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Electron microscope observations on the nuclear division in *Valonia ventricosa* (Chlorophyceae, Siphonocladales)

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The process of nuclear division in a coenocytic, marine green alga, *Valonia ventricosa* J. Agardh, has been examined in the electron microscope. The mitotic (or meiotic) spindle is centric and totally closed throughout the nuclear division. Kinetochores are evident on the chromosomes. Spindle microtubules converge on the centrioles located to one side of the hooked polar regions of the nucleus, but distinct polar openings are absent. The spindle is conspicuously asymmetric at metaphase and is transformed to a usual form at anaphase. Separation of chromosomes, resulting from spindle elongation and shortening of the chromosome-to-pole distance, is not distinctly synchronous. At telophase the spindle assumes a dumbbell shape and abscission of the interzonal spindle seems to be accomplished by a twist of the daughter nuclei. Microtubules which are expected to participate in orientation of the cytoplasmic net-work were not observed in the cytoplasm. Cytokinesis directly leading to partition of motile cells was not observed in the present material.

Introduction

The taxonomic system of the siphonous green algae currently employed primarily depends upon the structure of the vegetative thallus, nuclear condition, chloroplast characters, chemical composition of the cell wall and manner of septation. Since earlier workers recognized the siphonous green algae (e.g. Greville, 1830; Schmitz, 1878; Blackman & Tansley, 1902), the phylogenetic affinities of the assemblage have been variously discussed by many phycologists (for reviews, Feldmann, 1938; Fritsch, 1935, 1947; Egerod, 1952; Chapman, 1954; Nizamuddin, 1964), but 'disagreement on both composition and systematic arrangement of the member genera has persisted to the present' (Egerod, 1952). Thus, there remain problems that require further study, especially pertaining to their phylogeny. Fritsch (1947) says, 'There are no valid grounds for supporting that the multinucleate habit originated only once during the evolution of the Chlorophyceae, . . .'

Recently, new phylogenetic schemes in the Chlorophyceae have been proposed on the basis of the ultrastructural features of cell division and motile cells (Pickett-Heaps & Marchant, 1972; Pickett-Heaps, 1975; Stewart & Mattox, 1975), but the siphonalean algae were excluded due to paucity of information. Thereafter, ultrastructural studies on the cell division of the hemisiphonous algae, *Acrosiphonia* [Acrosiphoniales] (Hudson & Waaland, 1974) and *Cladophora* [Cladophorales] (McDonald & Pickett-Heaps, 1976), and the eusiphonous alga *Batophora* [Dasycladales] (Liddle, Berger & Schweiger, 1976), have been conducted, and these studies give us a little insight at the ultrastructural level into phylogenies of the complex. However, they are not enough to analyse this subject, because of lack of information on many other important members. In this context, we have taken interest in the ultrastructure of cell division in the coenocytic green algae, especially of the Siphonocladales.

Materials and Methods

Plant material

Mother plants (*Valonia ventricosa* J. Agardh) of the vesicles used here were originally collected by one of us (SE) at Ayamaru-misaki, Amami-oshima Island, in September, 1974, and were maintained in culture from generation to generation in Provasoli's Enriched Sewater (PES) (Provasoli, 1968) at 23–25°C, 16–8 h photoperiod, using cool-white fluorescent illumination (*c.* 1000–2500 lx).

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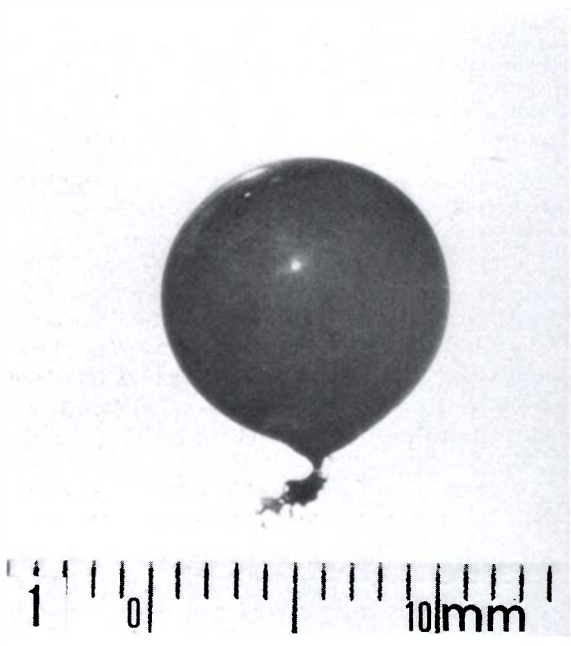
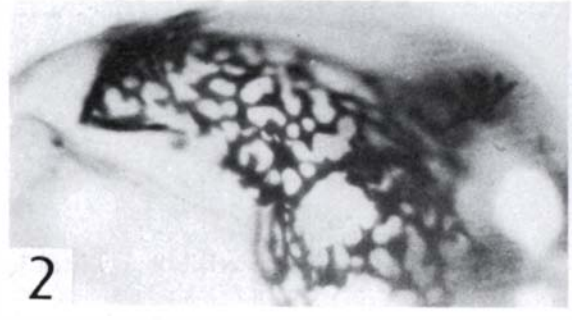
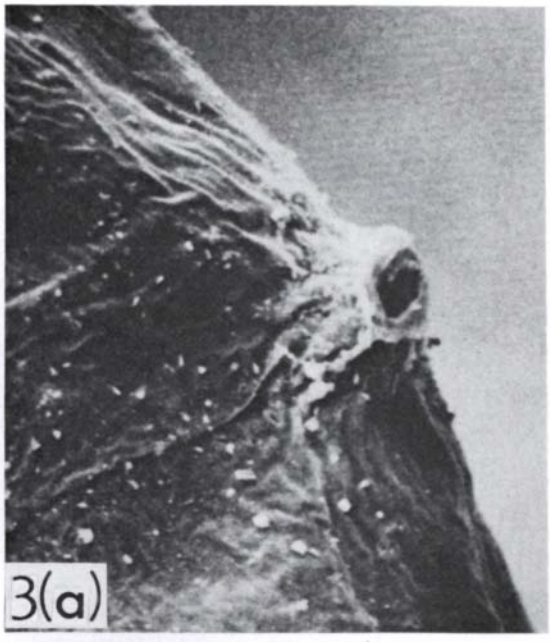
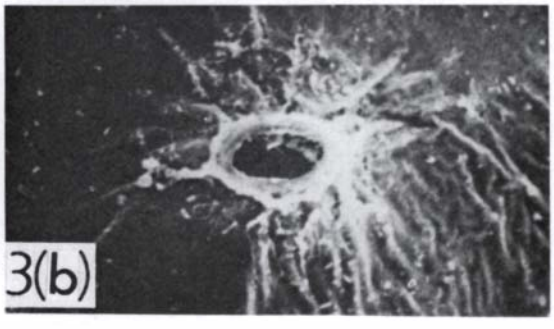
Soon after we planned to conduct an ultrastructural study of mitosis and cytokinesis in this alga, we fortunately had an opportunity to fix two fertile vesicles for electron microscopy. However, we could not test the condition of fixation, and had to employ a tentative fixation method which would ensure electron microscopical observation. Therefore, certain artifacts and a more variable degree of preservation were unavoidable (e.g. partial disruption of nuclear envelope).

A single, giant vesicle of *Valonia ventricosa* (Fig. 1), one of the simplest species of *Valonia*, contains an extremely large central vacuole and thin layer of peripheral cytoplasm. In the present study, vesicles, approximately 8 mm in diameter, were fixed when the cytoplasm had aggregated into a net-work (Fig. 2), leading to the production of sexual or asexual swarmers. That the formation of net-work was a sign of the reproductive phase, not of segregative division, was justified by the appearance of releasing papillae on the cell wall of vesicles (Fig. 3a and b).









FIGS 1–2. Light micrographs of *Valonia ventricosa*.

FIG. 1. A single living vesicle in vegetative phase.

FIG. 2. Part of cytoplasmic net-work in reproductive phase. Micrograph prepared from the EM block. Approximately × 14.

FIG. 3. Scanning electron micrographs of releasing pore produced on the cell wall.
(a) Side view. × 270.
(b) Oblique front view of the same pore shown in Fig. 3a. × 230.

Electron microscopy

Two free living vesicles were fixed for 4 h at room temperature in 5% unbuffered glutaraldehyde (prepared by adding a volume of culture medium to 50% biological grade glutaraldehyde, Fischer Scientific Company, New Jersey, U.S.A.). Following glutaraldehyde fixation the specimens were briefly washed in fresh PES culture medium, and one of two vesicles was processed for scanning electron microscopy in a manner as described below; the other one was put into 5 ml of fresh culture medium to which an equal volume of distilled water was added dropwise over 10 h for minimizing collapse of the vesicle. Finally the specimen was washed again by a mixture of equal volumes of culture medium and distilled water and was postfixed for 15 h in 2% osmium tetroxide (a mixture of the same volumes of culture medium and 4% osmium tetroxide in distilled water). After this the vesicle was transferred to a fresh mixture of culture medium and distilled water to which 50% ethanol was added dropwise over 5 h until final concentration of ethanol was 30%, and then the specimen was dehydrated stepwise through an ethanol series by 5% increments (each step is about 1 h) and put in absolute ethanol for 2 h (with three changes). Propylene oxide was added dropwise overnight until the ratio of propylene oxide to ethanol was approximately 9:1. The specimen was transferred to pure propylene oxide and left over 12 h (with three changes). A very gradual dehydration process prevented collapse of the vesicle, but when the first drop of a resin mixture of Epon 812, DER 736 and MNA (see Kushida, 1966; Hori & Chihara, 1974 for details) was added to the glass vessel which contains 5 ml of pure propylene oxide and the vesicle, the specimen began to collapse. Therefore we had to cut it open to prevent further collapse and consequent degradation of the cytoplasmic net-work. After this, the resin mixture was slowly added dropwise over 5 h, until the ratio of resin to propylene oxide was 2:1. The latter was evaporated off in a dessicator overnight, and the specimen was transferred to fresh resin mixture and was left to soak in resin for 1 day. Finally the resin mixture was polymerized in a 60°C oven for 72 h.

Thin sections were made by LKB ultrotome III, using a diamond knife and stained with saturated aqueous uranyl acetate (12 min), followed by Reynolds' lead citrate (12 min) (Reynolds, 1963), and viewed in an Hitachi HU-12A electron microscope.

For scanning electron microscopy, the vesicle fixed in glutaraldehyde as described above was washed in culture medium and was dehydrated in an ethanol series. The specimen was then transferred to amyl acetate and was put through the critical point drying procedure (Anderson, 1951; Horridge & Tamm, 1969), followed by gold sputter coating by ion beam bombardment apparatus (Eiko Ion Coater 1B-3) (see Akahori & Fukuoka, 1975; Takada Suzuki, Akahori and Yonehara, 1975 for details), and examined with an Hitachi-Akashi MSM-4 scanning electron microscope.

Results

Thin sections through the cytoplasmic net-work contain interphase and dividing nuclei, a huge number of electron dense globules, large pyrenoidal starch plates and other cell organelles (Fig. 4). A microtubular system which might be expected to contribute the formation of net-work is not observed in the cytoplasm, except for that radiating from the centriolar complex (Fig. 5). Mitosis in *Valonia ventricosa* is not synchronous within a net-work cytoplasm and is independent of cytokinesis (Fig. 4). Cytokinesis leading to swarmer formation has not yet occurred at this stage. Interphase nuclei of this alga are acentric and heterochromatic (Fig. 4).

Prophase-prometaphase

The first sign of incipient mitosis is an appearance of centriole(s) (Fig. 6). The nucleus is round and heterochromatic.

Figure 7 is a later stage where microtubules have proliferated in the nucleus and the chromatin is more condensed; increasing their density and electron translucent areas through which the continuous microtubules probably run past. The nucleolus appears to be more reduced.

Metaphase

The nucleus is asymmetrical; both polar regions are slightly hooked or twisted as they could not be lying in the same plane of section (Fig. 8). Thus, one can see a pocket of the nucleus at one pole, at the extranuclear lateral side of which centrioles are situated; and the other pole is more rounded (Fig. 9), because of a probable section obliquely through more distal portion of the hooked pole. Chromosomes become distinct, but a classical metaphase plate and the synaptinemal complex were

not found. In this alga, kinetochores are differentiated, to each of which more than ten microtubules are associated (Figs 8 and 10). Although some microtubules seem to have contact with the nuclear envelope, most converge to centrioles located outside the nucleus (Fig. 11). However, a clear polar fenestra was unexpectedly absent so that the spindle microtubules probably pass through tiny gaps in the nuclear envelope (Fig. 11). In many directions extranuclear microtubules extend from around the centriole complex area (Figs 10 and 12), which may be a MTOC (Microtubule-organizing-centre) defined by Pickett-Heaps (1969).

Anaphase

The spindle elongates considerably and the hooks have disappeared, but the nuclear envelope remains intact. Migration of chromosomes is asynchronous (Fig. 13), and chromosome-to-pole distance has decreased from what it was at metaphase. Daughter nucleoli had already reappeared at this stage and separate with chromosomes (Fig. 13).

Telophase

Centrioles were detected until chromosomes had migrated into the daughter nuclei (Fig. 14). The nucleus assumes a dumbbell shape (Fig. 15) and chromosomes are dispersed. Bundled microtubules of the interzonal spindle seem to develop for pushing the chromosomes into the daughter nuclei. They do not penetrate further inside the daughter nuclei (Fig. 15). Separation of the interzonal spindle from the daughter nuclei may be accomplished by abscission; when the interzonal spindle elongates to full length, the daughter nuclei are twisted up in opposite directions (Fig. 16), and thereby the interzonal spindle is split off from the daughter nuclei. Figure 16 shows a stage just after the completion of abscission. Therefore, most telophase figures show that both distal portions of the interzonal spindle are narrower than at the midregion and are obliquely associated with the daughter nuclei (Fig. 15).

The daughter nuclei seem to remain separate after loss of the interzonal spindle. However, it is very hard to distinguish which is a pair of newly formed daughter nuclei, due to simultaneous occurrence of many nuclear divisions in the net-work (Fig. 4). After unknown duration of interphase, each daughter nucleus at this stage is probably followed by the next cycle of the nuclear division, since the nuclei found in sections of the net-work cytoplasm appear to be still insufficient to provide those of swarmers that are produced in a vesicle.

Discussion

Reproduction and life history of the genus *Valonia* has been investigated in *V. macrophysa* (Kuckuck,

FIG. 4. Section of part of net-work cytoplasm shown in Fig. 2 containing interphase (IN) and dividing nuclei (DN) and fragment of the interzonal spindle (IS) among electron dense granules which probably resulted from the repeated divisions of chloroplasts. Arrow indicates the plasma membrane surrounding cytoplasmic net-work. At this stage of net-work a sign of cytokinesis directly leading to zooid formation was not observed. × 2900.

FIG. 5. Centrioles (large arrows) and radiating microtubules (small arrows) in cytoplasm. × 9600.

FIG. 6. Prophase. Cross section of a centriole viewed at pole (arrow). × 9000.

FIG. 7. Prometaphase. Spherical nucleus containing condensed chromosomes with many electron translucent areas. Arrow indicates dispersed nucleolar material. × 10 200.

FIGS 8 and 9. Metaphase.

FIG. 8. Asymmetrically deformed nucleus bearing upper curved polar region and lower rounded one. Centrioles at both poles are probably present out of the section. Arrow indicates a kinetochore. × 9100.

FIG. 9. Centrioles observed at both poles (arrows); upper one is situated to one side of tipped polar region and two centrioles are found at lower rounded end of nucleus. Nucleolus is indicated by arrowhead. Chromosomes have still preserved their association with inner nuclear membrane. × 9100.

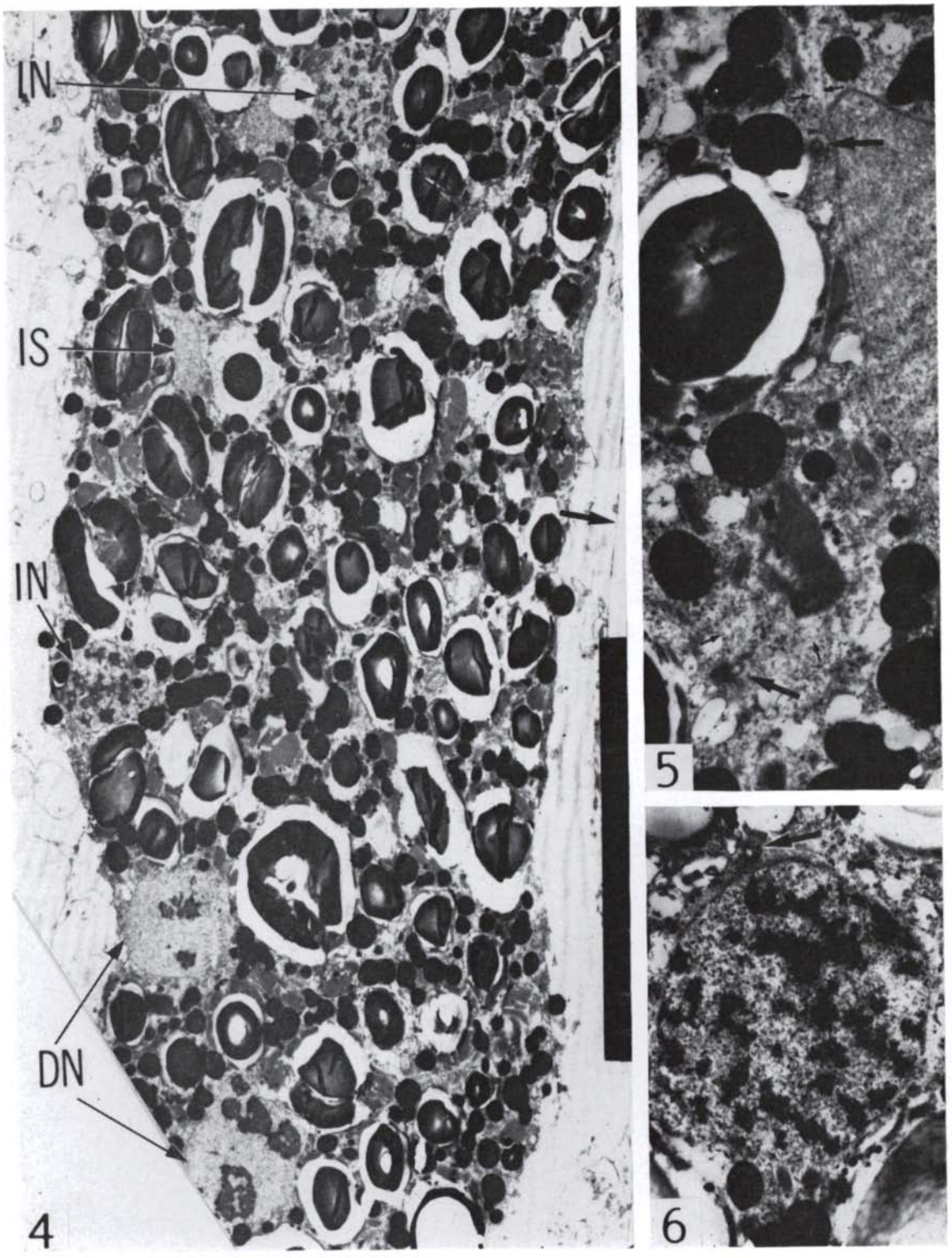
FIG. 10. At least ten microtubules converge to a differentiated kinetochore (arrow). Extranuclear microtubules (arrowhead) radiating from the probable MTOC (Microtubule-organizing-centre) around centriole complex (c) × 23 100.

FIG. 11. Spindle microtubules converge through nuclear envelope to centrioles (arrow) located outside nucleus, but a distinct polar opening could not be observed. × 14 400.

FIG. 12. Extranuclear microtubules (arrows) radiating from centriole (c) which is located to one side of pole. × 30 000.

FIG. 13. Anaphase. Chromosomes move towards poles in elongated nucleus. Arrow indicates daughter nucleolus. × 9 600.

FIG. 14. Centriole (arrow) situated at pole of telophase nucleus; lower region (IS) which has no chromosomes is distal end of interzonal spindle which is cut obliquely. × 14 700.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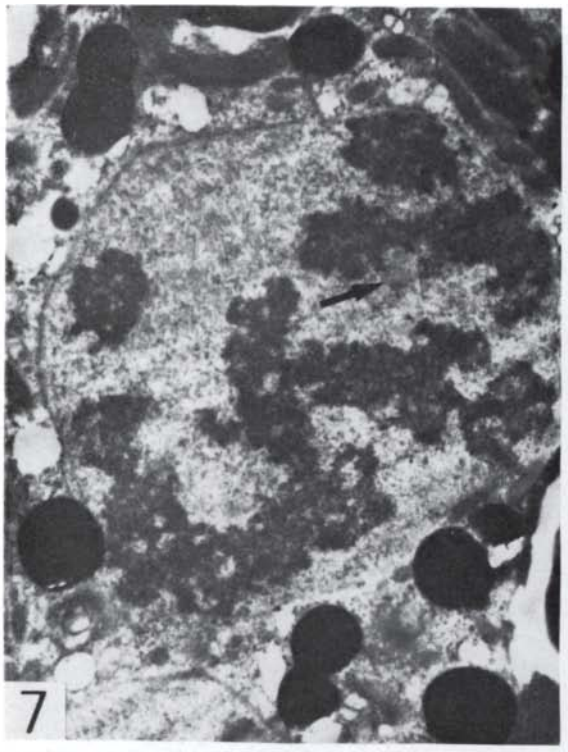
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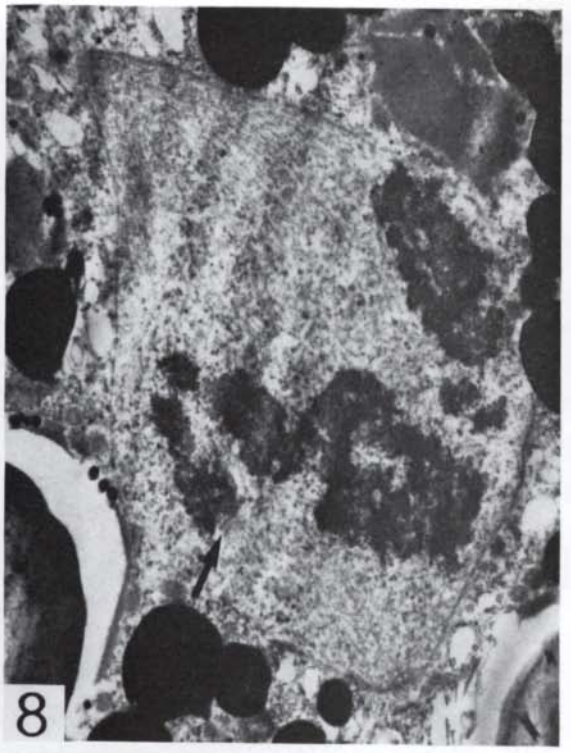
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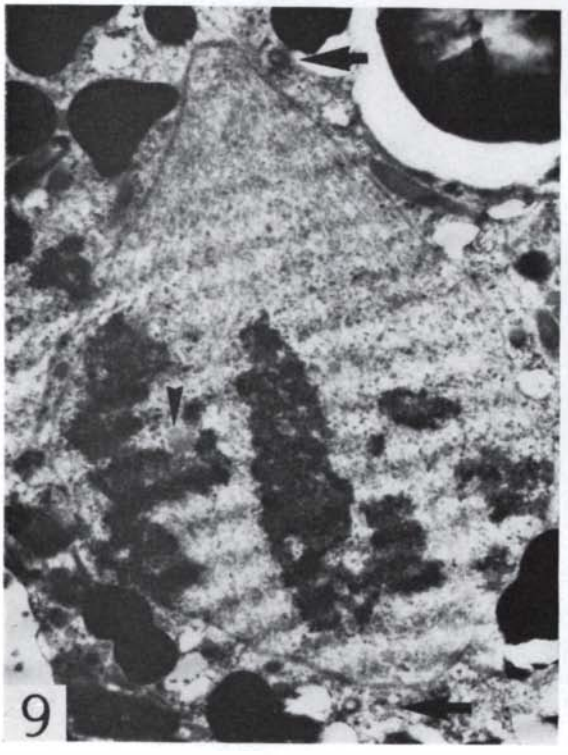
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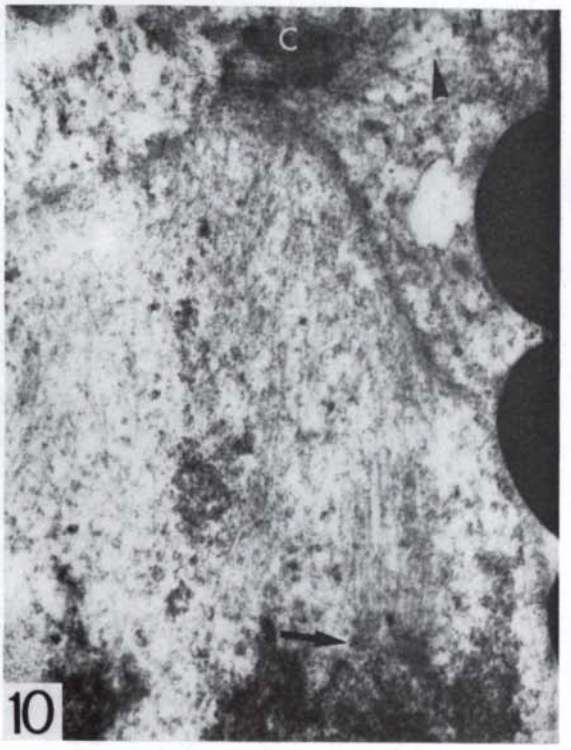
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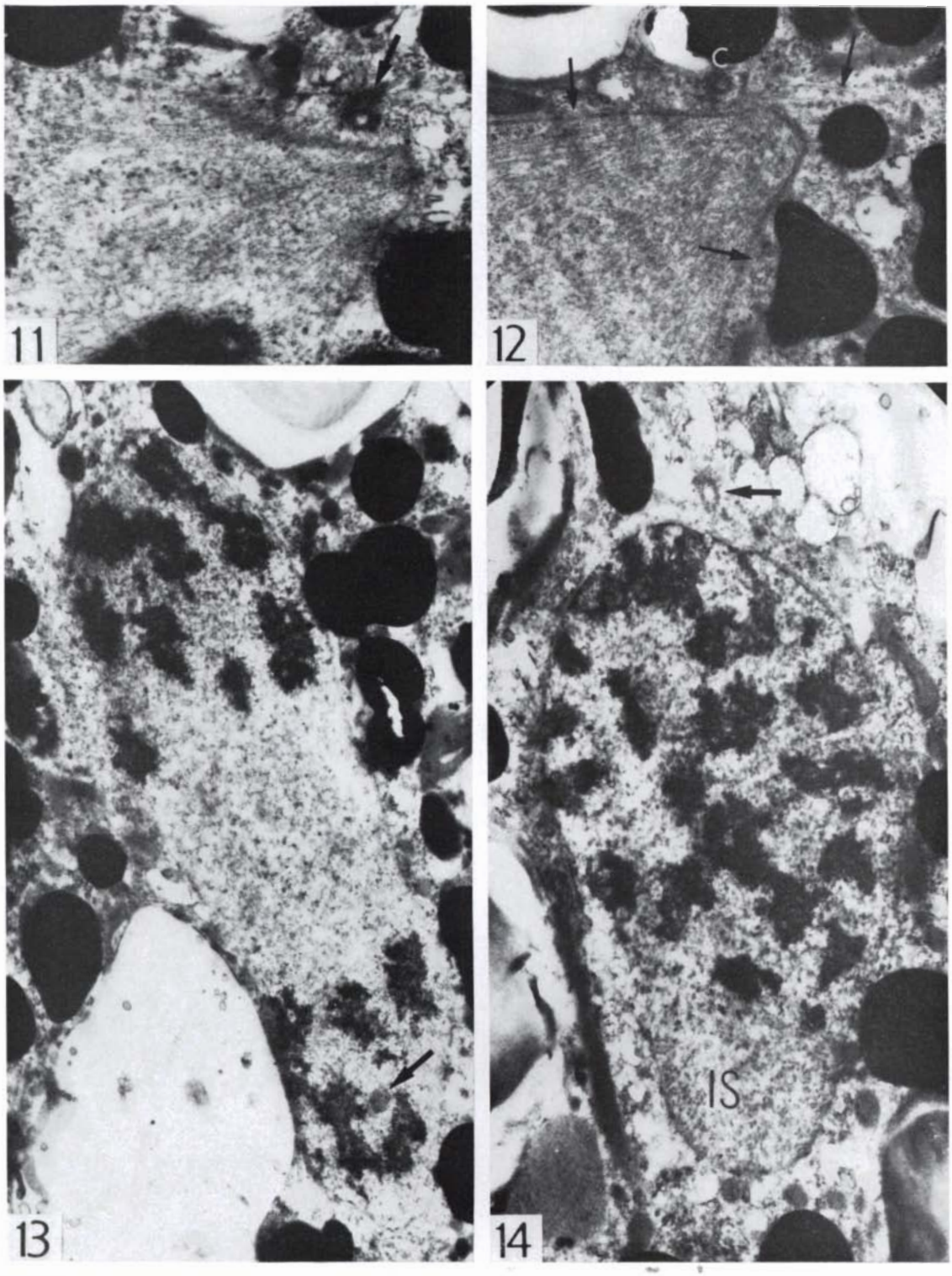
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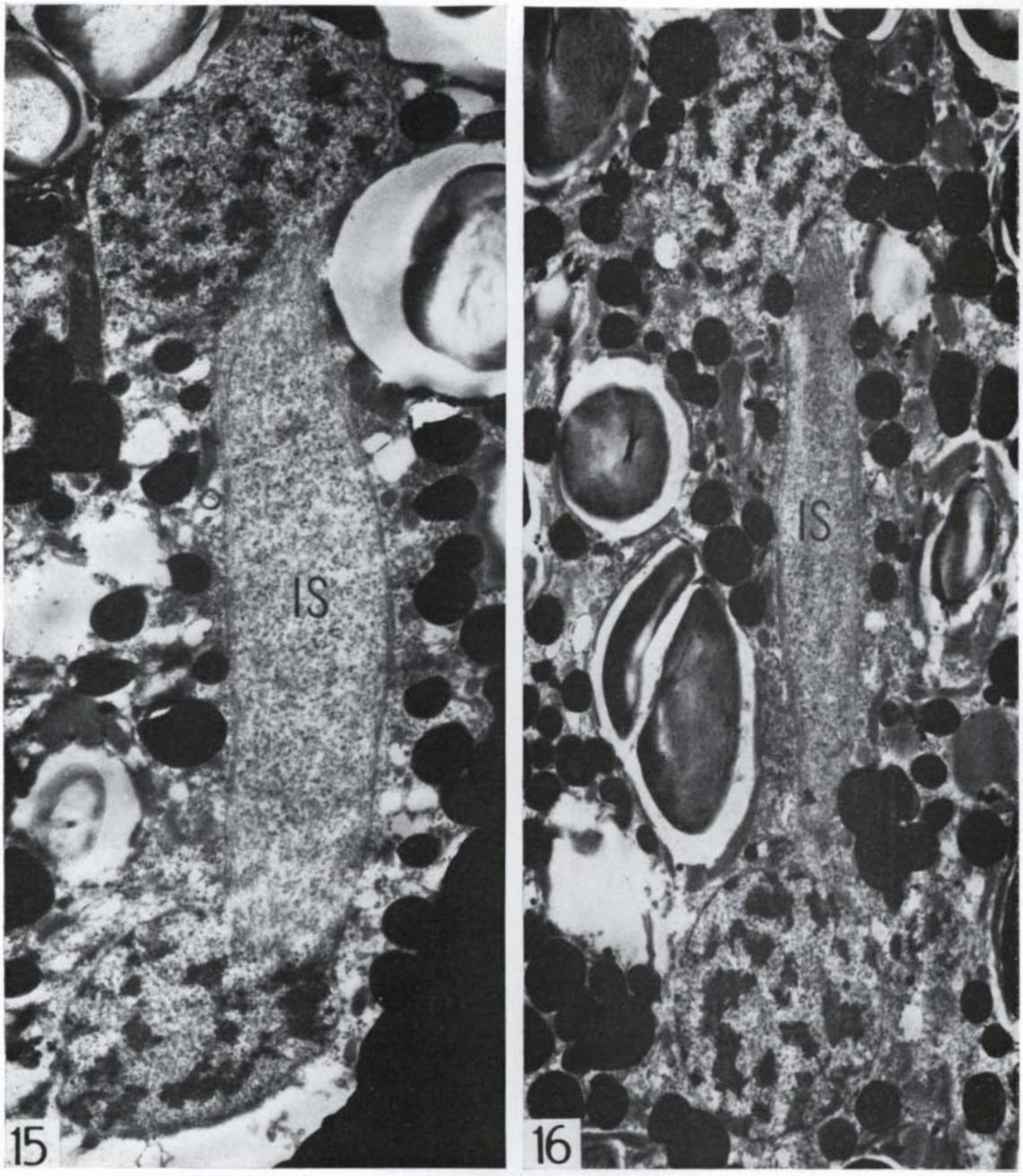

FIGS 15–16. Telophase.

FIG. 15. Two daughter nuclei connected by fully elongated interzonal spindle (IS) are slightly twisted. × 7900.

FIG. 16. Probable remnant of the interzonal spindle (IS) after completion of abscission. Daughter nuclei are twisted up in opposite directions. × 6600.

1907; Chihara, 1953, 1959) and *V. utricularis* (Famintzin, 1860; Kuckuck, 1907; Schechner-Fries, 1934; Schussnig, 1938). However, our present knowledge is still meagre in studying the systematic and phylogenetic affinities of this genus among the green algae.

Although the reproductive phase of *Valonia*, including *V. ventricosa*, has not yet been artificially and synchronously induced, cytological and ultrastructural study may be effected by chance. In 1976, we fortunately had an opportunity to fix maturing vesicles of *V. ventricosa* from laboratory cultures for electron microscopy. The present observation was inevitably restricted to a limited stage of the reproductive process, and whether it was sporogenesis or gametogenesis was not determined, because we fixed the vesicles at an early stage of the reproductive process and before the visualizing of difference in pigmentation of the net-work (Chihara, 1959).

So far as we are aware, nuclear division has been studied only in *V. utricularis* by Schechner-Fries (1934) and Schussnig (1938). They confirmed that meiotic division occurs during gametogenesis and mitosis during sporogenesis. Although not confirmed in *V. ventricosa*, spindle figures of anaphase (Fig. 13) and telophase (Fig. 15) in this alga resemble those from gametogenesis in *V. utricularis* which Schussnig (1938) illustrated from the light microscope. The telophase figure of his paper (Abb. 2–22) may correspond to Fig. 15. These resemblances possibly suggest the nuclear division observed in this study may be of gametogenesis.

Ultrastructural nuclear division in *V. ventricosa* closely resembles that described for *Cladophora glomerata* (McDonald & Pickett-Heaps, 1976); retention of the nuclear envelope throughout the division, absence of distinct polar fenestrae, non-typical alignment of chromosomes on an equatorial region, centric, presence of kinetochores, and some other resemblances. However, there are also some differences between them. Centrioles in *V. ventricosa* are situated to one side of the polar region of the nucleus, while in *C. glomerata* they are situated on the long axis of the spindle as in most other green algae. Spindle microtubules of *C. glomerata* converge to the intranuclear region adjacent to the centriole complex but never traverse through the envelope as in *V. ventricosa*.

Compared with *Acrosiphonia spinescens* (Hudson & Waaland, 1974), an organism closely related to *Cladophora*, there are some differences in major respects: presence of the distinct fenestrae, typical metaphase plate, and absence of kinetochore.

The nuclear envelope of *Batophora oerstedii* is retained throughout the nuclear division as in most other green algae, and perinuclear ER conspicuously surrounds it (Liddle, Berger & Schweiger, 1976). Metaphase and anaphase plates of chromosomes are distinctly observed in contrast to those of *Cladophora* and *Valonia*. Centrioles and polar openings are absent in *Batophora*. Differentiation of kinetochore is not known in this alga.

Although the present results seemingly suggest closer affinity of *V. ventricosa* with *C. glomerata* than with two other algae, phylogenetic conclusion should be made after further studies of other important taxonomically related algal groups, the members of the Siphonocladales, Codiales, Derbesiales and Caulerpales *et al.*, as well as the Cladophorales, Acrosiphoniales and Dasycladales.

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