

Morphology, Morphogenesis and Systematic Position of the Sorocarp Forming Ciliate *Sorogena stoianovitchae* Bradbury & Olive, 1980

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ABSTRACT. Reinvestigation of the type population of the sorocarp-forming ciliate *Sorogena stoianovitchae* Bradbury & Olive, 1980 using the Fernández-Galiano technique and various electron-microscopy techniques (scanning electron microscopy, freeze-fracture and ultrathin sections) expands the observations reported in the original description of the species. *Sorogena stoianovitchae* is a colpodid ciliate with oral ciliature consisting of 25 ciliated paroral dikinetids on the right and 3-5 small adoral organelles on the left of an elongated and domed oral slit, resembling that of the genus *Platyophrya*. *Sorogena stoianovitchae* divides in the free swimming condition and not in a division cyst, as is the case in the colpodids sensu stricto (s. str.), e.g. *Colpoda*, *Bresslaua*, or *Tillina*. As shown in a detailed light-microscopy study, morphogenesis in *S. stoianovitchae* is of the stomatic mode typical for certain colpodid ciliates. Based on the wealth of new information the phylogenetic position of *S. stoianovitchae* is discussed at some length and arguments are given in favor of the following classifications: *S. stoianovitchae* Bradbury & Olive, 1980 currently sole member of the family Sorogenidae Bradbury & Olive, 1980; order Sorogenida Foissner, 1985; subclass Colpodia Foissner, 1985; class Colpodea Small & Lynn, 1981. This investigation facilitates the discovery of further members of this genus reported primarily from the tropical and subtropical zone.

Key words. Colpodea, morphogenesis, oral apparatus, somatic cortex, sorocarp forming ciliate, *Sorogena stoianovitchae*, taxonomy.

IT was a great surprise for many protozoologists when Olive [33] discovered a ciliate that was capable of forming an aerial fruiting body or sorocarp. The life cycle of this "aggregative" ciliate as described by Olive & Blanton [34] shows a striking similarity to the life cycle of mycetozoa. Initially, this ciliate was regarded as a member of the Mycetozoa group [32], a mistake quite understandable to anyone who has seen the aggregation, culmination and sorocarp formation of this extraordinary ciliate [7-9]. The original description of *Sorogena stoianovitchae* by Bradbury & Olive in 1980 [10], together with an electron-microscopy (EM) study of the trophic ciliate, gave the first thorough treatment of its possible systematic position. Based on light-microscopy observations *S. stoianovitchae* was said to show a resemblance to *Enchelys* and in connection with the EM data it was tentatively placed in the order Haptorida, but within a newly erected family Sorogenidae to stress the clear difference from the known haptorids. Based on a reinterpretation of the electron micrographs presented by Bradbury & Olive [10], Small & Lynn [38] suggested that *S. stoianovitchae* might better be regarded as a colpodid ciliate. Foissner [22] supported this view and separated the Sorogenidae at the ordinal level. The availability of the original cultures maintained by one of us (R. L. Blanton) enabled us to perform a reinvestigation of *S. stoianovitchae* with the intention of supplementing its original description, studying its morphogenesis at light-microscopy level, and showing the true organization of the oral ciliature using ultrathin sectioning and freeze-fracture electron microscopy. These new data clearly show that *S. stoianovitchae* is a colpodid ciliate, although a rather special one. *Sorogena stoianovitchae* still requires an order of its own [22] and is a remarkable member of the Colpodea, a group of ciliates that shows an astonishingly wide spectrum ecologically as well as in terms of oral structures [22].

MATERIAL AND METHODS

The origin and the method of cultivation of *S. stoianovitchae* (ATCC 50031) has been described in detail elsewhere [34]. The ciliate has been reported primarily from tropical and subtropical areas (with the exception of one record from North Carolina [32]) where it lives on wet decaying plant material and feeds on terrestrial ciliates like *Colpoda steinii*. The stock used in this study (isolate PNG 76-73) is the same as the one used by Bradbury & Olive [10].

Living cells were studied with phase-contrast and Nomarski optics. For the demonstration of the silverline pattern the Chat-

ton-Lwoff technique was applied following the instructions given by Corliss [12]. The localization of the kinetosomes is seen best with the pyridinated silver carbonate technique of Fernández-Galiano [15] using the modification recommended by Augustin et al. [1]. Cells prepared according to the latter procedure were also used to study the morphogenesis of *S. stoianovitchae*. The biometric analysis was done with specimens stained with Protargol following a modification recommended by Foissner [20]. (For details of the biometric analysis see Berger et al. [6].) For thin-section electron microscopy a simultaneous fixation in 3% glutaraldehyde and 1% osmium tetroxide in 0.1 M phosphate buffer pH 7.0 [37] was used prior to embedding in Epon 812. The sections, stained with uranyl acetate and lead citrate, were photographed with a Siemens Elmiskop 102. For freeze-fracture the routine procedure as described by Bardele [3] was followed using a Balzers double replica device. For scanning electron microscopy cells were fixed in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer pH 7.4 for 30 min, washed thoroughly, postfixed with 2% osmium tetroxide for 2 h, and dehydrated in a graded series of ethanol (up to 70%). The cells were then mounted on round (12 mm diameter) glass cover slips coated with polylysine, further dehydrated in 85%, 95% and absolute ethanol, critical point dried in a Polaron CPD, and sputter-coated with gold-palladium. The scanning electron microscope (SEM) observations were made with a Cambridge Stereoscan 250 Mk 2.

All figures except Fig. 8 are printed or drawn as seen from outside the cell. The encircled arrowhead in the freeze-fracture micrographs indicates the direction of shadowing.

RESULTS

General morphology. A young theront of *S. stoianovitchae* recently escaped from a sorocarp cyst and not yet filled with too many food vacuoles measures 30-40 × 20-30 μm in vivo. The cell is more or less reniform and compressed laterally. The outline of its ventral side is sigmoid whereas the dorsal side is slightly convex (Fig. 1, 4). The trophic stage of *S. stoianovitchae* is almost circular in cross section. Depending on the degree of food intake the trophont measures 50-70 × 30-45 μm (Table 1). In both stages, the theront and the trophont, the elliptical cytostome is located on a domed elevation (Fig. 1, 3, 4, 13, 14) in a subapical position. In living cells (this holds also for fixed cells, see Fig. 2) the oral ciliature at the base of the oral dome is difficult to distinguish from the somatic ciliature. The cell has an almost spherical macronucleus, 15-18 μm in diameter and

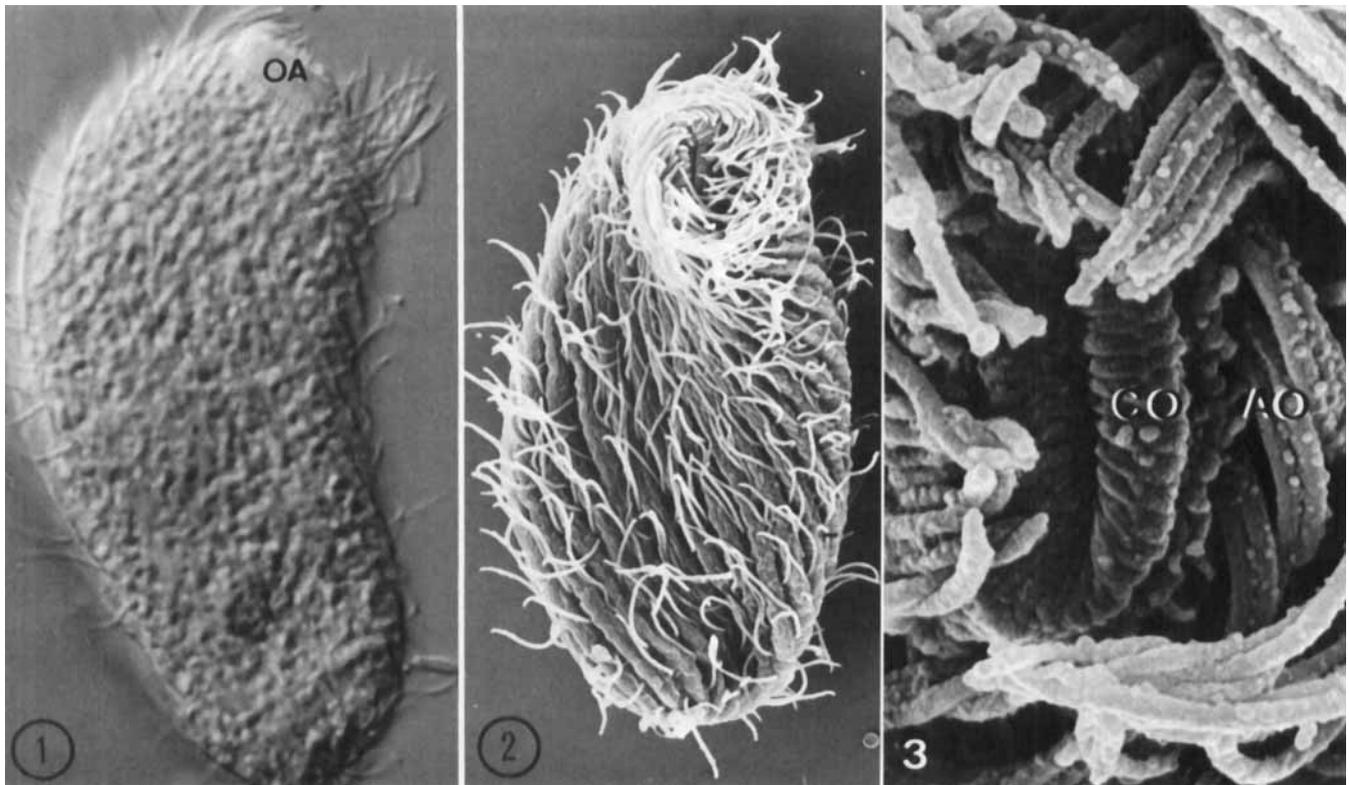


Fig. 1-3. Morphology of *S. stoianovitchae*. 1. Theront of *S. stoianovitchae* focused to the plane in which the collar-like dome of the oral apparatus (OA) is seen in sideview. Nomarski optics. $\times 1,800$. 2. Scanning micrograph of a theront of *S. stoianovitchae* to show the orientation of the somatic kineties. In fixed cells the somatic cilia are usually bent toward the anterior pole of the cell thus hiding the shorter oral cilia. $\times 2,300$. 3. Scanning micrograph of the oral slit at higher magnification. Corrugated rim of the collar-like dome of the oral apparatus (CO). Adoral organelles (AO). $\times 17,000$.

a single separate micronucleus, 2.5–3 μm in diameter. Both nuclei are located in the middle of the cell and may be difficult to detect in living trophonts due to the numerous food vacuoles (Fig. 4). There is one subterminal contractile vacuole with a single excretory pore on the right-ventral side near the posterior end of the cell as well as a slit-like cytoproct near the excretory pore two kineties to the left. The position of the contractile

vacuole and the cytoproct are shown in a camera lucida drawing of a silver nitrate impregnated cell (Fig. 5).

When swimming the ciliate rotates about its longitudinal axis. On the bottom of the culture dish *Sorogena* creeps. When slightly compressed under a cover slip, the cell shows a high degree of flexibility, performing an almost amoeboid movement.

The somatic cortex shows 18–21 kineties composed exclu-

Table 1. Biometrical data of *Sorogena stoianovitchae*.

Character	\bar{x}^a	\tilde{x}	s	s_x	v	Min	Max	n
Length (μm) ^{b,c}	52.3	52	6.1	1.4	11.7	41	64	19
Width (μm) ^c	31.1	31	4.7	1.1	15.2	21	39	19
Length of the oral area (μm) ^c	7.2	7	0.7	0.2	9.6	6	9	19
Distance from the anterior end of the cell to the beginning of the macronucleus (μm) ^c	22.5	22	3.4	0.8	15.2	15	32	19
Number of somatic kineties in the middle of the cell ^d	19.1	19	0.8	0.2	4.2	18	20	19
Number of dikinetids in a right-lateral somatic kinety ^d	27.3	29	4.5	1.0	16.6	20	34	19
Number of dikinetids in a left-lateral somatic kinety ^d	20.3	20	2.3	0.5	11.1	14	24	19
Number of dikinetids in the paroral membrane ^d	25.4	26	2.2	0.5	8.5	21	29	19
Number of adoral organelles ^d	4.3	4	0.7	0.1	15.2	3	5	19
Length of macronucleus (μm) ^c	18.1	18	3.1	0.7	17.3	11	24	19
Width of macronucleus (μm) ^d	15.0	15	2.2	0.5	14.9	10	19	19
Length of micronucleus (μm) ^d	3.1	3	0.4	0.1	13.6	2	4	19
Width of micronucleus (μm) ^d	2.5	2.5	0.4	0.1	14.4	2	3	19

^a Abbreviations: min = minimum, max = maximum, n = number of cells measured, s = standard deviation, s_x = standard error of mean value, v = variation coefficient, \bar{x} = arithmetical mean value, \tilde{x} = mode.

^b All data from cell lying on their right or left side, respectively.

^c After protargol preparations.

^d After silver carbonate preparations.

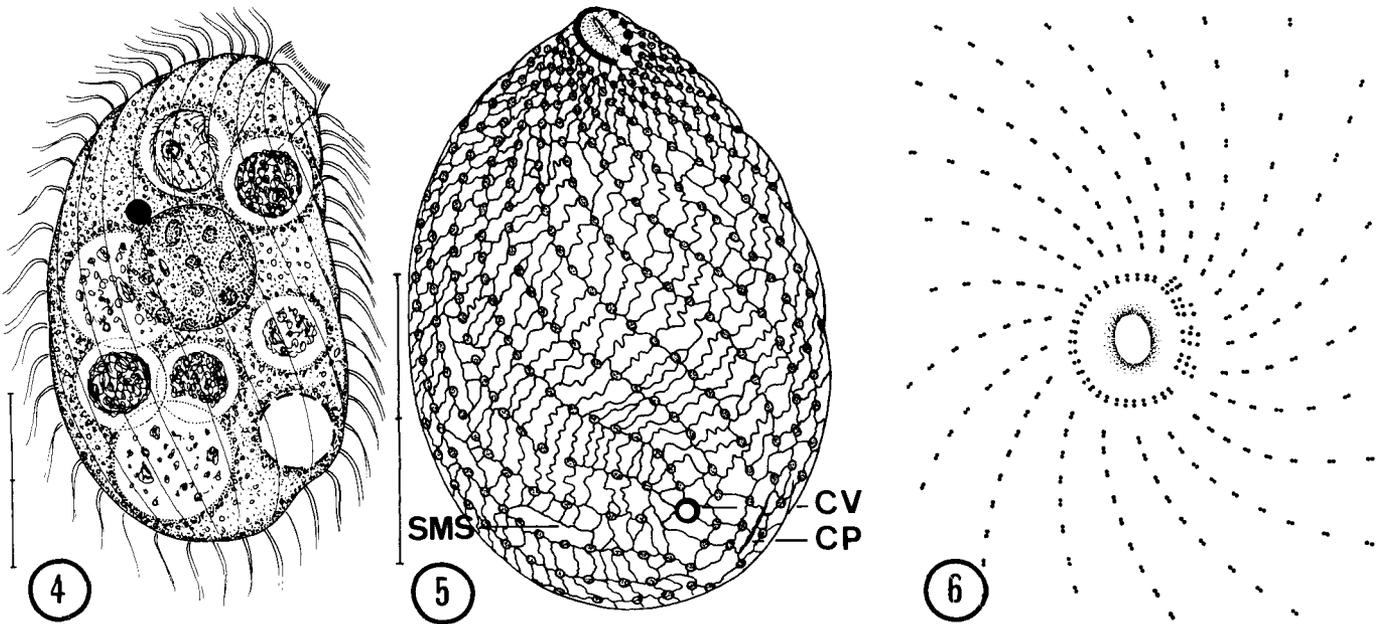


Fig. 4-6. Slightly schematic drawings of trophonts of *S. stoianovitchae*. 4. Right lateral view of an early trophont drawn from life. The micro- and the macronucleus, seven food vacuoles in various stages of food digestion and the contractile vacuole in the lower right corner are shown. Bar = 25 μ m. 5. Drawing of a Chatton-Lwoff silver nitrate impregnated cell. The cytoproct (CP), the excretory pore of the contractile vacuole (CV) and a semimedial silverline (SMS) are marked. Bar = 25 μ m. 6. Drawing to show the array of the somatic and oral ciliature in the anterior half of a cell drawn after a silver carbonate stained specimen.

sively of dikinetids. The kineties on the right side of the cell are slightly shorter than those on the left side and terminate in front of the pore of the contractile vacuole (Fig. 5). The somatic cilia measure 7-8 μ m in length. As seen clearly in the scanning micrographs, two adjacent kineties are separated by a cytoplasmic crest as described by Bradbury & Olive [10]. The somatic kineties take a spiral course. Seen from outside of the cell, and from the anterior to the posterior pole, the kineties take a counterclockwise turn (Fig. 2, 5, 7, 9). The drawing of *S. stoianovitchae* given by Bradbury & Olive [10] is incorrect in showing the somatic kineties running in a clockwise orientation, a mistake that is easily made by focusing to the "inappropriate" plane. Our Fig. 8 also shows a clockwise orientation of the somatic kineties. In this particular case it had to be focused *through* the cell to picture the oral apparatus, which was lying "underneath the cell." Only when seen from inside the cell the somatic kineties run in a clockwise orientation.

Sorogena stoianovitchae has a colpodid silverline system (Fig. 12, and shown for the entire cell in a camera lucida drawing in Fig. 5). Vertical silverlines connect the somatic dikinetids. At higher magnification an elliptical silverline is visible around every dikinetid. It is mostly from these dikinetid territories that highly wavy horizontal silverlines pass to the neighboring vertical silverlines. In small irregularly placed areas of the somatic cortex short semimedial silverlines are seen in addition (for a comprehensive treatment of the silverline terminology see [16]).

The oral ciliature is located around the outer base of the naked oral dome seen most clearly in freeze-fracture replicas (Fig. 13, 14). The paroral ciliature consists of a double row of fairly short cilia, about 5 μ m in length, and arranged in a C-shape, thus encompassing the dorsal and the ventral part of the oral dome. On the left side of the oral dome there are 3-5, but most often 4 adoral organelles. Silver-stained specimens show that the adoral organelles consist of 6-8 kinetosomes (Fig. 6, 9), but as seen in freeze-fracture replicas only 4-5 of these kinetosomes are ciliated (Fig. 14).

Fine structure of young trophont. *Sorogena stoianovitchae* is extremely difficult to prepare for ultrathin sectioning because the numerous extrusomes cause the cells to explode almost immediately when fixed with either glutaraldehyde or osmium tetroxide alone, or with various mixtures of both fixatives. We have not been able to obtain better preservation of the somatic cortex than Bradbury & Olive [10]. The extrusomes are of the mucocyst type and can be stained with methylgreen-pyronin [17]. They are ellipsoidal in shape and measure 1-2 \times 0.5-1 μ m. In freeze-fracture replicas the attachment sites of the mucocysts to the plasma membrane are characterized by the occurrence of the well-known attachment rosettes (Fig. 15), a prerequisite for extrusome extrusion via fusion of the organelle's membrane with the plasma membrane. In vivo the numerous mucocysts may be responsible for the silvery refractile appearance of cells first described by Bradbury & Olive [10].

The somatic ciliature only consists of dikinetids. Usually both kinetosomes are ciliated, but at times only the posterior one bears a cilium. A line diagram of the kinetid pattern of *S. stoianovitchae* drawn by Lynn [29] from the micrographs published by Bradbury & Olive [10] is shown in Fig. 29. The anterior kinetosome has a prominent tangential transverse microtubule ribbon and probably a single postciliary microtubule. The posterior kinetosome has a steep kinetodesmal fiber, a postciliary ribbon of four microtubules, a transverse ribbon of about six microtubules and an electron-dense transversal fiber on the left side of the posterior kinetosomes which has been mistaken as kinetodesmal fiber by Bradbury & Olive [10]. The freeze-fracture aspect of the proximal part of the ciliary membrane is shown in Fig. 16 and is of certain interest for phylogenetic considerations [2]. As seen in this micrograph, *S. stoianovitchae* has a rather inconspicuous necklace area and there are no ciliary plaques as found in typical colpodids like *Colpoda*, *Bresslausa* or *Tillina* [2], all belonging to the order Colpodida [22].

The oral area is better preserved in fixation, probably because it contains fewer extrusomes. We agree with Bradbury & Olive

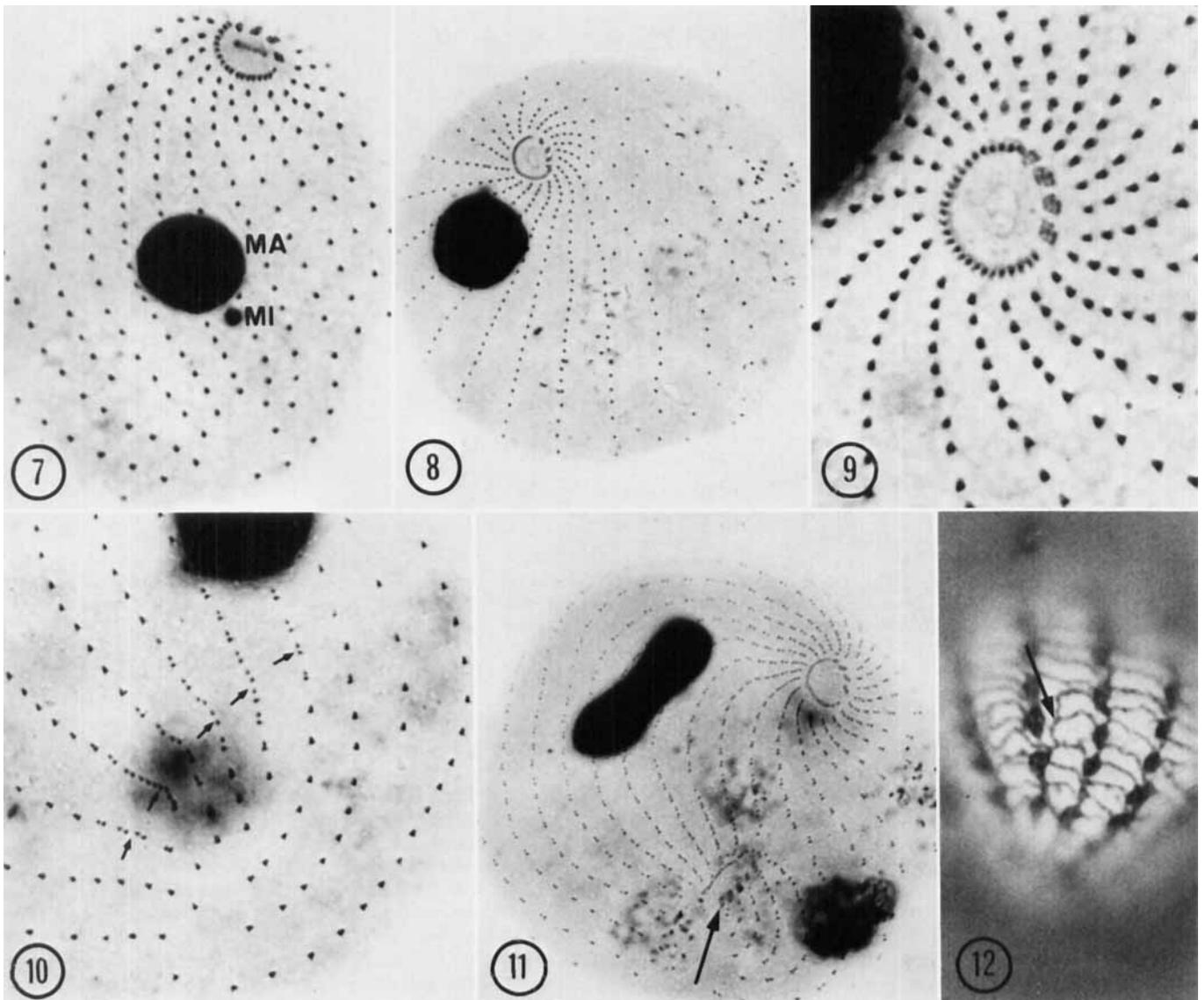
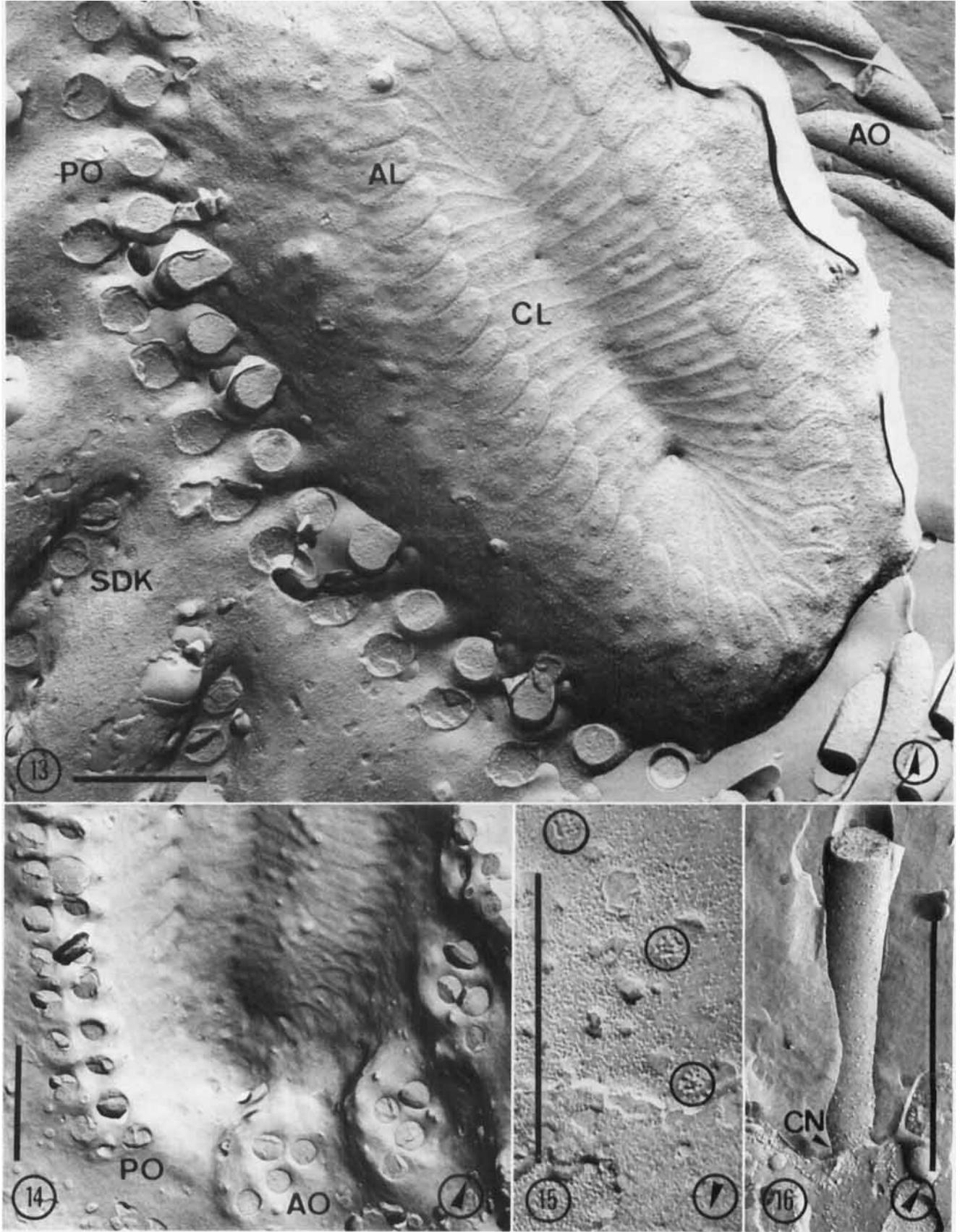
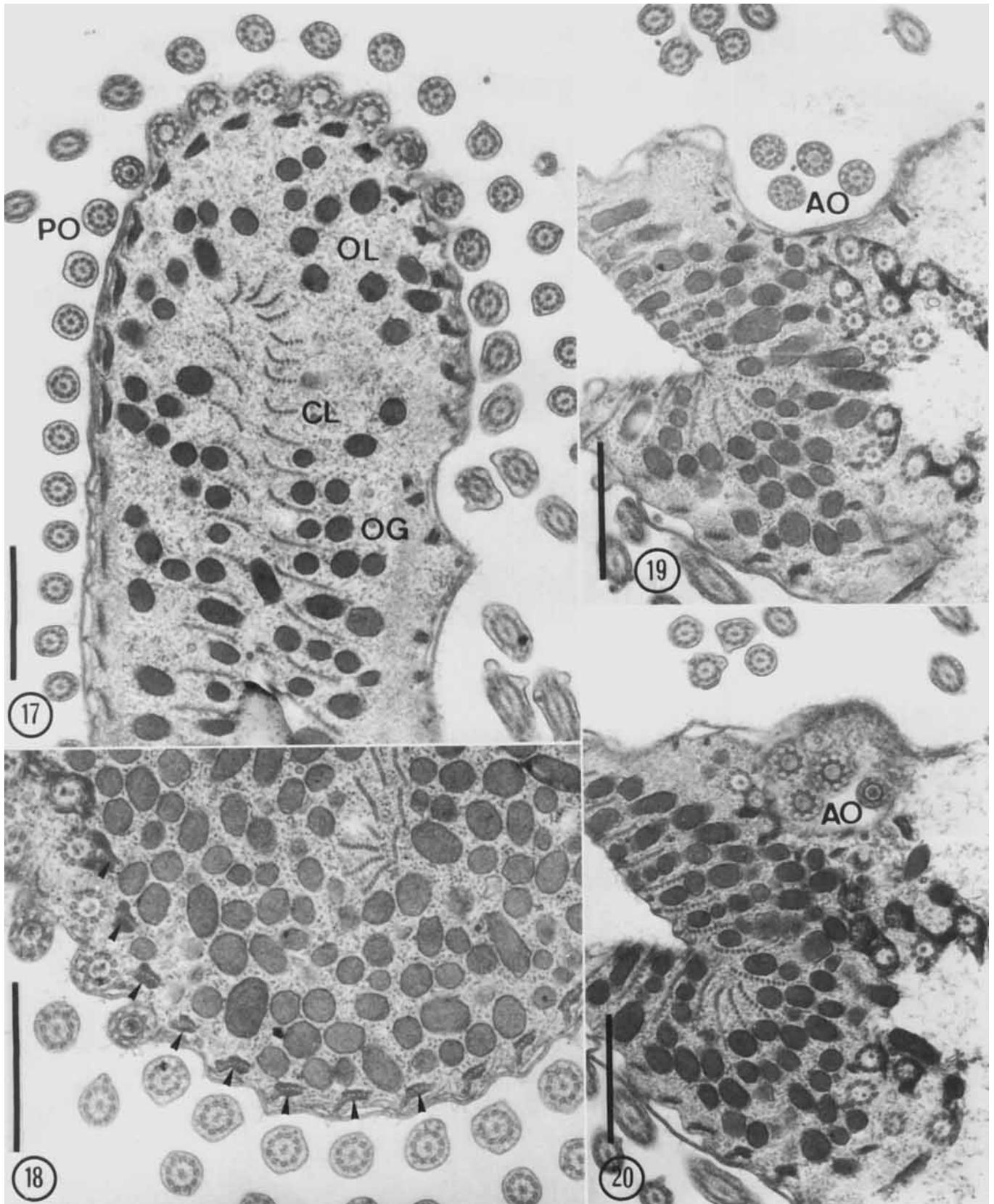


Fig. 7-12. Photomicrographs of silver carbonate and Chatton-Lwoff stained specimens of *S. stoianovitchae*. 7. The array of the somatic kineties is shown. The oral structures are seen in the upper right. The dark round structures in the center are the macro- and micronucleus (MA, MI). 8. Figure 8 allows counting of the somatic kineties and shows the condensed ciliature around oral apparatus. This is a view as seen from inside the cell, thus the somatic kinetids show a clockwise orientation. 9. Oral structures are shown at higher magnification as seen from outside the cell. Since the specimen is squashed the oral area appears more rounded than it is in reality. 10. Early stage of kinetosome proliferation (arrows). 11. Later stage of morphogenesis corresponding to the drawing in Fig. 21. Arrow marks newly formed adoral organelles (Fig. 6-11 are purposely without scale bars since the applied staining technique leads to unavoidable distortions of the cells which would give meaningless measurements). 12. Chatton-Lwoff preparation showing part of the silverline system. Arrow marks semimedial silverline. $\times 4,000$.

Fig. 13-16. Fine structure of *S. stoianovitchae* as seen with the freeze-fracture technique. 13. A replica showing the cytostomial area and the broken off cilia of the paroral dikinetids (PO). The micrograph shows the protoplasmic face of the plasma membrane. Note the conspicuous particle arrays that line the most distal parts of the underlying pellicular alveoli (AL). The particle rows directed toward the center of the cytostome may mirror the position of the cytopharyngeal lamellae (CL). Somatic dikinetids (SDK) and cilia of the adoral organelles (AO) are labeled. Bar = 1 μm . 14. Exoplasmic face of the plasma membrane of the oral area with the paroral dikinetids (PO) and four adoral organelles (AO). The micrograph is printed in reverse to facilitate its interpretation. Bar = 1 μm . 15. Attachment rosettes (encircled) of mucocysts in the protoplasmic face of the plasma membrane. Bar = 1 μm . 16. Protoplasmic face of the ciliary membrane of a somatic cilium to show the absence of ciliary plaques. CN ciliary necklace. Bar = 1 μm .





[10] that glutaraldehyde may lead to an exaggeration of the protrusion of the oral apparatus. However, glutaraldehyde fixation gives a more natural appearance of the elongated oral apparatus compared to Fernández-Galiano's preparations, where compression of the cells causes an artificial rounded appearance of the oral ciliature. From an examination of a large number of thin sections of the adoral organelles we conclude that usually the first dikinetid (the one close to the oral dome) is ciliated completely. Since in the next two dikinetids only the anterior kinetosomes carry a cilium, there are four cilia from three dikinetids and there are in addition one or two nonciliated dikinetids in every adoral organelle. Two serial sections in Fig. 19 and Fig. 20 help to resolve the seeming difference between the silver-stained cells, which show all kinetosomes (ciliated as well as nonciliated), and the freeze-fracture replicas, which show only the position of broken off cilia.

The paroral ciliature, which surrounds roughly two-thirds of the oral apparatus, consists of about 25 ciliated dikinetids. It is the posterior kinetosome (not the anterior one as reported in [10]) of each dikinetid that gives rise to a nematodesma and to a ribbon of postciliary microtubules. At a short distance from the kinetosome these microtubules become sandwiched between electron-dense material (Fig. 17, 18). These microtubule ribbons (as well as those coming from the adoral organelles), first course upward into the oral dome where they can be seen underlying the anteriormost projections of the pellicular alveoli (Fig. 18 arrowheads). Increasing in microtubule number, these ribbons turn downward to line the oral slit, and finally form the cytopharyngeal lamellae. Deeper in the cytopharynx the individual microtubular lamellae join laterally to form an almost closed cytopharyngeal tube described already by Bradbury & Olive [10]. The cytoplasm of the cytostomial dome shows numerous electron-dense granules, but hardly any membranes that might be involved in food vacuole formation. Further investigations are needed to clarify whether these osmiophilic granules are involved in food digestion or whether they contain membrane precursors as suggested for similar inclusions in the suctorian tentacle [5].

In the freeze-fracture micrographs (Fig. 13, 14) 30–38 particle rows originate from an equivalent number of bowed particle rows. We interpret these particle rows as the attachment sites of the cytopharyngeal lamellae to the plasma membrane that covers the actual cytostome. The bowed particle rows most likely represent the attachment of the distal ends of the pellicular alveoli to the plasma membrane. There are not exactly twice as many cytopharyngeal lamellae as there are somatic kineties because the oral ciliature is not formed by a single interposition of an additional pair of kinetosomes between the anterior ends of the somatic kineties as will be shown in the description of stomatogenesis. Instead, the number of the cytopharyngeal lamellae seems to correspond to the number of both the paroral dikinetids and the number of the dikinetids that form the adoral organelles. In other words, it seems to be the posterior kinetosome of each of these oral dikinetids that sends its postciliary microtubules to become part of the cytopharyngeal lamellae.

Morphogenesis of *Sorogena stoianovitchae*. Vegetative reproduction in *S. stoianovitchae* takes place by bipartition. Contrary to colpodid ciliates s. str., cell division in *S. stoianovitchae* does not occur in a division cyst but in the free swimming condition. It is only during the last stage of division immediately prior to the separation of the two daughter cells that the dividing cell rests motionless on the bottom of the culture dish. It is now widely recognized that a comprehensive understanding of ciliate morphogenesis from light microscopy is an absolute prerequisite for the study of this process by electron microscopy. As the first step we have undertaken a careful study of stomatogenesis and cell division of *S. stoianovitchae* using pyridinated silver carbonate impregnated cells (Fig. 10, 11). The Fernández-Galiano method is a powerful technique to disclose every single kinetosome, but it should also be noted that owing to compression by the coverslip, the cell shape is often distorted in photographs. The entire process, shown in a series of camera lucida drawings (Fig. 21–28), is described as follows.

As the first sign of a beginning cell division a proliferation of kinetosomes is seen in 5–6 right lateral somatic kineties close above the excretory pore of the contractile vacuole (Fig. 22). These zones of kinetosome proliferation become larger and while the macronucleus begins to elongate they split into an anterior and a posterior portion (Fig. 23). Both portions will give rise to the oral ciliature of the opisthe. In the next step, which shows a dumbbell-shaped constriction of the macro- and the adjacent micronucleus, the future adoral dikinetids in front of their somatic kineties have become arranged in 4–5 compact adoral organelles (Fig. 11, 24). At the same time the anterior portion of the oral dikinetids is aligned in three or four broken rows, which lie perpendicular to the adoral organelles. In Fig. 25 a single bowed argentophilic structure is seen and is easily identified as the paroral ciliature of the opisthe. Meanwhile an oblique cleavage furrow appears in front of the oral ciliature of the opisthe (Fig. 26, 27). It cuts the parental cell into two daughter cells as soon as the nuclear division is finished. As seen in Fig. 26 the division of the micronucleus is finished before the division of the macronucleus. The final separation of the daughter cells seems to be brought about by a kind of rotational movement of the proter relative to the opisthe about 45° from the plane of division. In the latter stage of division the paroral dikinetids become visible as separate entities. No signs of reorganization are to be seen in the parental oral structures and in the silverline system.

The mode of kinetosome proliferation in the somatic cortex deserves an additional remark. The process starts with a separation of the two kinetosomes of a somatic dikinetid, then in front of the anterior kinetosome a new kinetosome appears, resulting in typical triads (Fig. 11, 24). A short time later a fourth kinetosome appears in front of the posterior parental kinetosome, thus resulting in a quadrupling of kinetosomes that later separates to form two dikinetids. What we regard to be the new kinetosomes usually appear as smaller argentophilic dots compared to old kinetosomes. During kinetosome proliferation the new kinetosomes are not seen in straight line with the course

Fig. 17–20. Ultrathin sections of the oral area of *S. stoianovitchae*. 17. Slightly oblique section through the oral collar showing the cilia of the paroral dikinetids (PO). The oral lip cytoplasm (OL) contains numerous osmiophilic granules (OG) of unknown function. For comments on the suggested connection between the cytopharyngeal lamellae (CL) with the structures labeled by asterisks, see the discussion. 18. The microtubules sandwiched in electron-dense material are postciliary mt arising from the paroral dikinetids. 19, 20. Two serial sections showing the adoral organelles (AO). While the upper two adoral organelles in both micrographs show 5 resp. 4 free cilia, the other adoral organelles are cut at a more proximal level showing up to nine cross sections of kinetosomes. Part of these kinetosomes are arranged in pairs. Bar = 1 μm.

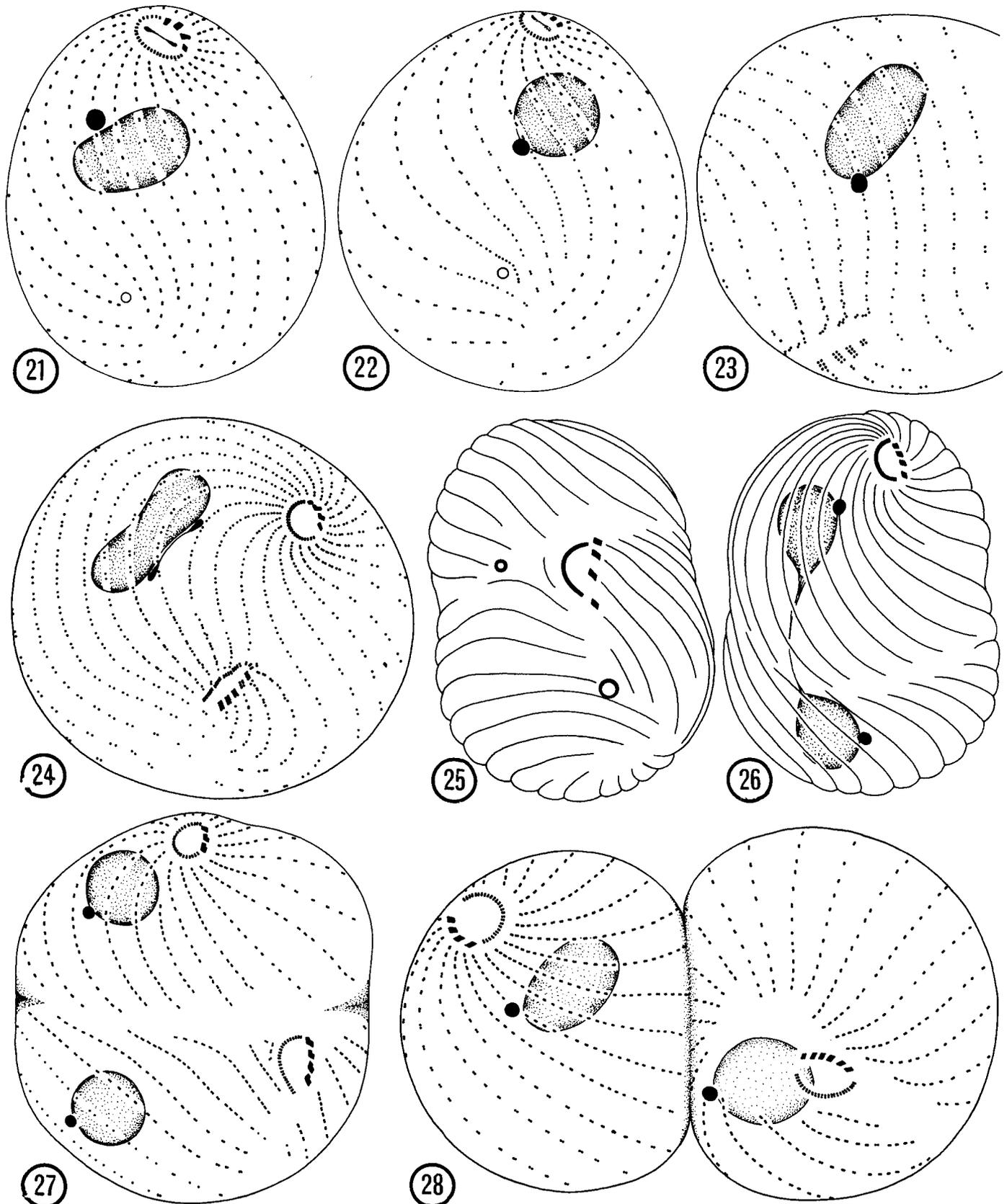


Fig. 21-28. Morphogenesis of *S. stoianovitchae* drawn after silver carbonate (Fig. 21-24, 27, 28) and Chatton-Lwoff (Fig. 25, 26) stained specimens. 21. Left side of a non-dividing cell. 22. Very early divider. Kinetosome proliferation for the oral primordium occurs in several right-lateral somatic kineties in front of the contractile vacuole pore. 23. Early divider. While oral structures begin to differentiate kinetosome proliferation

of the kinety, but are shifted slightly to the anterior left quarter in front of their parental inducer. Finally, the two new dikinetids, being the result of a “semi-conservative” mode of replication, separate further and arrange themselves in a straight line with the somatic kinety. Because of the restricted resolution of the light microscope not all the details of kinetosome proliferation could be detected, but it appears that more than one round of kinetosome replication takes place to cope with the demand for a higher number of kinetosomes in the future oral apparatus.

DISCUSSION

The ability of a ciliate to form an aerial sorocarp similar to those of myxobacteria and eukaryotic mycetozoa is a fascinating example of convergent evolution among single-cell organisms. It seems to be one possible adaptation to the temporary lack of water in the habitat of these organisms. Despite the fact that this adaptation is apparently of no phylogenetic significance, the systematic position of the sorocarp-forming ciliate is of utmost interest by itself.

Somatic cortex. The drawing of *S. stoianovitchae* given by Bradbury & Olive [10] is incorrect in showing the somatic kineties running in a clockwise orientation, a mistake that is easily made by focusing to the inappropriate focal plane. According to Lynn [28, 29] the somatic dikinetid in colpodid ciliates is characterized by a fairly steep and short kinetodesmal fiber arising (as in all other ciliates; the rule of desmodexy) from the right anterior quadrant of the posterior kinetosome. The structure labeled kd in Fig. 6 and Fig. 8 in the paper by Bradbury & Olive [10] originates from the left side of the posterior kinetosomes and therefore cannot be a kinetodesmal fiber, but represents a so-called transverse fibril first described for *Woodruffia* [25] and found in two other members of the order Cyrtolophosidida [13], *Platyophrya* [14] and *Kuklikophrya* [31]. In the latter genus the transverse fibril on the left side of the dikinetid was also misinterpreted as a kinetodesmal fiber [31]. The “sheet of microtubules” arising from the anterior kinetosomes of somatic dikinetids in *S. stoianovitchae* (labeled sm in Fig. 6 and Fig. 8 in [10]) is identified in this paper as the anterior ribbon of transverse microtubules. There is a second transverse ribbon associated with the posterior kinetosome. This “posterior” transverse ribbon runs in a posterior direction and may overlap with the next posterior transverse ribbon forming a sort of “left post-ciliodesma” in many colpodids. Thus the somatic dikinetids of *S. stoianovitchae* (Fig. 29) are very similar to those of *Kuklikophrya*, *Platyophrya*, and *Woodruffia*. Schematic drawings of somatic dikinetids of the latter three species are given by Lynn (Fig. 5 in [28]).

Oral apparatus. The anarchic field shown in Fig. 12 and Fig. 14 in the paper by Bradbury & Olive [10] in reality are the kinetosomes of an adoral organelle. The group of free cilia seen in the upper-right corner of their Fig. 12 can be regarded as a cross-section of another adoral organelle. We are aware that much better electron-microscopy preparation will be necessary to obtain more detailed information on the fine structure of both the somatic and buccal cortex. More detailed fine structural information must await better preparation techniques, but our observations establish the existence of a colpodid oral ciliature in *Sorogena* and enough details to relate this genus to the other colpodid genera. The paroral ciliature of *Sorogena* resembles

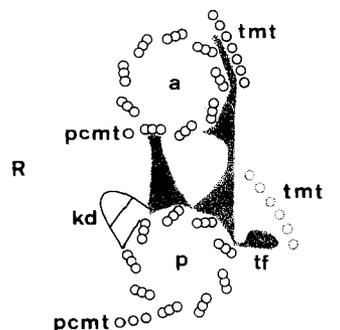


Fig. 29. Somatic kinetid pattern of *Sorogena* as seen from outside of the cell, reproduced with permission from [29]. Anterior kinetosome (a), posterior kinetosome (p), kinetodesmal fiber (kd), postciliary microtubule(s) (pcmt), transverse microtubules (tmt), transverse fiber (tf), right (R), left (L).

that of *Platyophrya* [14] in being a single row of paroral dikinetids with both kinetosomes ciliated. The paroral ciliature of *Cyrtolophosis* is somewhat different since it has been shown to consist of two parts, an anterior and a posterior one, a situation that may originate from an incomplete union of the paroral anlagen, which are derived from the anterior part of several stomatogenic kineties. Moreover, there are clear differences in the adoral organelles. In *Cyrtolophosis* those kinetosomes lying close the oral slit are nonciliated [13]. In the adult cell they do not seem to be so closely associated with the ciliated kinetosomes of the adoral organelles. These barren kinetosomes may have lost contact with the adoral organelles and now form what usually is shown (in a somewhat exaggerated manner) as an additional line of argentophilic granules between the paroral and the adoral ciliature [30]. This structure, which has caused much confusion in colpodid literature, may originate from a sculpturing process of the adoral organelles. No such barren kinetosomes were observed adjacent to the adoral organelles in *Sorogena* or *Platyophrya*.

Morphogenesis. The process of morphogenesis during cell division is of great significance for the understanding of the origin of the ciliate's kinetosome, which has proven to be the most telling character of ciliate diversity and so far the only basis for ciliate classification. It has recently been argued that a true understanding of ciliate morphogenesis at the ultrastructural level may also help to clarify ciliate evolution [4].

Many terrestrial colpodids divide in cysts and dedifferentiate the oral structure of the tomont that is going to divide, two or four new oral structures (depending on the number of cells formed in the division cyst) have to be formed [35]. In contrast to this mode of division, other colpodids [27, 36], such as *Woodruffia*, *Platyophrya*, *Bryometopus*, *Bryophrya*, *Bursaria*, and *Sorogena*, divide as free-swimming individuals without dedifferentiation of the oral apparatus of the proter [22]. Morphogenesis in *Sorogena* is very similar to morphogenesis in *Platyophrya* [26], *Cyrtolophosis* [11], *Microdiaphanosoma* [19] and *Bryometopus* [39]. It is somewhat different from *Woodruffia* [36], in which the paroral of the proter is involved in kinetosome proliferation; this is not the case in *Sorogena* where all new kinetosomes arise within somatic kineties.

←

is shifted to the somatic kineties. 24. Middle stage of division. Oral structures are fully differentiated but are still lying obliquely with respect to the longitudinal axis of the cell. 25, 26. Line diagrams of the infraciliature of a late divider. 27. Another late divider. Oral structures of opisthe have turned to left by about 45°, lying parallel to the longitudinal axis of the cell. 28. Very late divider immediately before separation of daughter cells.

Systematic position of *S. stoianovitchae*. In the original description of *S. stoianovitchae* the ciliate was regarded as a member of the Haptorida. Bradbury & Olive recognized that *S. stoianovitchae* did deviate in several aspects from typical haptorids even though it resembles the genus *Enchelys* in some respects [10]. There is the absence in *S. stoianovitchae* of toxicysts, extrusomes typically found in the voracious haptorids. It was noted that the numerous inclusion bodies located around the anterior part of the cytopharynx differed considerably from typical toxicysts, although they may be involved in the digestion of the prey as the authors suggested [10]. Mucocysts do exist in great number in *S. stoianovitchae*, but are extremely difficult to preserve. The majority of the foamy-looking areas described by Bradbury & Olive [10] as "inflated cisternae of the endoplasmic reticulum" seem to be extrusomes, exploded within the cell. Mucocysts were found to play a central role in the process of sorocarp formation [7, 8]. Bradbury & Olive noticed the absence of a fibrous stratum, which in many haptorids separates the bulk of the endoplasm from a cortical layer of ectoplasm. Moreover, *S. stoianovitchae* lacks the clavate cilia typical for many haptorids. Although these and other features (e.g. the somatic dikinetids) did not fit with the diagnosis of the Haptorida, *S. stoianovitchae* was regarded as an atypical member of the Haptorida placed in a new family, the Sorogenidae [10]. Bradbury & Olive regarded this ciliate as a proper gymnostome equipped with a continuous perioral ciliature. The naked lip, the comparatively simple cytopharynx and the circular array of nematodesmata seemed to justify the proposed systematic position.

Now what are the characteristics that favor the assignment of *S. stoianovitchae* with the Colpodea? As first noticed by Small & Lynn [38] somatic dikinetids of *S. stoianovitchae* do not show the haptorid, but instead show the typical colpoid pattern. The silverline pattern of the somatic cortex of *S. stoianovitchae* resembles the silverline pattern of the colpoid ciliates *Cyrtolophosis* [16], *Sagittaria* [23], and *Colpoda* [22]. The somatic and oral infraciliature of *S. stoianovitchae* is almost identical to the infraciliature of *Sagittaria* [18] and *Platyophrya* [16, 23]. The similarity between *Sorogena* and *Platyophrya* is particularly pronounced. There is only a single characteristic that in vivo distinguishes the two genera; the domed oral area in *Sorogena*, which is lacking in *Platyophrya*. Otherwise the oral ciliature and minute details seen in freeze-fracture replicas are identical. Moreover, morphogenesis in the somatic cortex of *Sorogena*, at least at the light microscopy level, is similar in every detail to the somatic morphogenesis in *Platyophrya* [14, 26], *Cyrtolophosis* [11], and *Microdiaphanosoma* [19, 22]. The general pattern of morphogenesis in *Woodruffia*, another cyrtolophosid colpoid, is similar to *Sorogena* but differs in the detail of the reorganization of the paroral of the proter. The significance of this difference is hard to estimate until more detailed electron microscopy data on the stomatogenic events in the proter of other colpoid ciliates become available. In general, it is a shortcoming of many morphogenetic studies that comparatively little attention is paid to the developmental processes in the proter. This is based on the wide-spread but incorrect impression that the proter often takes over the seemingly unchanged oral apparatus of the parental cell.

The Colpodea is remarkable among all groups of ciliates for the number of taxa transferred to it from other groups. These taxa include among others the genera *Bryometopus*, *Kreyella*, and *Bursaria* [21, 22]. The typical dikinetid pattern mentioned above with a transverse ribbon arising from the posterior kinetosome is regarded as a characteristic feature of the colpoid ciliates. One may doubt that such a complex pattern developed more than once and thus all ciliates with such a pattern are regarded as a monophyletic group. Nevertheless, there are some

differences in the somatic cortex of the "classical" colpoids, which encompasses *Bresslaua*, *Colpoda*, *Tillina*, and the "more recent acquisitions" to that group, such as *Platyophrya*, *Woodruffia*, and *Sorogena*. The latter genera have no ciliary plaques and usually one parasomal sac per dikinetid, while the former have ciliary plaques and three parasomal sacs per dikinetid. Because of the difference in the patterning of the particles in the proximal part of the ciliary membrane the senior author [2] still has some doubt that the colpoids form a monophyletic group. It is recalled that the most recent revision of the class Colpodea by Foissner [22] recognized two subclasses, the Bryometopia with the order Bryometopida and the subclass Colpoda with the orders Sorogenida, Bryophryida, Cyrtolophosidida, Grossglocknerida, Colpodida, and Bursariomorphida. The establishment of the order Sorogenida at that time seemed justified by the quite different looking "haptorid" oral ciliature of *Sorogena*. Foissner's classification of the Colpodea was mainly based on the recognition of three major silverline patterns: the kreyellid, the platyophryid, and the colpoid silverline pattern. But there exist mixtures of these, for instance the kreyellid and the platyophryid pattern in the Bryometopidae [22], and the silverline pattern may also change during morphogenesis, as in *Cyrtolophosis* [16]. Other criteria were the oral ciliature and the mode of life. Now that it is realized that the oral ciliature of *Sorogena* is so similar to that of *Platyophrya*, one may consider an assignment of the Sorogenida to the order Cyrtolophosidida. But then what is the systematic value of the conspicuous union of both the micro- and the macronucleus within a common outer nuclear membrane, which is observed in the cyrtolophosid genera *Woodruffia*, *Kuklikophrya*, *Platyophrya*, *Cyrtolophosis*, and probably *Sagittaria*, but not in *Sorogena*? *Sorogena* seems not to be closely related to the Colpodidae and the Grossglockneridae [24], which divide in cysts. For the time being the mosaic combination of so many features found in other colpoid orders as well as the "mycetozoan way of life" seems to justify a separate order Sorogenida.

So far the genus *Sorogena* is monospecific, but since it is likely that further species may be found a more detailed redescription of *S. stoianovitchae* seemed necessary. To our knowledge rotting plant materials of temperate zones have not yet been scanned systematically by nonmycologists. We hope that with the help of the biometric data given for *S. stoianovitchae* other researchers will be able to recognize further new and undescribed species of the family Sorogenidae.

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