in the large toad of California, *Bufo halophilus*. This *Opalina* seems to be an undescribed species somewhat intermediate between *O. ranarum* and *O. obtrigona*. I do not here describe and name it, reserving this until I publish a more general systematic study of the Opalinas. In my notes I am calling it *O. intermedia*, and for convenience will so refer to it here, but the name may not stand.

In the resting condition the nuclei of this species resemble those of *O. ranarum*, *O. obtrigona*, *O. dimidiata*, *O. flava* and other as yet

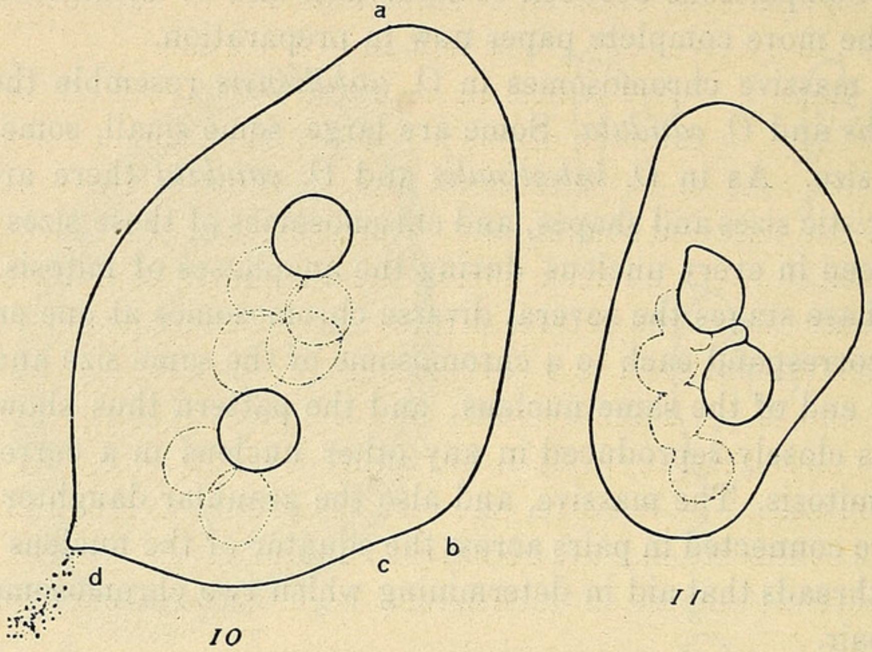
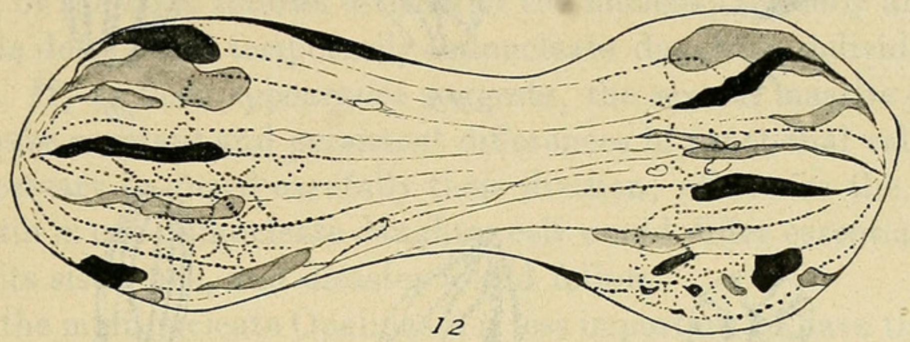


Fig. 10. An individual of *Opalina antilliensis*, showing some of the axial vacuoles of the excretory system, also the slight posterior protuberance, and a trailing mass of excreta. × 280 diameters.

Fig. 11. An individual of *O. antilliensis*, showing excretory vacuoles. × 280 diameters.

undescribed multinucleate species I have studied. There is a network of chromatin beneath the nuclear membrane, the nodes of the network being enlarged into irregular masses of unequal size. In addition there are large clumps of chromatin clinging to the inside of the nuclear membrane. As mitosis approaches, the chromatin network resolves itself into lines of granules which appear later, in the spindle stage, as linear granular chromosomes resembling those of the granular type in *O. antilliensis*, as described above, and they separate to the daughter nuclei in the same way. The coarse chromatin masses on the other hand, do not form chromosomes at all. There are no massive chromosomes in the multinucleate Opalinas. Upon the spindle, along with the linear granular chromosomes, one finds from one to four or five irregular masses of chromatin, which do not divide during the mitosis, but are carried undivided into one or the other of the daughter nuclei. It is rather usual to find such a chromatin mass at or near each end of the spindle, but in numerous nuclei no such arrangement is observed. Occasionally one sees a dividing nucleus in which a mass of chromatin lies at the equator of the spindle, and sometimes this chromatin mass fails to divide or promptly to pass intact into either daughter nucleus. In fig. 20 is shown one such chromatin mass which appears as if it might fail to be included in either daughter nucleus. No care is taken in the multinucleate Opalinas



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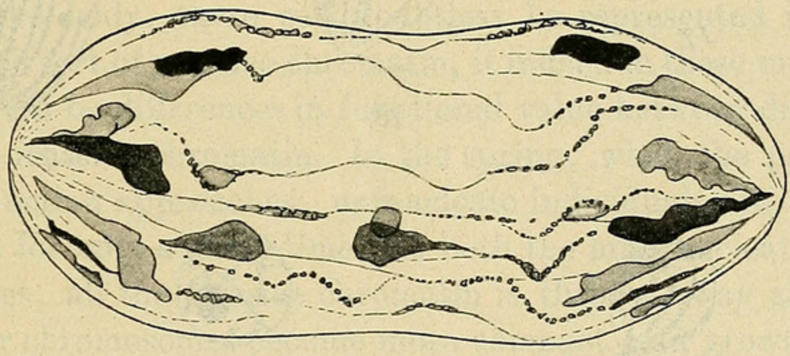

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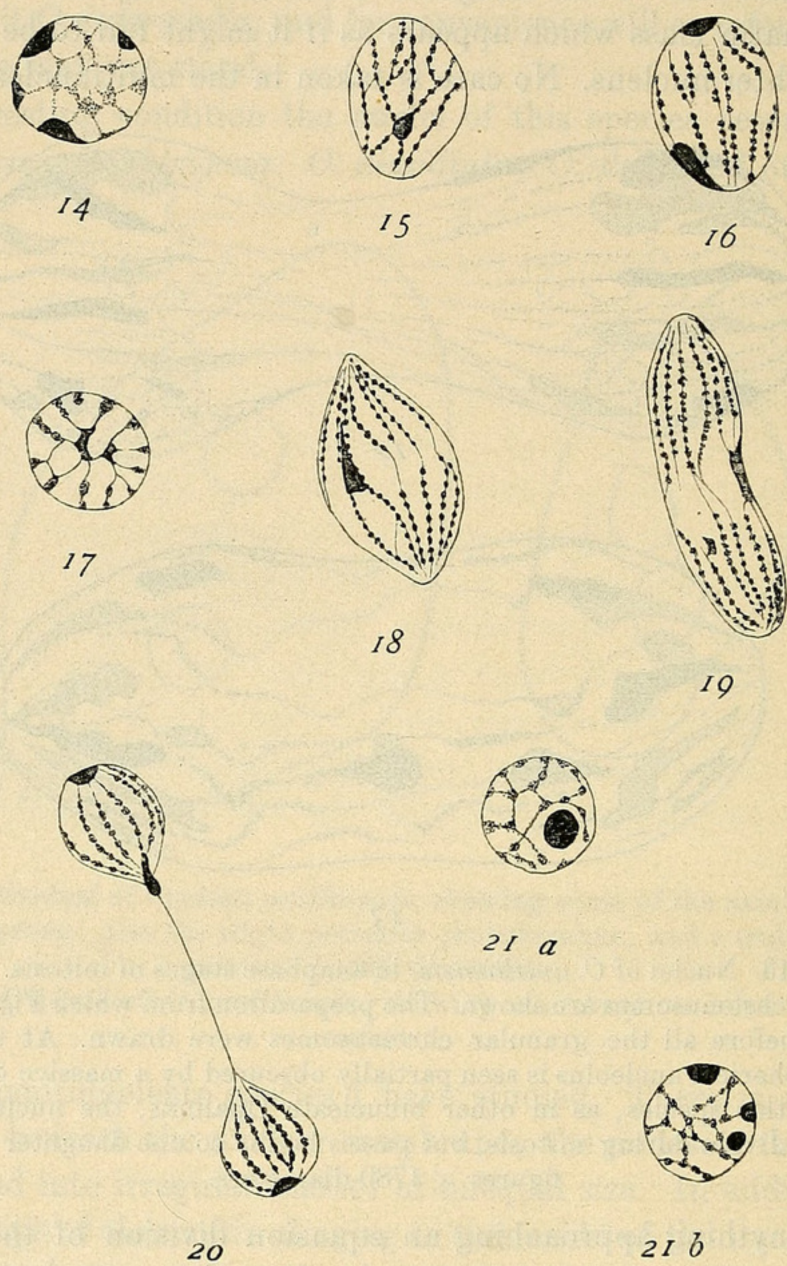
Figs. 12 and 13. Nuclei of *O. antilliensis*, in anaphase stages of mitosis. Both massive and granular chromosomes are shown. The preparation from which Fig. 13 was taken was injured before all the granular chromosomes were drawn. At the equator of Fig. 13 the spherical nucleolus is seen partially obscured by a massive daughter chromosome. In this species, as in other binucleate Opalinas, the nucleolus does not disappear or divide during mitosis, but passes bodily to one daughter nucleus. Both figures × 1780 diameters.

to secure anything approaching an equasion division of the chromatin masses.

Comparison of the resting nuclei in multinucleate Opalinas shows the apparent homology between the chromatin masses in the two groups, while study of the mitotic phenomena in the two groups indicates the similarity of the granular chromosomes in the two. The multinucleate and the binucleate forms take equal and very similar care of the granular chromatin in mitosis, but they differ greatly in the attention paid to the massive chromatin in the division of the nuclei. The binucleate forms manipulate the chromatin masses as carefully as the granular chromosomes, dividing and distributing them as accurately, and retaining among them the characteristic diverse forms and sizes always recognisable in the massive chromosomes during the anaphases of mitosis. The multinucleate Opalinas, on the other hand, neglect the chromatin masses, making no provision for their division or for the presence of even ap-



Figs. 14 to 21. Resting nucleus and mitosis in *Opalina intermedia*. × 1780 diameters. Fig. 14, a resting nucleus. Four masses of chromatin are seen clinging to the inside of the nuclear membrane. A more delicate network, also just beneath the nuclear membrane, is shown. The achromatic structures are not drawn. Fig. 16, a nucleus entering upon mitosis. One bit of massive chromatin is seen on this, the upper, surface of the nucleus, also numerous granules arranged in lines. Fig. 16, a nucleus in an early anaphase stage of mitosis. Fig. 17, an end view of a nucleus similar to that shown in Fig. 16 or Fig. 18. Fig. 18, a later anaphase. Fig. 19, a still later anaphase. The nucleus is beginning to constrict at the equator. Fig. 20, a very late anaphase, the two daughter nuclei being almost separated. Fig. 21, two daughter nuclei, *a* and *b*, in the resting condition.

proximately equal quantities of massive chromatin in the two daughter nuclei.

The phenomena observed in *Opalina intestinalis* and *O. caudata* suggest that Hertwig's distinction between trophochromatin and idiochromatin may here apply. The massive chromatin, which is thrown away bodily before the sexual phenomena are completed, seems plainly to be not reproductive. Accepting Hertwig's term we will call it trophic.

In the binucleate Opalinas, from time to time thruout the year division of the body follows division of the nucleus, tho only after considerable delay, and temporarily uninucleate daughter individuals are formed. If, as their appearance suggests, the several massive chromosomes have constant and persistent differences in functional value, then it is necessary to guard carefully their division; otherwise the massive chromatin in one uninucleate daughter cell would differ essentially from that of its sister cell, and disaster would follow.

In the multinucleate Opalinas it is less important to have the nuclei all exactly equivalent, for among the many nuclei, in spite of occasional division of the body, there will doubtless be represented in sufficient quantity each sort of massive chromatin, if indeed in these multinucleate Opalinas there be differences in functional value between different portions of the massive chromatin. In the spring, when the period of sexual reproduction approaches, uninucleate individuals are formed but they persist for only a short time. In both the multinucleate and binucleate species, all the massive chromatin is thrown away at this time, the granular chromosomes become more compact, and growth processes are at a low ebb, until the readjustments resulting from the sexual process are accomplished. Then new massive chromatin is formed from the granular chromatin, much as in Paramecium the trophic macronucleus arises from an originally idiochromatic micronucleus. The difference in the care exercised by binucleate and multinucleate species in dividing the trophic massive chromatin is probably due to the less need of exact equivalence of trophic qualities between the nuclei of forms which for all but a week or so in the year live in a multinucleate condition.

Further discussion of these conditions and relations I prefer to postpone until I shall have followed both sets of chromosomes in *O. antillien-sis* thru the sexual reproductive phases of the life cycle.

II. *Opalina antilliensis*, new species.

In the rectum of the large toad, *Bufo agua*[1], of Jamaica, West Indies, are generally many individuals of an unusually interesting *Opa-*

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[1] I am greatly indebted to Professor E. A. Andrews of Johns Hopkins University for half a dozen living specimes of this toad, as will as for other Amphibian material from Jamaica, containing Opalinas of much interest.

lina. In the accompanying paper, "Chromosomes in *Opalina*", some of the features of its nuclear structure are figured and described. A brief description of its diagnostic characters, with figures, is here given.

Opalina antilliensis is a binucleated, much flattened species resembling the less flattened *O. macronucleata* Bezzenberger, and very closely resembling *O. binucleata* Raff. Raff figures the lines of cilia as longitudinal in her species, instead of oblique, or more properly spiral, as in all other species. The nuclei as figured by Raff are much smaller than in *O. antilliensis*, but Raff's statement of the dimensions agrees with what I find in *O. antilliensis*. If the stated dimensions, rather than those figured, in Raff's paper are correct, and if the lines of cilia were spiral instead of longitudinal as figured, the agreement with *O. antilliensis* would be exact. But, of course, Raff's description must be taken as it stands. Even if there were no observable differences in structure between the two species, one from *Bufo agua* in Jamaica, the other from *Limnodynastes dorsalis* and L. *tasmaniensis* from Australia, my study of the genus as a whole and of its tendency to most completely intergrading speciation would lead me to doubt the consanguinity of these two forms. I think even practical identity of structure would not be a sufficient indication of consanguinity in forms of such diverse and widely separated habitats. This question, however, must be reserved for discussion in a more comprehensive study of the taxonomy in this genus.

In form *Opalina antilliensis* varies between the limits shown in the accompanying figures. Individuals that have recently come from longitudinal division, i. e. division parallel to the lines of cilia[2], may be broadly triangular (fig. 1). Individuals recently come from transverse division, i. e. division transverse to the lines of cilia, may be much more slender (fig. 2). The usual shape is in general broadly oval (fig. 4), but one familiar with the form of other species of *Opalina* recognises several features whose significance is best brought out by comparison.

In all the Opalinas, the anterior end of the body is bent to one side. In the cylindrical forms, whether binucleate like *O. intestinalis* (fig. 5), or multinucleate like *O. dimidiata* (fig. 7), this is very evident. In the flattened forms this bending of the anterior end to one side is obscured by the flattening and by the fact that the anterior end is usually very broad (fig. 9). In figure 8 the anterior end is the portion between *a* and *b*. It is demarcated from the rest of the body, on one side, by a more or less evident indentation *c*. The posterior end is shown at *d*. The lines of cilia are slightly oblique, i. e. spiral, as in all holotrichous Ciliata.

[2] Usually called oblique division.

On one side of the posterior end, in many individuals, is a more or less well developed point (fig. 10) similar to that seen in some individuals of *O. caudata* (fig. 6) or *O. obtrigona*, or to the larger protuberance sometimes observed beside the posterior end in *O. zelleri* (fig. 8). It is directly at the base of this protuberance, when present, that the pore of the excretory vacuole opens to discharge its granular and liquid contents. Parts of the vacuole itself have been observed in a number of individuals (figs. 10 and 11) and in many more the sticky discharged contents have been seen attached to the posterior cilia and trailing behind as the animal swims (fig. 10).

The number of rows of cilia in this, as in all Opalinas varies with the size of the individuals. They are accurately drawn in fig. 4, which shows them forty-eight in number at the anterior end. In eight cases the branching of lines of cilia is shown. In other individuals, of course, the number of lines of cilia would be different.

Dimensions of large individual: Length of body, measured from *a* to *d* (fig. 4), 0,2 mm.: width of body, measured at right angles to this line at the widest place, 0,114 mm.: diameter of resting nuclei in full sized individuals, 0,0243 mm. The flattened form of the body is shown in the section, fig. 3, in which the diameter of the nuclei is seen to practically equal the thickeness of the endosarc, the dotted line in this figure indicating the boundary between endosarc and ectosarc.

The nuclei are more nearly central than in most binucleated forms. In early stages of mitosis the long axes of the nuclei lie nearly transverse to the lines of cilia. Later they come to lie more nearly parallel to them. The character of the chromosomes in this species is shown in the accompanying paper upon Chromosomes in *Opalina*.

In the specimens of *Bufo agua* I have had for study *Opalina antilliensis* has been the only *Opalina* present.

3. Zur Kenntnis der Harpacticidengattung Epactophanes Mrázek.

Von Erich Keßler, Leipzig.

(Mit 6 Figuren.)

eingeg. 14. April 1914.

Ehe ich auf meine eignen Untersuchungen, die Gattung *Epactophanes* betreffend, eingehe, möchte ich kurz auf einige Tatsachen, die Menzel (4) in seiner Arbeit in dieser Zeitschrift (Bd. XLIII, Nr. 13 vom 17. März 1914) festgestellt hat, hinweisen.

Menzel versucht an der Hand der Beschreibung von *Moraria muscicola* zu zeigen, daß wir es bei diesem von Richters (7) 1900 beschriebenen Harpacticiden, »auch was die allgemeine Körpergestalt anbelangt, mit einem typischen Vertreter der Gattung *Moraria* Scott (=