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Notes on a New Boveria Species, *Boveria labialis* n. sp.

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With 21 textfigures.

In the summer of 1914, one of the authors discovered two different parasitic ciliates, belonging to the genera *Boveria* and *Licnophora* and co-inhabiting the respiratory trees of a *Cucumaria* species and also of *Stichopus japonicus*. The genus *Boveria* was erected recently by STEVENS (1901) for a parasitic ciliate (*Boveria subcylindrica*) found in the respiratory trees of *Holothuria californica*, in Monterey Bay, California. Later (1904) the same authoress[1] described from two species of Neapolitan bivalves, *Tellina exigua* and *Capsa fragilis*, another form of *Boveria*, which she regarded as a variety of *Boveria subcylindrica*, calling it *B. subcylindrica* var. *neapolitana*. She gave a detailed description of the external characters, the binary fission, and the nuclear division of the two forms, yet left such important matters as the conjugation and

1) STEVENS, N. M. Further studies on the ciliate Infusoria *Licnophora* and *Boveria*. Arch. f. Prot. K., Bd. III, 1904.

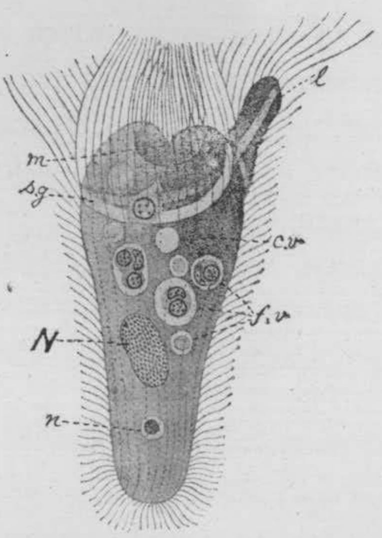
the encystment entirely untouched. In the *Boveria* species studied by us, we have been fortunate enough to make observations on these phenomena, the results of which seem to deserve publishing. As to the *Licnophora* species, we have not yet finished our studies, so that we will reserve its description until a future occasion.

Before going further, we feel it our pleasant duty to express our hearty thanks to Professor Ijima for his kind advice and criticism during the writing and publishing of this paper. We are also much indebted to Mr. B. Aoki, formerly of the Anatomical Laboratory of the Kiu-Shiu Imperial University, for his kind help in the matter of obtaining some of the relevant literature.

I. Description of *Boveria labialis* n. sp.

The present species of *Boveria* lives in abundance not only in the respiratory trees of the holothurian hosts mentioned before, but also in the gill-lamellæ of a *Tellina* species. The animals (fig. 1) are of the shape of a trumpet, being 31—100 μ in length. The aboral end, with which the animals are usually found adhering to the inner surface of the respiratory organs of the host, is rounded and measures, as ascertained on preparations fixed with Schaudinn's solution, 8—16 μ in diameter. The broader, slightly elevated end, measuring 16—26 μ in width, represents the peristomal disc. Appended to it on one side, is an anteriorly directed rod-like

Fig. 1.



An individual 60 μ long. Del's. hæmatoxylin. *c.v.*, contractile vacuole; *f.v.*, food vacuoles; *l.*, lip-like process of peristomal disc; *m.*, mouth; *N*, meganucleus; *n*, micronucleus; *s.g.*, spiral groove.

process, the lip (*l*), 10—15 μ long. The mouth (*m*) is a shallow but tolerably wide oval depression placed nearly at the centre of the peristomal disc. The margin of the disc is bordered by a narrow and very shallow groove (*s.g.*), which starts from the very tip of the lip, and after taking a spiral course of about $1^5/_6$ turns, ends in the mouth-depression. The two ridges bordering this spiral groove carry each a single series of strong cilia, which are about $^1/_3$ as long as the body-length. The remainder of the body-surface is covered by finer and much shorter cilia arranged in 20—26 longitudinal rows,

The contractile vacuole (*c.v.*) may be seen in the living specimens as a small clear vesicle, lying generally beneath the peristomal ciliated groove. The mouth is not followed by a cytopharynx. Nor is the finely granular lenticular disc present, which is said to exist in the aboral region of *Boveria subcylindrica*. There are several food-vacuoles of varying sizes, mostly situated in the anterior half of the body. Of the two nuclei, which are hardly visible in the fresh state, the elliptical meganucleus (fig. 1, *N*) is situated usually in the middle of the body, while the vesicular micronucleus (*n*) lies near the aboral end, The meganucleus in the resting state appears, when examined in the fixed and stained state, as a thick mass of minute, homogeneously deeply stained granules on a very delicate network of linin, the whole structure being bounded against the entoplasm by a faintly stainable nuclear membrane. But, in individuals which are in the process of fission or of conjugation, the meganucleus reveals a structure very different from that mentioned above; that is, while the majority of the nuclear granules, which seem to represent plasmosomes, are contracted into a slightly stainable central mass, thus giving rise to a narrow clear area between this mass and the nuclear membrane, a few, which are deeply stained with hæmatoxylin and no doubt consist of chromatin, are separated from the central granular mass and attached to the nuclear membrane. The micronucleus in the resting state is vesicular and contains the chromatin massed together in the centre, thus a clear peripheral space being produced inside the nuclear membrane. When the micronuclear division sets in, the central chromatin mass is transformed into chromosomes, which are always four in number.

The present species in question differs markedly from the known American species as well as its Neapolitan variety in possessing the prominent lip on the peristomal disc. From the Neapolitan variety it further differs in the rounded, instead of the pointed, shape of the aboral end and in the larger extension of the oral ciliary spire. The following table shows other points of differences between the three forms of *Boveria*.

	Host	Body length	Breadth of oral end	Breadth of aboral end	Form of aboral end	Oral ciliary spire	Lenticular disc	Lip
Californian form	*Holothuria californica*	54 – 81μ	18—21μ	9—15μ	rounded	1 turn and 290°	present	absent
Neapolitan form	*Tellina exigua*, *Capsa fragilis*	37—102μ 65—121μ	20μ (on an average)		pointed	1 turn and 210°	absent	absent
Japanese form	*Stichopus japonicus*, and *Tellina* sp.	32 – 100μ	18—26μ	8—16μ	rounded	1 turn and 290°	absent	present

We feel fully justified in regarding the Japanese form as a new species, for which we propose the name of *Boveria labialis*.

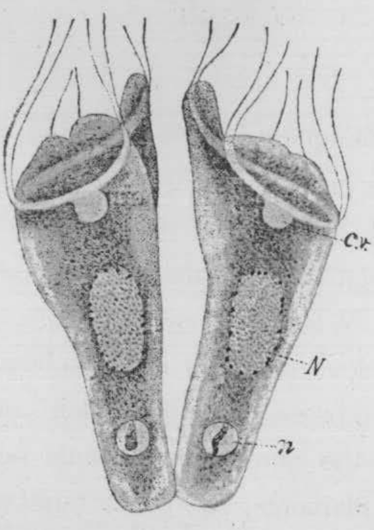
II. Conjugation.

In all well investigated ciliates, conjugation is known to take place after fissions frequently repeated during a prolonged period. This is also the case with the present species, the conjugation occurring periodically. As the large number of individuals living inside the respiratory tree of the same host must have originated from a single or a few progenitors and should have passed approximately the same number of generations, they may reasonably be assumed to be at a given moment all nearly the same in their physiological condition. Probably this explains to a degree why in *Boveria labialis* the majority of individuals in the same host are found simultaneously in conjugation, if they are found at all in that stage. It is, therefore, necessary to take several holothurians infested by the ciliates, in order to obtain the different stages of this reproductive phenomenon. Good permanent preparations were made by lightly smearing the respiratory tree containing the parasites on slips, and by treating with SCHAUDINN's solution, afterwards staining with iron-hæmatoxylin and orange-G. We also prepared sections, making use of various combinations of fixing and staining reagents, but with no specially recommendable results.

The conjugation of *Boveria* belongs to the isogamous type. Nevertheless, it presents some interesting points of deviation from the common type attributed to the Ciliata. To mention some of the most remarkable points; (1) the two conjugants come into mutual attachment with their aboral ends, instead of with the oral; (2) the meganucleus in each conjugant does not disappear, as it usually does, but persists after conjugation, eventually becoming coalesced with derivatives of the original synkarion; (3) the micronucleus of each conjugant undergoes divisions, of which the first division is invariably amitotic and the second and third mitotic, these latter giving rise to numerous fine chromatin threads, while in ex-conjugants the micronucleus divides always mitotically and gives rise to four distinct chromosomes. The third point seems to be particularly noteworthy, since it involves facts which seem to suggest an explanation as to the mutual relation of mitosis and amitosis both ontogenetically and phylogenetically. It is interesting to note that LAPAGE and WADSWORTH[1] have recently reported from *Dendrocometes paradoxus* an interesting case of the heterogeneity of micronuclear division, which is, however, less complicated than the present case. According to the authors, distinct chromosomes arise in the divisions during conjugation, while in the divisions during bud-formation there appear simple minute chromatin granules instead of chromosomes.

To begin with the changes of the micronucleus, leading to the formation of the conjugation-spindle, When two conjugants come into mutual attachment, the micronucleus (fig. 2, *n*), which is still in its original position, begins to swell. Then the entire micronucleus gradually elongates, first into an elliptical shape and later into that of a bow with swollen ends (fig. 3, *n*). The micronucleus thus transformed finds its place always on one side of, and closely to, the meganucleus (*N*), which has up to this moment remained unchanged in position and

Fig. 2.



Figures 2–13 relate to the conjugation. Iron-hæmatoxylin.

1) LAPAGE, G., and WADSWORTH J. T.—On *Dendrocometes paradoxus ;* Part II, Reproduction. Q. J. M. S., Vol. 61, 1916.

structure. This stage of the micronuclear division seems to last for a comparatively long time, since it is very often met with in conjugating individuals. The two ends of the bow-like micronucleus become more and more swollen, while the intermediate parts are thinned out and finally become cut off in the middle. Neither spindle fibres nor chromatin threads make their appearance in the

Fig. 3.

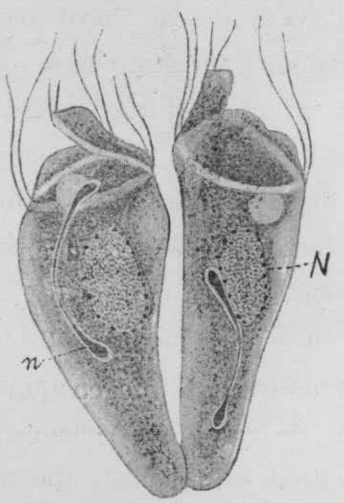
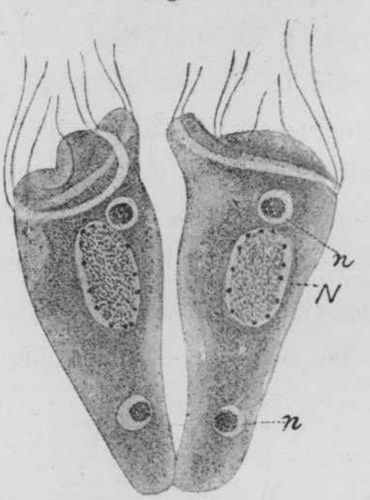


Fig. 4.



meanwhile. After the division, both daughter nuclei reassume a spherical shape and enter into the resting stage (fig 4, *n*), when all the chromatin substance is massed together in the centre of a clear space which is bounded all round by a delicate nuclear membrane.

The second division of the micronucleus proceeds in the following way. The spherical nucleus first becomes slightly swollen and afterwards spindle-shaped; meanwhile the chromatin contents become arranged into fine and numerous filaments, the exact number of which cannot be made out (fig. 5, *n*). The spindle thus formed takes in the sequel the shape of a dumb-bell, the two ends of which contain each a group of fine chromatin threads. When the

Fig. 5.

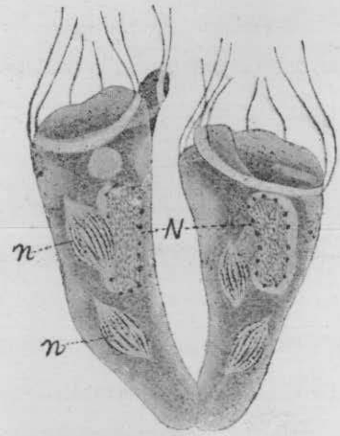
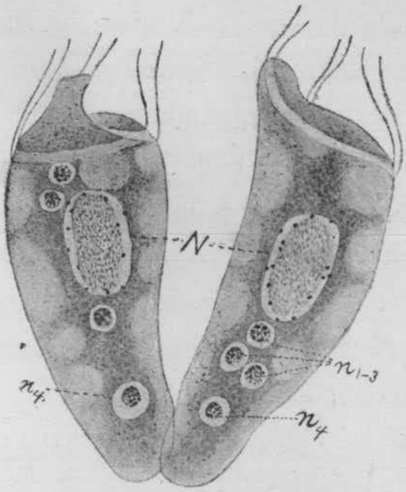


Fig. 6.



second division is over, there are produced in each conjugant four spherical micronuclei (fig. 6, n_1,—n_4), each consisting, as before, of a central chromatin mass and a clear peripheral space. Three of these micronuclei (fig. 7, n_1—n_3) are destined to degenerate and disappear sooner or later, while the remaining one (n_4) persists and undergoes further changes preparatory to the third or the ripening,

Fig. 7.

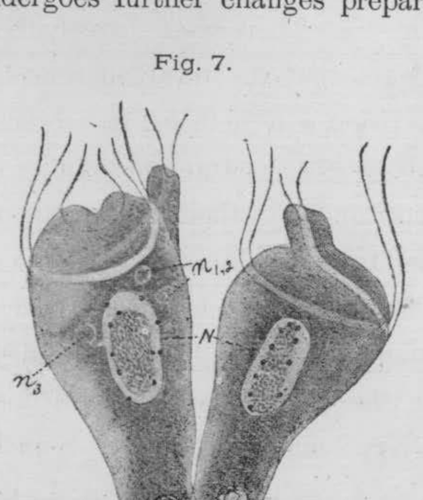
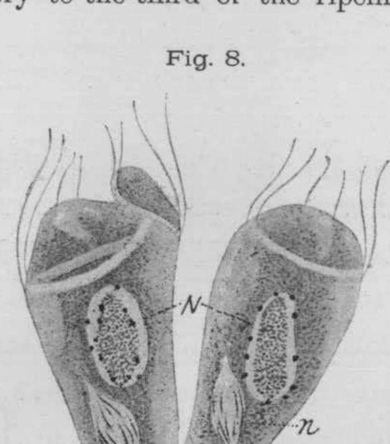
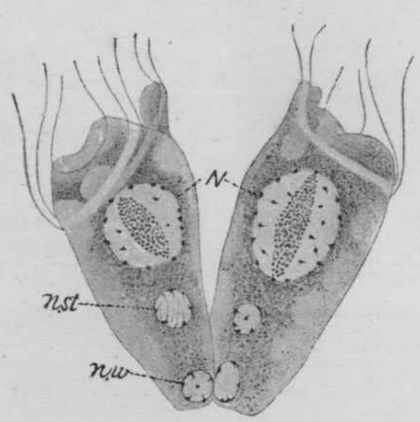


Fig. 8.



division. In this case, the hitherto compact chromatin mass gradually loosens and dissolves itself into separate granules, which soon rearrange themselves into short threads irregularly disposed in the clear micronuclear space (see n_4 in fig. 7). After that the micronucleus takes the shape of a spindle of the usual appearance (fig. 8, *n*). The chromosomal threads are now seen to consist each of a linear series of minute granules. They are so fine and so closely set together that they cannot be counted, nor the mode of their distribution exactly be made out. The two spherical micronuclei (fig. 9, *n. st.* and *n. w.*), resulting from the third division, closely resemble in structure the mother nucleus (fig. 7, n_4) before the spindle formation, except in one important fact, that the chromatin structures contained in either one are noticeably less both in number and in quantity than in the mother nucleus (*cf.* fig. 7 with fig. 9). There can scarcely be a doubt that the third micronuclear division is a reducing one, brought about by the chromosomal threads in the maturation spindle not undergoing a splitting of any kind, and by their becoming distributed in equal parts between the two daughter micronuclei. It is to be mentioned that R. Hertwig (1888) has made out in ***Paramecium aurelia*** that its sexual nucleus contains about half as many "Chromatin-elemente" as the micronucleus of ordinary individuals. This seems to be very similar to the reducing process in the present case.

Fig. 9.



The two micronuclei resulting from the above ripening division in each conjugant are the stationary and the wandering micronuclei. In both the chromatin never concentrates, accordingly the peripheral clear space inside the nuclear membrane does not appear. The wandering nucleus shifts its position to the aboral end of the body, *i.e.*, to the point of adherence of the conjugants, and the two corresponding nuclei (fig. 9, *n.w.*) stand side by side without moving for a relatively long period. This seems to be due, in part at least, to the slowness with which the plasmic fusion is accomplished between the two conjugants. Unfortunately we have not succeeded in directly observing the interchanging migration of the wandering nuclei. But no doubt this really occurs, followed by the coalescenee of the immigrated nucleus with the stationary nucleus in each conjugant, as the result of which process the synkarion is formed (fig. 10, *sy.*). This is about twice as large as either of the two coalescing micronuclei, and contains numerous chromatin threads which run on the whole parallel to one another. Soon afterwards the chromatin threads condense and eventually become rearranged into a single, moderately thick and continuous ring twisted in the manner of the figure 8 (figs. 11, *a*). This ring lies close to the nuclear membrane. The entire synkarion now begins to elongate so as to acquire the shape of a spindle; in the meanwhile the 8-shaped chromatin thread becomes discontinuous at one point and gives rise to a long and irregularly coiled thread (fig. 11, *b*) which takes a central position. This single chromatin thread divides twice transversely,

Fig. 10.

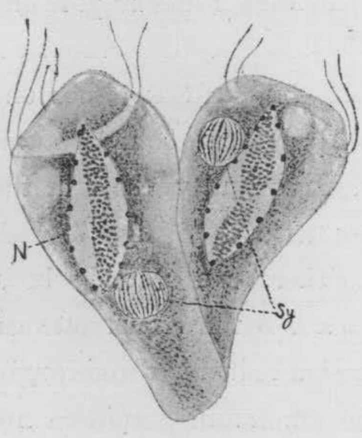
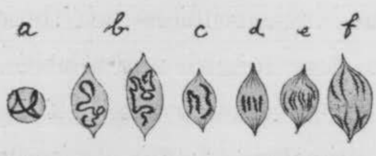


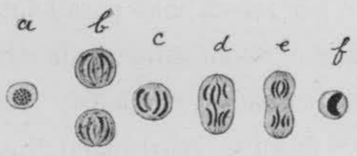
Fig. 11.



giving rise to four chromosomes of about equal length (fig. 11, *c* and *d*). These chromosomes arrange themselves in equatorial parts of the spindle, all disposed parallel to one another and to the spindle axis. In this position each of them splits longitudinally, thus giving rise to eight slender chromosomes (fig. 11, *e*). After that, half the number of the chromosomes—that is to say, four of them—travel to either pole of the spindle (fig. 11, *f*). A set of fine diverging fibres, extending from the poles into the interior of the spindle, is visible in properly manipulated prep rations. We have not detected in the neighbourhood of the poles any structure which may be construed to represent the centrosome. The entire spindle now stretches out extensively; its middle parts gradually thin out, until it is there cut off into two, thus bringing the first division of the synkarion to an end.

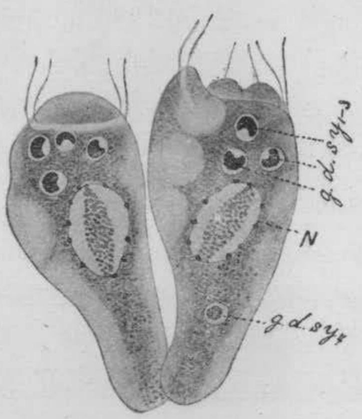
Each of the two daughter synkarions soon afterwards enters into a resting or reconstruction period (fig. 12, *a*). During this period the chromosomes disintegrate into separate granules, which are aggregated together not very densely in the central parts of the now spherical daughter synkarion. This state seems to last only for a short time. Then, the chromatin granucles are loosened and rearrange themselves into an entangled spireme-like band (fig. 12, *b*). This band becomes discontinuous at three places, and by condensation brings forth four longish and relatively thick chromosomes lying parallel to one another (fig. 12, *c*). Now the daughter synkarions are each transformed into a spindle figure (fig. 12, *d*) similar to that of the first synkarion division. The spindle gradually elongates, in the sequel becoming constricted in the middle. In this second division of the synkarion the chromosomes do not perform longitudinal splitting, as they did in

Fig. 12.



the first division, but divide transversely, so that there arise two sets of four parallel chromosomes, the sets soon afterwards to be divided between the resulting two grand-daughter synkarions (fig. 12, *d* and *e*). The chromatin contracts into a compact mass in all these four nuclei of a conjugant. In three of them, this mass assumes the shape of a crescent or a comma (fig. 12, *f*, and fig. 13, *g. d.* sy_{1-3}), while the remaining one is peculiar in having the chromatin mass of a spherical form (fig. 13, *g. d.* sy_4). It may here be mentioned at once that the latter is the one which persists as the micronucleus in the ex-conjugant. Now the process of conjugation is at an end, and the two conjugants begin to detach themselves from each other.

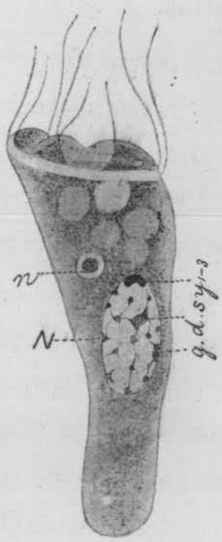
Fig. 13.



We will now turn our attention to the meganucleus in each conjugant. During the early stage of conjugation the meganucleus shows little change in its appearance, except in one important point: that the granular nuclear plasm has differentiated into two parts, that is, the majority of the granules, which are only lightly stained with hæmatoxylin, and a few others which lie around the former and are stained very deeply with hæmatoxylin (fig. 2, *N*). In our opinion, the granules of the former kind represent plasmosomes, while those of the latter kind consist of chromatin. About the time when the micronucleus of the conjugants has first divided into two (fig. 4), the meganuclear granular plasm begins to contract, leaving a clear space between it and the nuclear membrane. This clear space grows gradually wider (*cf.* figs. 5—9, *N*), excepting at two opposite ends of the major axis of the ellipitical meganucleus, where the meganuclear granular plasm is directly, or almost directly, attached to the meganuclear membrane (figs. 9, 10, and 13, *N*). The above contraction process of the granular plasm occurs on the outset equally in both the plasmosomic and the chromatin granules (figs. 4 and 5). But this is not so later, when the intranuclear peripheral clear space has grown wider than before (figs. 6—10, *N*), then the peripherally situated chromatin granules cease to partake in the further contraction of the plasmosomic granules, and begin to liberate themselves from the last mentioned eventually to attach themselves to the meganuclear membrane. Although it is very difficult to decide if the granules so disposed represent the entire chromatin in the meganucleus, yet we are inclined to believe that to be the case, since in preparations treated with differential stains, there exist in the central granular mass so far contracted no bodies which show staining reactions for chromatin. No further change takes place in the meganucleus during the process of conjugation. It never manifests any sign of disintegration, which, in other infusorians, usually befalls the meganucleus at a certain period of conjugation. On the contrary, in the present species, the conjugant meganucleus persists in the state described above not only during conjugation but even after it. In this respect the species offers a remarkable exception to the general rule among the infusorians.

The formation of nuclei in the ex-conjugants goes on in the following way. Of the four small nuclei resulting from the twice repeated division of the synkarion, those three, in which the chromatin has acquired a comma-like shape (fig. 13, *g. d.* sy_{1-3}), undergo degenerative changes and cease to exist as independent structures, but attach themselves to, and then become incorporated with the meganucleus. In the meganucleus of an ex-conjugant represented in fig. 14, there are found three relatively large and deeply stained dots (*g. d.* sy_{1-3}), which are conspicuously larger than the ordinary

Fig. 14.



An ex-conjugant just after the conjugation. Iron-hæmoxylin. In the interior of the meganucleus (*N*) there exist the three quarter-portions of the synkarion (*g. d.* sy_{1-3}). The plasmosomic granucles in the meganucleus are not represented.

meganuclear chromatin granules visible there —in this figure the deeply lying plasmosomic granular mass is not represented. They are nothing else, in our view, than the three quarter-portions of the synkarion above referred to and incorporated with the meganucleus. We are not quite clear about the true significance of this fusion of the three quarter-portions of the synkarion with the old meganucleus, unless it be an indication of the physiological rejuvenescence or reinforcement of the latter. As an indirect evidence of the above view, it may be pointed out that, as soon as the amalgamation of the nuclei is over the ex-conjugant begins to take food and grows a little larger, while the renovated meganucleus returns to its normal appearance, *i.e.*, the state in which the central granular mass expands and occupies the whole interior of the nuclear membrane, and the peripherally situated chromatin granules are lost to sight as such and sink in between the plasmosomic granules. It is a highly interesting fact that, in the first and second fissions which occur after the conjugation and details of which will be given in the next section, the three quarter-portions of the synkarion incorporated with the meganucleus reappear in the dividing meganucleus (figs, 15 and 16, *g. d.* sy_{1-3}). This phenomenon never occurs in the ordinary fission of the animal, and may therefore be said to be peculiar to the first two fissions of ex-conjugants.

III. Fissions following the conjugation.

The most prominent cytological changes accompanying the first fission of the ex-conjugant are presented by both its micronucleus and meganucleus. The process of the first micronuclear division is entirely similar to that described by STEVENS in the Neapolitan form of *Boveria*. The chromatin mass first contracts into a crescent-like shape and subsequently divides, by two cleavage planes crossing each other at right angles, into four comparatively thick chromosomes which later are so disposed as to be parallel to one another. Then the entire micronucleus is transformed into a long spindle, the four chromosomes taking the equatorial position as usual. The subsequent processes are on the whole similar to those before described in the divisions of the synkarion. The two daughter micronuclei (fig. 15, *n*) on entering the resting stage, separate from each other and place themselves one at each end of the now elongated meganucleus. The meganucleus elongates more and more in the direction of the body-axis; meanwhile, its granular contents contract and divide into three nearly equal-sized masses, lying one behind the other. In the two clear spaces between the middle and the other two masses, there are found in all three chromatin granules (fig. 15, *g. d.* sy_{1-3}), which are conspicuously larger than any of the darkly staining chromatin granules in each of the three meganuclear granular masses. They vary somewhat in shape, but are in most cases crescentic. As pointed out before,

Fig. 15.

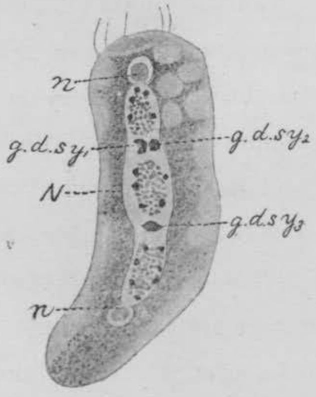


Figure 15—17 relate to the first binary fission after the conjugation. Iron-hæmatoxylin. All the reference letters are the same as those in the preceding figures.

these cannot be anything else than the three grand-daughter synkarions which were taken up by the persisting meganucleus.

The meganucleus now begins to divide by simple constriction at the two clear spaces between the three granular masses. The result of the division: three meganuclei of a spherical shape and approximately uniform size, are simultaneously produced each including, besides its own small chromatin as well as plasmosomic granules, one of the three large chromatin masses derived from

Fig. 16

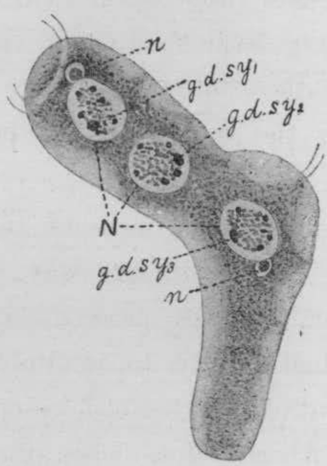
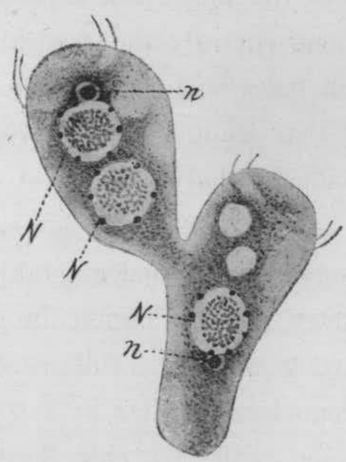


Fig. 17.



the original synkarion (see fig. 16). After this trinal division of the meganucleus, the oblique transverse binary fission of the ex-conjugant commences. As to the morphological changes which the cytoplasmic structures undergo during this fission, there is an essential agreement with those taking place in the ordinary binary fission of the species (see later) and also with those described by STEVENS in *Boveria subcylindrica*, so that their full description may here be dispensed with. Only one peculiar feature may be pointed out, *viz.*, the fact that in the first binary fission of the ex-conjugant, one of the three newly arisen meganuclei passes over into the lower individual, while the other two go to the upper (fig. 17). Now in this upper individual the micronucleus divides into two, and then follows the binary fission of the body, whereby each of the two resulting individuals receives both one micronucleus and one meganucleus. The result is, that the three individuals arising from the trinal fission of an ex-conjugant contain each a meganucleus and a micronucleus. Thus they have now returned to the condition of the resting stage.

STEVENS has fully described the ordinary binary fission in *Boveria subcylindrica*. In that case, it is said that the granular part of the meganuclear plasm divides into three masses, two polar and one central; the central one again divides into two, each of which fuses with one of the polar, so that, ultimately, there exist only two granular masses representing the meganuclei of the two individuals that shall arise from the fission. In *Boveria labialis*, we have never come across a case of the division of the ex-conjugant meganucleus taking place in a similar way. The ordinary binary fission in the present species proceeds in the following way. In full-grown individuals ready to multiply, the micronucleus divides in the manner already described, essentially agreeing with STEVENS' description. At the time when the long drawn out micronuclear spindle approaches the likewise elongated meganucleus, the granular contents of the latter are found in the contracted state, the chromatin granules being found scattered on the surface of the large mass consisting of the plasmosomic granules. About this time the organism loses its cytostome as well as the oral ciliary spire. When the micronuclear division is finished, the two daughter micronuclei enter into the resting stage and come to be situated nearly at opposite poles of the meganucleus. Meanwhile, the first sign of the fission makes its appearance as light and incomplete ring-furrow running obliquely around the middle of the body. Before the fission is completed, both the upper and the lower individuals become provided with a mouth and a low oral ciliary spire. At this stage the meganucleus is already constricted into two of equal size and of quite a similar structure. The chromatin granules now leave the surface of the central plasmosomic mass and adhere to the inner surface of the nuclear membrane, exactly as they do in the individuals arising from the first fission after conjugation (see fig. 17).

From the foregoing, it will be clear that, in the present species, there may be distinguished two types of meganuclear division, namely, the trinal and the binary. The former takes place in the two successive fissions following the conjugation, the latter in the ordinary binary fission. We are of the opinion that the first binary fissions of the ex-conjugant are but a modification of trinal fission. This seems plain, not only from the trinal divison actually undergone by the ex-conjugant meganucleus, but also from the presence within the meganucleus of the three quarter-parts of the synkarion chromatin. This mode of fission of the ex-conjugant may be regarded as a process by which asexual reproduction, and at the same time, a regulatory distribution of the meganuclear and micronuclear substances are accomplished. In this sense th's modified trinal fission in question may be called the regulatory fission.

We are inclined to think that similar sorts of the two types of meganuclear division and of fission possibly exist also in the species studied by Stevens, to which the present species is closely related in many other respects.

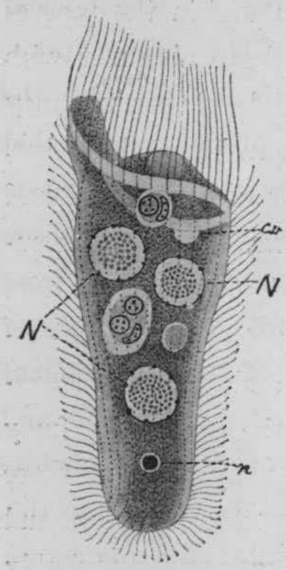
IV. Fragmentation of the meganucleus and its physiological significance.

In *Boveria labialis*, the occurrence of a single meganucleus and a single micronucleus is to be looked upon as the normal state, but frequently one meets with individuals possessing 2—6 meganuclei and a single micronucleus. Such polymeganuclear individuals are in percentage roughly as follows:

Individuals with 2 meganuclei 5%; those with 3 meganuclei 90%; those with 4 meganuclei 4%; those with 6 meganuclei 1%.

We see that individuals with 3 meganuclei (fig. 18, *N*) are by far the most prevalent. As far as our observations go, the polynuclear condition is brought about by fragmention of the single original meganucleus, which process takes place mostly after, and rarely before or during, the conjugation. We have ascertained that all the supernumerary meganuclei sooner or later disappear, and only one persists. From the indefiniteness of the period in which the fragmentation occurs, and from the persistence of the conjugant meganucleus, it is evident that the present case of meganuclear fragmentation is a process physiologically entirely different from that generally known in infusorians.

Fig. 18.

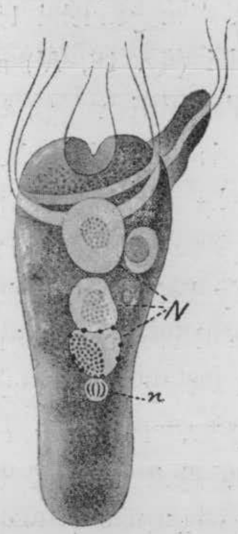


This figure and the next show individuals with supernumerary meganuclei (3 in both). Iron hæmatoxylin.

According to our opinion, the present case is to be regarded as a regulatory process of the organism, which sets in when the normal karyoplasmic relation tends to be disturbed in HERTWIG's sense, or, more precisely, when the meganuclear substance has made an excessive growth. In the present species, the conjugant meganucleus persists and moreover fuses with three of the four daughter synkarions. These facts seem, in our opinion, to provide reasons for why there must take place in the meganucleus an occasional throwing off of the nuclear matter.

It is interesting that we have frequently met with individuals with supernumerary meganuclei, in which the micronucleus (fig. 19, *n*) was found to contain four distinct chromosomes, though showing no distinct karyokinetic figure. In the individual shown in fig. 19, of the three meganuclei (*N*) present, two have their contents remarkably less strongly stained than the third; this probably indicates that the two are in the process of degeneration. We have also met with individuals in which the supernumerary meganuclei are degenerating still further, while the micronuclear contents are found in the normal state, *i.e.*, forming a compact mass. Now, judging from the fact that in the ordinary fission the micronucleus divides first and then the meganucleus in its turn, the above case may be interpreted in this way, that in those individuals the micronuclear division and consequently the fission of the body have been suppressed probably owing to a certain abnormality in the physiological condition of the organism, and this has caused the fragmentation of the overgrown meganucleus.

Fig. 19.



In this individual, the micronucleus (*n*) is forming 4 chromosomes, but shows no other signs of division; 2 faintly stained meganuclei are in the process of degeneration

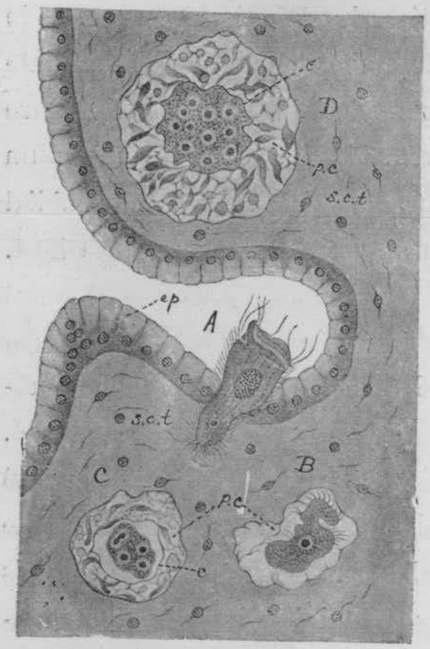
Not infrequently the meganucleus of the present species is seen to perform chromidia formation. In this case the meganucleus contains an unusually small number of chromatin granules in the periphery, and in the central parts very large compact chromatic masses numbering more than two. These central chromatic masses later leave the meganucleus and come into the cytoplasm in which they are probably absorbed in the end.

In passing the bacterial infection of the *Boveria*, which is occasionally observed, may be mentioned. We have here to do with a *Bacillus* form, of which large numbers generally accumulate in the neighbourhood of the meganucleus. The parasites are strongly stained by DELAFIELD's hæmatoxylin and by iron-alum hæmatoxylin, and are easily liable to be mistaken for chromidial bodies. They do not seem to cause any serious harm to the host.

V. Encystment.

Boveria labialis was observed by us to encyst in the subepithelial connective tissue of the respiratory tree of the host. The encystment seems to occur when the parasites have had to live under certain unfavourable conditions, for instance, when the water in the respiratory organ has become poor of oxygen. This may be occasioned by the host long remaining in a forcibly contracted state or in the air during low tide, or when a shortage of food has set in, probably owing to excessive multiplication of the parasites.

Ordinarily the encystment proceeds in the way to be described below and illustrated semidiagrammatically in fig. 20. Any individual about to encyst first attaches itself always with its aboral end to the inner epithelium of the respiratory tree. Then it penetrates the epithelium and begins to sink gradually deeper in the subepithelial connective tissue (see the individual *A*). After having completely burrowed into the connective tissue, the organism gradually and strongly contracts its own body (the individual *B*.) During this contraction period the organism loses the peristomal disc, the contractile vacuole, the meganucleus, and food vacuoles, consequently the micronucleus and the cilia on the general body-surface represent the only visible organs. The cilia not only persist but become gradually longer and later even thicker, Fig 20.



Four stages of the encystment, conbined for convenience into one figure; full explanation is given in the text. Fixed with picro-acetic acid solution, sectioned, and stained with iron hæmatoxylin and orange-G. *c.*, cyst; *ep.*, inner epithelium of the respiratory tree; *p.c.*, pericyst cavity; *s.c.t.*, subepithelial connective tissue.

and lie as a whole within a tolerably wide cavity (*p.c.*) developed around the body of the parasite. This last mentioned cavity, which may be called the pericyst cavity, is a space in the connective tissue, which no doubt has arisen through a destructive disturbance caused by the encysting parasite on the surrounding tissue. The cavity is bordered all around by a more or less dense sheet of the matrix of the tissue. In a later stage of the encystment (the individual *D*), some of the tissue cells enter into the substance of this bordering wall of the pericyst cavity. To this wall as well as to the thin pellicular wall of the organism the cilia are attached with both their distal and proximal ends. It is true, however, that the proximal or inserting ends of the cilia are, in most places of the body of the organism, easily torn off by the knife-blade during microtomising, as is represented in cyst *B*.

At the stage represented by the individual *C*, the organism is found completely contracted into a roundish body, in the interior of which the original single micronucleus has just started direct division. A little prior to this micronuclear multiplication, the organism secretes the cyst which is often widely separated from the body, as is represented in the figure. The cilia are now transformed each into a thick spindle, the slender terminal portions of which often anastomose with those of the neighbouring cilia.

Finally there comes the stage represented by the individual *D*, which is the most advanced we could observe. Here the entire cyst has grown enormously, measuring about 85 μ in diameter, about two fifths of which length belong to the diameter of the cyst-proper. The wide pericyst cavity is traversed, as was the case in preceding stages, by innumerable modified cilia. The anastomosis between the last mentioned has now become notoriously intricate. The micronuclear multiplication has advanced very much, the resulting minute nuclei being produced not less than fifty in number.

We have not been enabled to observe further changes of the cyst lastly described. But it is conceivable that the cyst contents ultimately divide into as many uuinucleate bodies as there are nuclei, and the latter are set free probably by dehiscence of the surrounding tissues of the respiratory organ.

We have not infrequently met with another type of encystment, which we venture to attribute also to *Boveria labialis*. According to our observations this sort of encystment takes place when the parasites have fallen into the body-cavity, owing probably to breaking off, by some cause or the other, of some portions of the respiratory tree. The above stated seems to be well born out by the fact that the cysts of this sort are most abundantly discovered in detached branches of the respiratory tree, freely floating in the cœlomic fluid. The parasites penetrate into the connective tissue of the respiratory tree, a peculiarity of this encystment, through the outer lining epithelium of the organ. After penetration the organism contracts its body into a small roundish mass and secretes the inclosing cyst. During this contraction period all visible organellæ excepting the micronucleus are lost to sight. Now the micronucleus begins to multiply amitotically. As the micronuclear multiplication advances, the entire cyst grows larger and larger until it measures about 85 μ in diameter. The inclosed cynthytial mass becomes at first irregular-shaped, and later complicately lobed and reticulated, recalling the plasmodial body of a certain mycetozoan.

Fig. 21.

Cyst of the second type. Technique the same as in the preceding figure.

Fig. 21 represents a cyst grown to maximum size, in which the polynucleate and reticulated appearance of the cyst contents is shown in the most advanced state. In this case of encystment too, we have not been able to ascertain the fate of the cyst.

Summary.

1. *Boveria labialis* mainly differs from *B. subcylindrica* STEVENS in lacking the cytoplasmic lenticular mass in the aboral region and in possessing a lip-like appendage on the peristomal disc. From *B. subcylindrica* var. *neapolitana* STEVENS it further differs in the rounded, instead of the pointed shape of the aboral end, and in the larger extension of the oral ciliary spire.

2. The conjugation is isogamic. Two individuals come into attachment with their aboral ends.

3. The micronucleus in the ordinary fission and also the synkarion form four distinct chromosomes in the same way. During the conjugation, the micronucleus divides in two different ways; *i.e.*, its first division is amitotic, while its second and third divisions are mitotic and give rise to numerous chromatin threads instead of four chromosomes. The third micronuclear division seems to be a reducing division.

4. The conjugant meganucleus persists as such in the ex-conjugant. This persistent nucleus absorbs three quarter-portions

of the synkarion.

5. The meganucleus often undergoes fragmentation, leading to its partical degeneration. The phenomenon seems to support HERTWIG's karyoplasm theory.

5. The meganucleus is occasionally seen throwing off a portion of its chromatin as chromidia, which sooner or later disappear in the cytoplasm.

7. Two sorts of cysts may arise according to the circumstance of the encystment. In the one, the cilia persist, but in a highly modified state, and the cyst contents appear as a roundish polynucleate plasmic mass; in the other, the cilia are lost, and the cyst contents appear as a highly reticulated body consisting of the polynucleate plasm.

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