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Mitosis, cytokinesis and multinuclearity in a Xanthonema (Xanthophyta) isolated from Antarctica

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Mitosis, cytokinesis and multinuclearity in a *Xanthonema* (Xanthophyta) isolated from Antarctica

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The ultrastructure of a *Xanthonema* strain featuring multinucleate cells was investigated by transmission electron microscopy. An important specific feature of the organisation of the photosynthetic apparatus in this strain is its association with mitochondrial profiles. The chloroplast girdle is composed of two different U-shaped lamellae, one peripheral and one subcentral. Multinuclearity is observed as often as the uninucleate state. The transition from the uninucleate to the multinucleate stage is connected to disturbances in the normal division pattern of the parietal chloroplast-mitochondria complex during interphase. As a result mitosis is not coordinated with cytokinesis. The return to the uninucleate stage occurs as a result of asynchronous cytokinesis or by aplanospore formation. Mitosis is of the semi-closed type, as in *Tribonema*. Centrioles replicate in early interphase, after the end of karyokinesis and progeny nuclei separate with the aid of CER invagination. Filament fragmentation takes place between neighbouring cells where two U-shaped segments adjoin, resulting in fragment ends being rounded rather than 'zweispitzig'. The taxonomic significance of various ultrastructural features for the classification of filamentous Xanthophyta is discussed.

Key words: chloroplast, cytokinesis, girdle lamellae, mitochondria, mitosis, multinuclearity, *Xanthonema*, Xanthophyta

Introduction

Recent investigations (e.g. Bailey & Andersen, 1998; Negrisolo *et al.*, 2004; Zuccarello & Lokhorst, 2005) have shown that the traditional, morphologically-based, classification of the Xanthophyceae, is often incongruent with molecular data and that a new classification system is needed. For example, molecular data do not support the monophyly of the traditional order Tribonematales, containing *Tribonema* Derbès *et* Solier, *Xanthonema* Silva and *Heterococcus* Chodat but ultrastructural investigations have been important for determining the taxonomic and phylogenetic relationships in the algae. Studies in the Xanthophyceae (Hibberd, 1980, 1989) revealed that they are characterized by chloroplasts with four membranes, photosynthetic lamellae with three thylakoids, and a layer of chloroplast ER that is continuous with the outer membrane of the nuclear envelope. They differ from other chromophytes by the presence of a girdle lamella and DNA concentrated in genophores.

Most ultrastructural investigations of the Xanthophyceae have dealt with the vegetative cell (Hibberd & Leedale, 1971), the chromatophore (Böger & Kiermayer, 1974; Hesse, 1980), spermatogenesis (Ott & Brown, 1978), zoospores (Massalski & Leedale, 1969; Lokhorst & Star, 2003), and flagellar hair formation (Leedale *et al.* 1970; Deason, 1971). Mitosis and cytokinesis have, however, been poorly documented, although the significance of these features for phylogenetic and taxonomic understanding has been demonstrated for other algae, especially chlorophytes. The only complete ultrastructural studies of the cell cycle in the Xanthophyceae are of *Vaucheria litorea* C. Ag. (Ott & Brown, 1972) and *Tribonema regulare* Pascher (Lokhorst & Star, 1988). There are no published data on mitosis, cytokinesis and cell-wall formation in *Xanthonema*.

The genus *Xanthonema* Silva is a filamentous, unbranched alga lacking obviously H-shaped cell

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wall segments when viewed by light microscopy (LM; Ettl, 1978). During a preliminary investigation of a *Xanthonema* strain isolated from the Antarctica, we noticed the presence of a relatively high number of multinucleate cells, despite reports that *Xanthonema* is generally considered to be uninucleate. The objective of this investigation was to study the ultrastructure of this isolate, with special emphasis on its mitosis, cytokinesis and the formation of multinucleate cells. The results were compared to data on other xanthophycean genera to evaluate the taxonomic significance of mitotic and cytokinetic features at genus and species level.

Materials and methods

Soil samples were collected in 1993 by M. Olech from the moraines of Ecology Glacier (62°09'S; 58°28'W) during the XVI Polish Antarctic Expedition to the Henryk Arctowski Station (King George Island, South Shetland Islands). Soil samples were first moistened with distilled water and algal samples were inoculated onto 1.5% agar solidified with Bristol's medium (Bristol, 1920). Cultures were maintained at 20°C under a 16-h : 8-h light–dark cycle at a light intensity of 3000 $\mu E.m^{-2}.s^{-1}$ provided by 40 W cool fluorescent tubes.

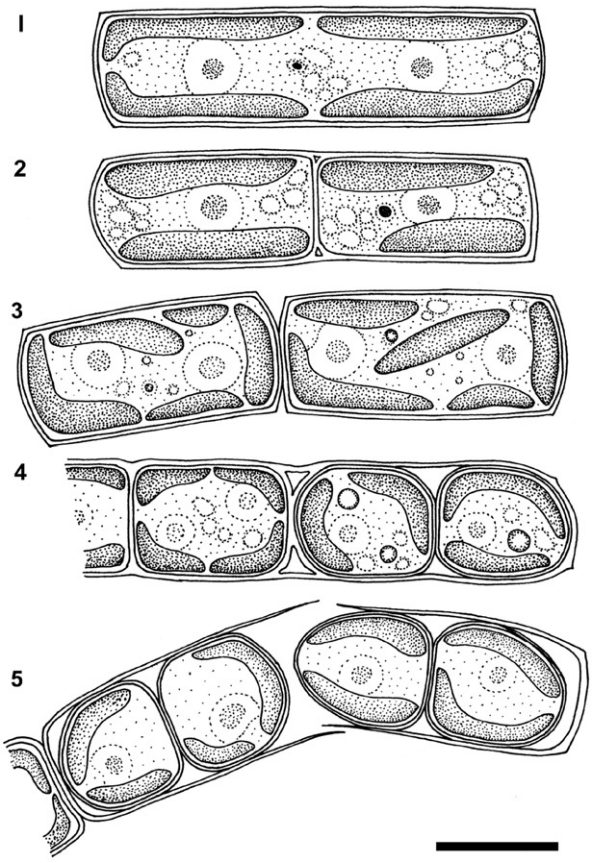
For transmission electron microscopy (TEM), filaments were removed from the surface of agar cultures with a spatula and fixed for 1 h at room temperature with 3% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. The filaments were then washed three times for 10 min in phosphate buffer, and fixed for 2 h at room temperature with 1% osmium tetroxide in 0.05 M phosphate buffer. After two washes of 10 min in the same buffer and dehydration in an ethanol series, the cells were infiltrated overnight with a 1 : 1 mixture of propylene oxide and Spurr's medium (Spurr, 1969), and then embedded in fresh Spurr's medium. The samples were polymerized at 70°C for 12 h. Sections were cut with glass knives on a Reichert–Jung Supernova ultramicrotome and collected on unsupported 400-mesh copper grids, which were subsequently double stained for 3 min with 5% uranyl acetate in 50% ethanol, followed by 1 min with a lead citrate solution (Reynolds, 1963). Over 200 cells were examined and photographed with a TESLA BS 500 electron microscope.

Results

Light microscopy observations

In 2-month old agar cultures, filaments are short, usually 2–4 cells long, and easily disintegrate into single cells. Filaments are (3.7−)4.2–5.0(−5.3) μm wide and cylindrical or slightly constricted near the transverse cell walls. The cell wall is thin, smooth, and about 0.14–0.25 μm thick.

The terminal cells are rectangular, rounded, lack the 'H' endings but occasionally, display small,



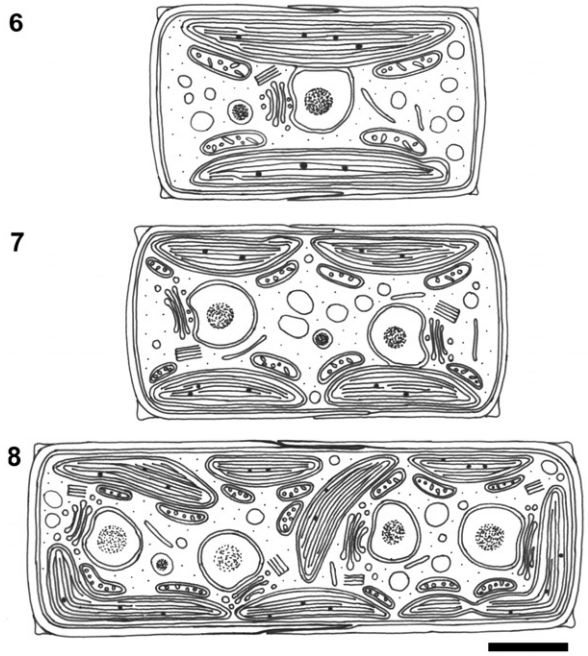
Figs 1–5. Light microscope drawings of *Xanthonema*. Fig. 1. Short filament with uninucleate cells containing two parietal chloroplasts. Fig. 2. Binucleate cell with four parietal chloroplasts. Fig. 3. Fragmentation of the filament with binuclear cells containing parietal and internal chloroplasts. Fig. 4. Filament with two terminal akinetes. Fig. 5. Fragmentation of the filament with akinetes. Scale bar: 5 μm.

wart-like protrusions at the corners. Two types of cells are present: (i) short (5.7–9.0 (−11.2) μm long) cells with 2–4 parietal, plate-like chloroplasts (Figs 1, 2), and (ii) elongated (10.2–18.1 (−20.0) μm long) cells with 4–8 (−10) chloroplasts, both parietal and internal (Fig. 3). A variable number, generally six to 30, of small vacuoles can be observed near the transverse cell walls or scattered throughout the cell cytoplasm.

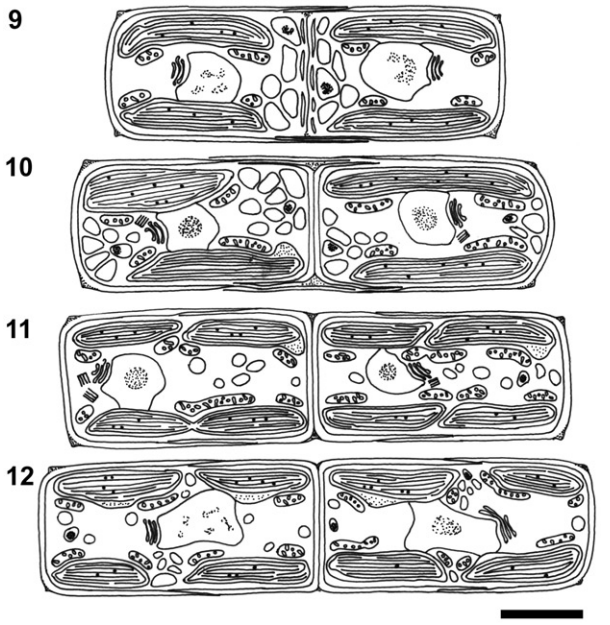
Reproduction is by fragmentation (Fig. 3) or the formation of akinetes, which are mainly produced in pairs (Figs 4, 5). Akinete-like cells with thickened cell walls, which germinate into single non-motile cells, were also observed.

Transmission electron microscopy observations

Vegetative cells have a variable number of nuclei, and can therefore be characterized as mono-, bi- or multinucleate (with 3–4 nuclei). The mononucleate cells are always short (cell type 'a', Fig. 6), whereas the multinucleate cells are always long (cell type 'b', Fig. 8). The binucleate cells can be either



Figs 6–8. Drawings to show different types of cells of *Xanthonema* during interphase. Fig. 6. Short uninucleate cell with two chloroplasts (type a). Fig. 7. Short binucleate cell with four chloroplasts (type a). Fig. 8. Elongated multinucleate cell with numerous both parietal and internal chloroplasts (type b). Scale bar: 2 μm.



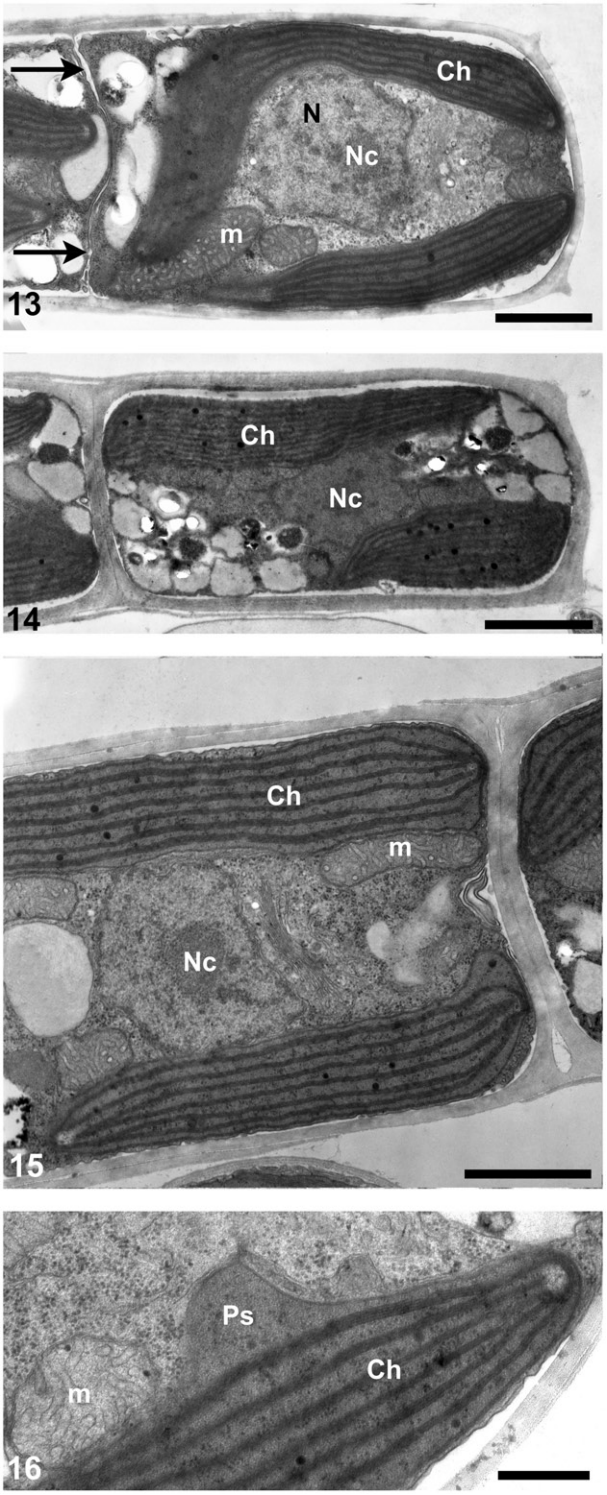
Figs 9–12. Drawings to show uninucleate cells of *Xanthonema* during interphase. Fig. 9. Cells in early interphase with primary transverse cell wall. Fig. 10. Cells in the middle interphase with almost completed secondary cell wall. Fig. 11. Late interphase: chloroplast division completed but mitochondria still dividing; vesicles have moved. Fig. 12. Cells in late interphase (top) and early prophase (bottom). Scale bar: 3 μm.

short (type 'a', Fig. 7) or elongate (type 'b', fig. 8). Bi- and multinucleate cells represent around 20–30% of all cells.

The ultrastructure of mononucleate cells varies during the cell cycle. In early interphase (Figs 9, 13), the transverse cell wall is thin and often wavy. Several small or 3–5 large vacuoles, occasionally with small aggregates of electron dense material, were observed near the cell wall. In young cells, a single nucleus with a central spherical nucleolus is located in the centre of the cell. The nucleus has an indentation on one side in which the Golgi body is situated. A single centriole is located beside the Golgi body in early interphase. Some cells with a thin transverse cell wall have two centrioles, which are parallel to each other but at different levels in the cell. There are two parietal plate-like chloroplasts, each of which has an envelope of four membranes and contains 5–7 lamellae, each of which usually comprises three thylakoids. A girdle lamella extends under the plastid envelope. A genophore is present at each plastid pole. Individual, electron-dense, plastoglobules are present between the lamellae. The external chloroplast membrane (CER) of at least one of the chloroplasts is continuous with the nuclear membrane. There are two mitochondrial profiles associated with each chloroplast. These are situated at the poles of the chloroplasts on the side facing the centre of the cell. Mitochondria have tubular cristae.

In middle interphase (Figs 14, 15) all mononucleate cells have transverse cell walls of equal thickness. The nucleus is central and the nucleolus is well visible. Vacuoles accumulate near both transverse cell walls and some are also located towards the cell centre. The number of lamellae in the chloroplast increases to seven to nine. The mitochondrial profiles elongate, some of them reaching the connection between the CER and the nuclear membrane.

In late interphase (Figs 11, 17), the small vacuoles are irregularly distributed throughout the internal cell space; the chloroplasts divide and the cells have four chloroplasts. During chloroplast division, one mitochondrial profile overlaps the two progeny chloroplasts, on the inner side. The division of the mitochondrial profiles accompanies separation of the progeny chloroplasts. It is possible that the chloroplast is involved in the division of the mitochondrial profiles. The number of chloroplast lamellae decreases to six to seven. The centrioles move in opposite directions from a more or less parallel position towards each side of the Golgi body. The nucleolus starts to disappear at the end of late interphase (Fig. 12).



Figs 13–16. Electron micrographs of uninucleate cells of *Xanthonema* during interphase. Fig. 13. Cell with two parietal chloroplasts, four mitochondrial profiles associated with chloroplasts, and thin transverse cell wall (arrows). Figs 14, 15. Cell in middle interphase with two chloroplasts and terminal pyrenoid-like body; mitochondrial profiles associated with CER, Golgi body adjacent to the nucleus with well defined nucleolus, thick transverse cell wall. Fig. 16. Detail of pyrenoid-like body associated with the chloroplast. Scale bars: 1.5 μm (Figs 13–15); 0.75 μm (Fig. 16). Abbreviations: Ch: chloroplast; m: mitochondrion; N: nucleus; Nc: nucleolus; Ps: pyrenoid-like body.

Mitosis in mononucleate cells

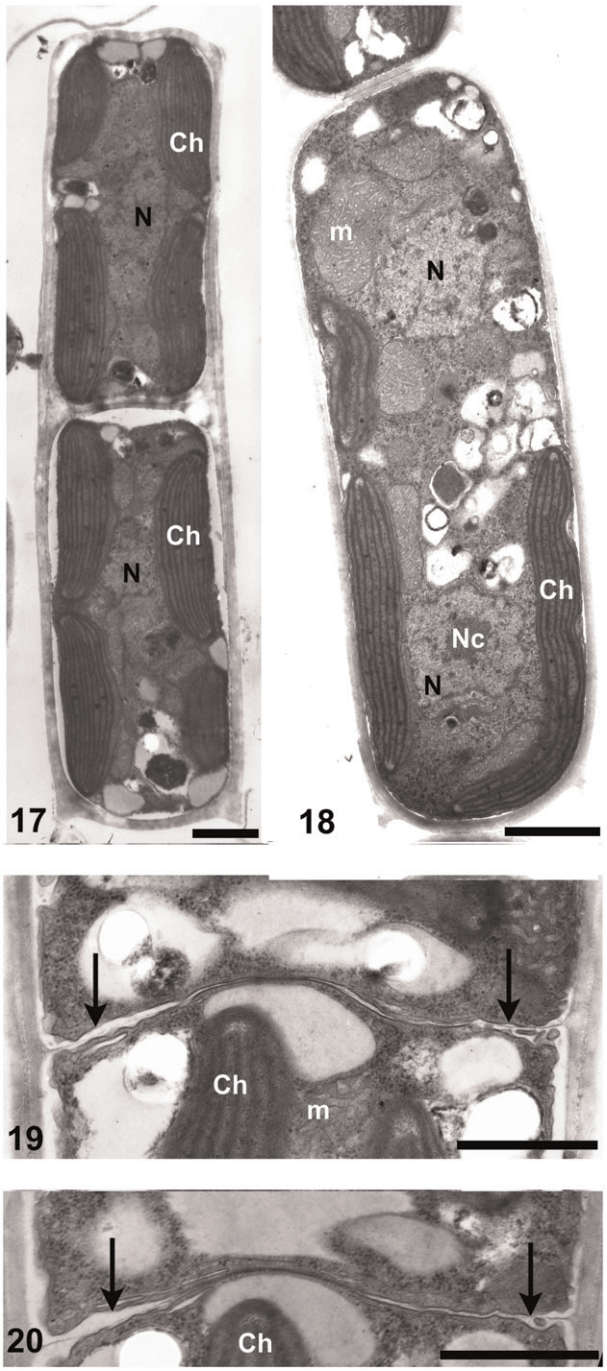
In prophase, mononucleate cells have four parietal chloroplasts and eight mitochondrial profiles, two of which, at the poles of each chloroplast, face the centre of the cell (Fig. 12). In early prophase the mitochondrial profiles increase in size, the parietal vacuoles concentrate in the equatorial plane of the cell where the future transverse cell wall will be formed, and the centrioles are positioned diagonally on opposite sides of the nucleus. Each centriole is perpendicular to the future mitotic spindle. At this stage, a Golgi body is positioned beside each centriole. One of the Golgi bodies is large, whereas the other is relatively small. The developmental mechanism of the second Golgi body remains unknown. A large perforation, through which the mitotic spindle microtubules enter the nucleus, appears in the nuclear membrane near each centriole at each nuclear pole.

During metaphase (Figs 25, 27) chromosomes are present on the equatorial plate. The Golgi bodies are located parallel to the nuclear membrane, with a centriole at each Golgi pole, at an angle of about 90° to the cisternae. A bundle of mitotic spindle microtubules appear in the perforation zone of the nuclear membrane beside the top end of the centriole. The spindle microtubules are intranuclear; mitosis is of the semi-closed type. Small vesicles can be observed in the space between the Golgi body and the nuclear membrane. Some of these appear to originate from the proximal Golgi cisternae, whereas others originate from the external nuclear membrane.

Chromosomes diverge towards opposite poles during anaphase (Figs 28, 29), and a large invagination appears in the nuclear membrane near its connection with CER (Fig. 20). In telophase (Fig. 26), the CER invagination divides the progeny nuclei and the mitotic spindle disappears. Vesicles, possibly containing building material for the cell wall, concentrate between the progeny nuclei in the plane of the future transverse wall and cytokinesis begins.

Cytokinesis and the formation of transverse cell walls

Cytoplasmic division in parent cells takes place as a result of fusion of lateral vesicles with the plasmalemma and fusion of central vesicles with each other. Central vesicles are bigger than lateral ones, and are characterized by the presence of electron dense material. During central vesicle fusion their contents become denser (Fig. 31) and a thin electron dense plate, the primary transverse cell wall, appears in the space between future progeny cells



Figs 17–20. Electron micrographs of cells from late interphase to start of primary wall formation. Fig. 17. Cell in late interphase with four chloroplasts, mitochondrial profiles divided. Fig. 18. After caryokinesis the different types of vesicles begin to concentrate in the equatorial plane of the binucleate cell. Figs 19, 20. Serial sections of the start of primary cell wall formation. Thin primary cell wall is situated between the plasmalemmae of daughter cells; vesicles, some with electron dense contents, are concentrated on both sides of the primary wall (arrows). Scale bars: 1.5 μm (Figs 17, 18); 0.75 μm (Figs 19, 20). Abbreviations: Ch: chloroplast; m: mitochondrion; N: nucleus; Nc: nucleolus.

(Figs 32, 19, 20). Progeny cell protoplasts then form their plasmalemmae from membranes of central vesicles and a thin primary transverse cell wall appears (Figs 33, 21); vesicles originating from the Golgi body continue to fuse with the plasmalemmae of new progeny cells, leading to the appearance of a plate-like structure at the centre on each side of the primary cell wall (Figs 34, 22, 23). These plate-like structures gradually extend centrifugally to form the new U-shaped segment of the progeny cell walls (Figs 35, 36). Each cell forms one U-shaped segment of secondary cell wall. The pair of U-shaped segments is joined by the primary cell wall, thus forming an H-like structure.

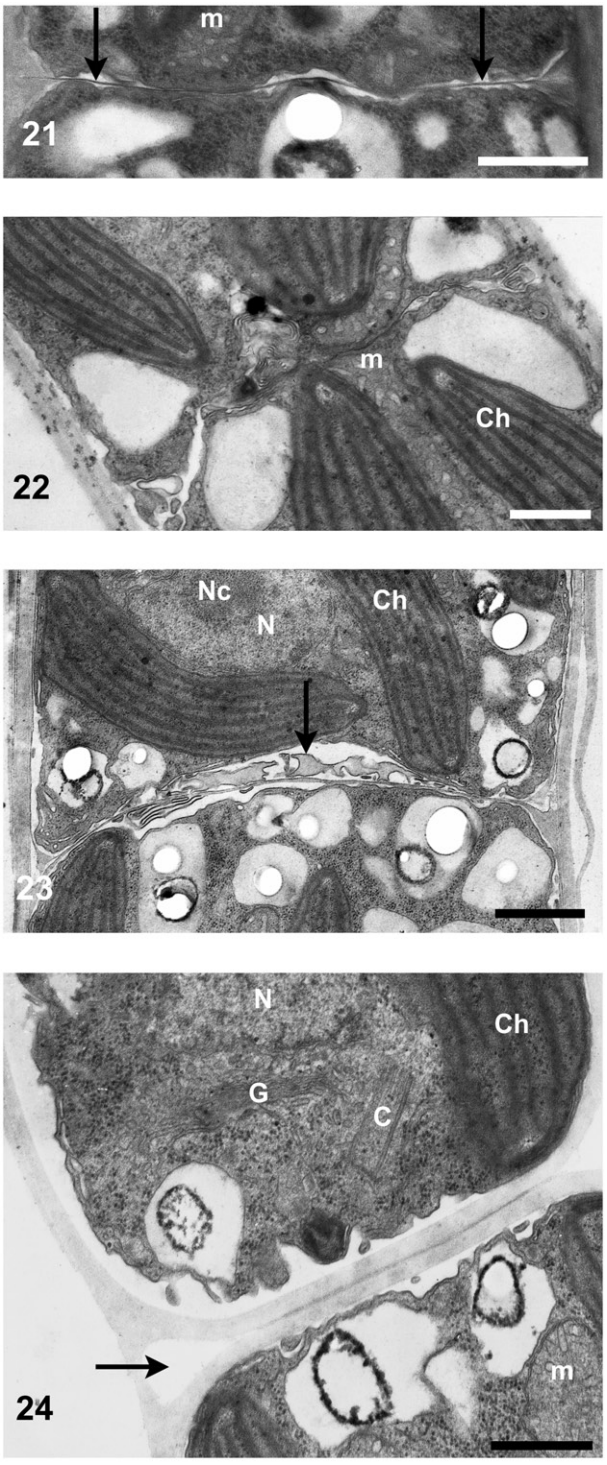
The formation of the secondary cell wall takes place centrifugally and results in the elongation of progeny cells. At the same time, the primary cell wall width decreases, gradually depolymerizing centripetally (Figs 35, 36, 24). After depolymerization of the primary cell wall, the remnants of the primary cell wall hold the progeny cells together. If the primary cell wall disappears entirely, the progeny cells separate and fragmentation of the filaments occurs. In the latter case, 'H'-shaped cell endings are not formed but there are small thickenings at the corners of the cells. Each progeny cell has one nucleus and two chloroplasts.

Chloroplasts and their movements in the different phases of the cell cycle

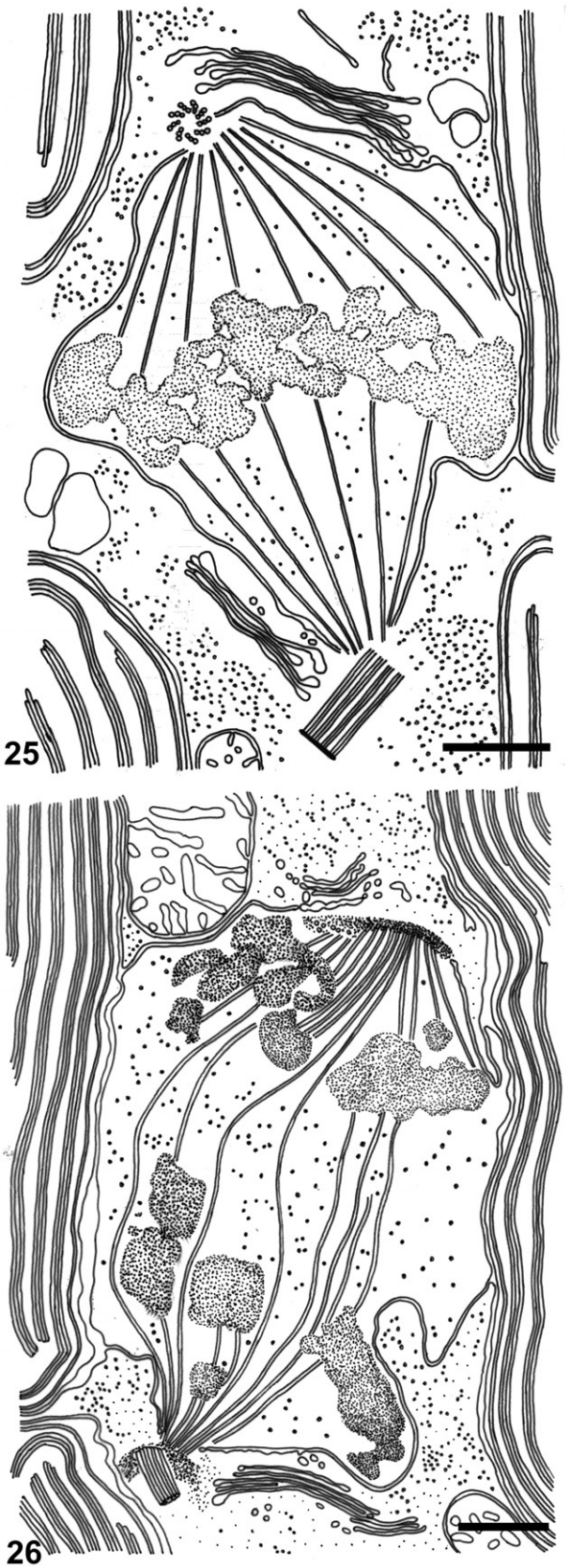
The chloroplast lamella girdle is not closed, but U-shaped with two arms. One arm is peripheral, situated just under the chloroplast envelope membrane (Fig. 37). The second arm is subcentral situated close to the longitudinal axis of the chloroplast. The latter is separated from the chloroplast envelope by the peripheral branch of the second girdle lamella and also often by one or two free interior lamellae.

The chloroplast usually possesses five to seven lamellae in early interphase, but at late interphase, a constriction appears in the middle of the chloroplast. The lamellae are fragmented at the constriction, and progeny genophores may be visible in the thylakoid-free zones. In early interphase of mononucleate cells, both arms of the girdle lamellae usually comprise three thylakoids, whereas in the curved zone, only one (Fig. 38) or two thylakoids are present. Free interior lamellae usually contain three thylakoids, and one of their ends often anastomoses with the peripheral branch of the girdle lamella.

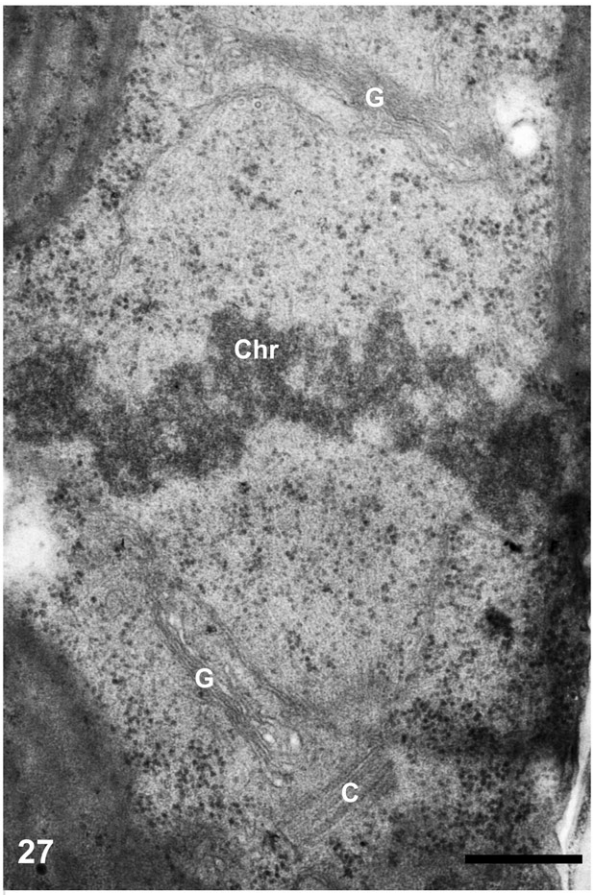
In middle interphase, new lamellae can form through fragmentation, subsequent separation, growth and reassociation of thylakoids leading to an increase in the number of lamellae to seven to nine. During this stage, the curved zones of girdle lamellae comprise three thylakoids.

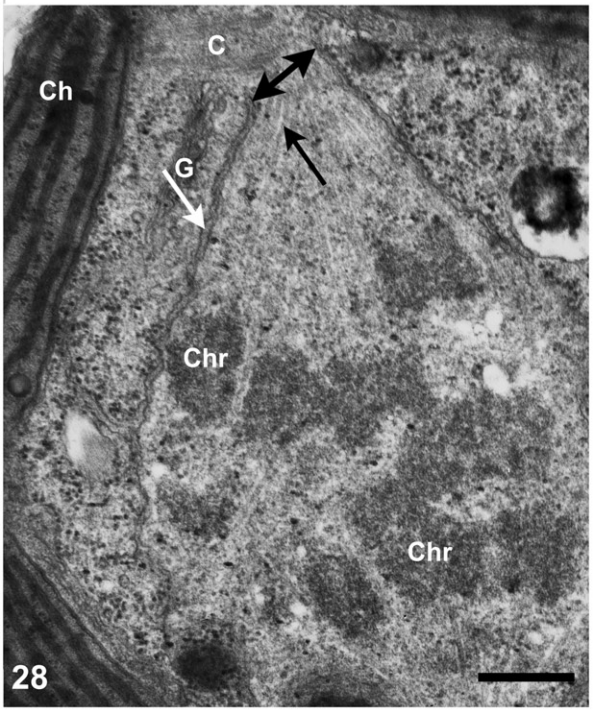


Figs 21–24. Electron micrographs of cell wall formation. Fig. 21. Completed primary cell wall spanning the parent cell walls (arrows) Fig. 22. Beginning of secondary cell wall formation. Fig. 23. Later stage of secondary cell wall formation (arrows). Fig. 24. Empty spaces between the mature cell wall segments of two daughter and one parent cell appear as the primary cell wall disintegrates (arrow). Scale bars: 0.75 µm (Figs 21–23); 1.5 µm (Fig. 24). Abbreviations: C: centriole; Ch: chloroplast; G: Golgi apparatus; m: mitochondrion; N: nucleus; Nc: nucleolus.

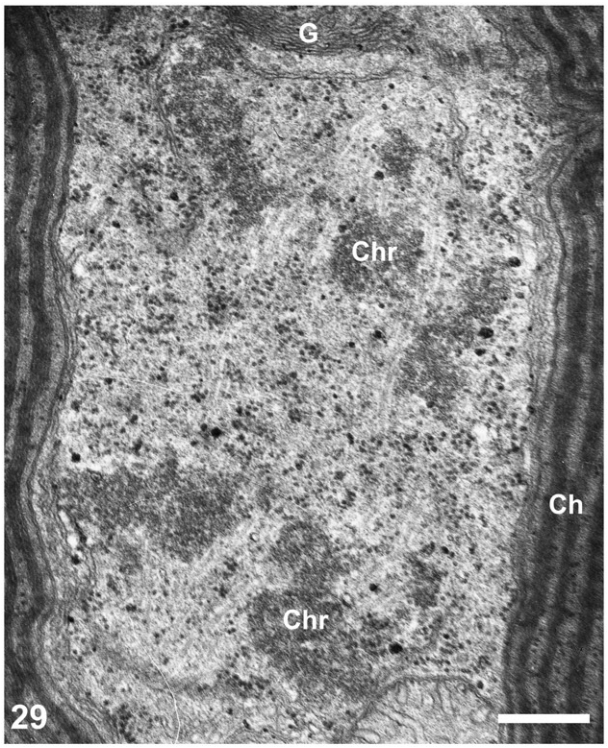


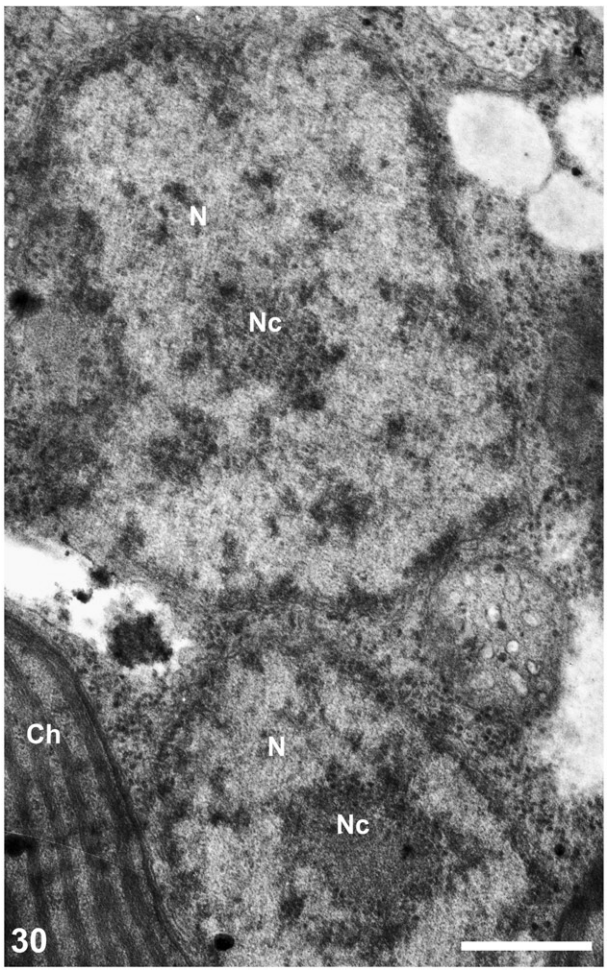
Figs 25, 26. Drawings of mitosis in *Xanthonema*. Fig. 25. Semi-closed mitosis with internal spindle, Golgi body adjacent to centrioles, chromosomes form metaphase plate. Fig. 26. Anaphase; invagination of CER begins to separate daughter nuclei. Scale bar: 0.5 µm



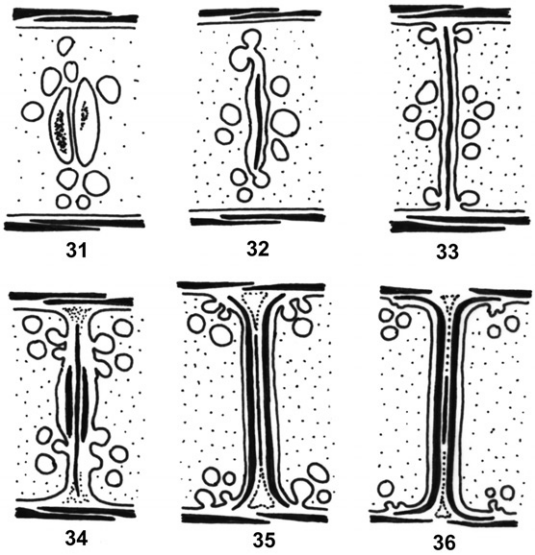


Figs 27, 28. Electron micrographs of mitosis in *Xanthonema*. Fig. 27. Metaphase with chromosomes in equatorial plane, two Golgi bodies on opposite sides of nucleus, longitudinally sectioned centriole. Fig. 28. Early anaphase showing chromosomes being pulled apart by spindle microtubules (black arrow), intact nuclear envelope (white arrow) with polar opening (double arrow) near the oblique, longitudinally sectioned, centriole. Scale bar: 0.75 µm. Abbreviations: C: centriole; Ch: chloroplast; Chr: chromosome; G: Golgi apparatus; m: mitochondrion.





Figs 29, 30. Electron micrographs of mitosis in *Xanthonema*. Fig. 29. Late anaphase. Fig. 30. Telophase, chromosomes no longer discernable, nucleolus beginning to appear. Scale bar: 0.75 µm. Abbreviations: Ch: chloroplast; Chr: chromosome; G: Golgi apparatus; N: nucleus; Nc: nucleolus.



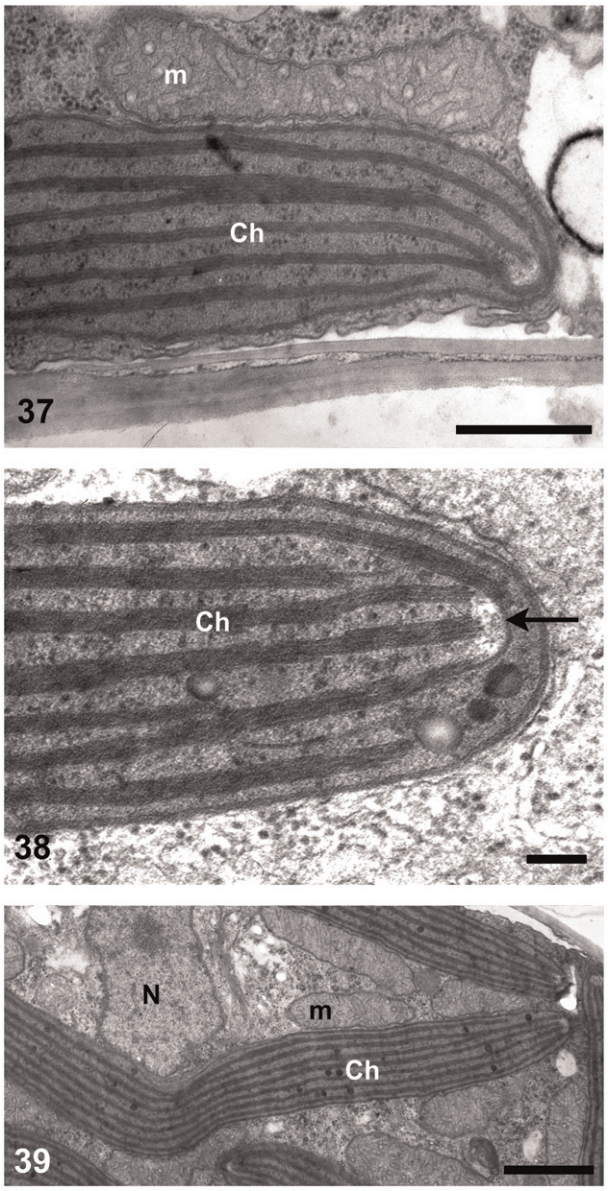
Figs 31–36. Schematic drawings of transverse cell wall formation in *Xanthonema*. Fig. 31. The vesicles lie in the plane of the future transverse cell wall; some vesicles contain granular electron-dense material for primary cell wall formation. Fig. 32. Medial vesicles fused and primary cell wall appearing. Fig. 33. Fusion of terminal vesicles with plasmalemma and separation of descendant cell protoplasts. Fig. 34. Beginning of formation of U-shaped segment of secondary cell wall. Figs 35, 36. Growth of U-shaped segments and disintegration of primary cell wall.

Formation of multinucleate cells

Abnormal chloroplast or mitochondrial divisions disturb the translocation of Golgi vesicles in bi-nucleate cells. As a result, progeny plasmalemmae, primary transverse cell walls, and U-shaped segments of the secondary cell wall are not formed. Thus, karyokinesis is not followed by cytokinesis, and vegetative cells containing two or more (three to four) nuclei are formed. We observed several variants.

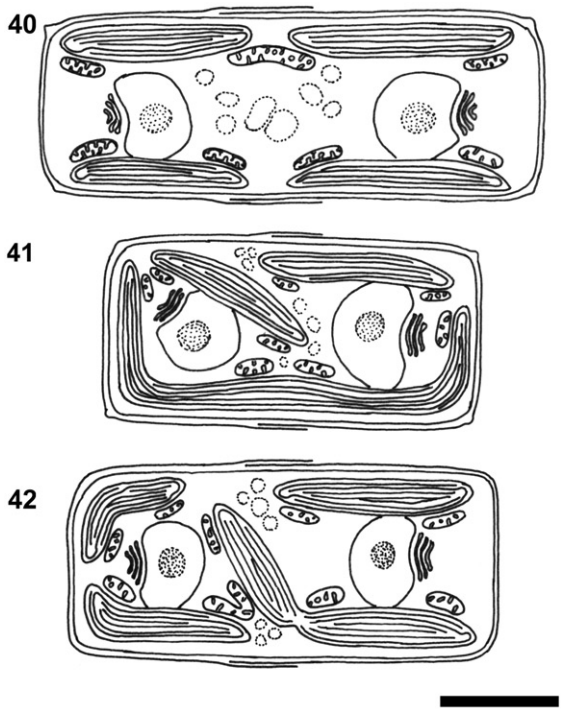
Variant A. Mitochondria do not divide after chloroplast division in late interphase and, as a result, a mitochondrial profile bridges two progeny chloroplasts during karyokinesis (Figs 40, 43). When nuclear division is complete, the undivided mitochondrial profile is situated on the equatorial plane of the cell, where the cell wall is usually formed, thus preventing lateral translocation of vesicles to the plasmalemma causing cessation of cytokinesis. An elongated, bi-nucleate vegetative cell with four parietal chloroplasts is formed. If the nuclei of this cell undergo new mitoses, in cells with three or four nuclei and four to seven parietal chloroplasts result. All chloroplasts are parietal in such cells

Variant B. Chloroplast division is disturbed at the beginning of late interphase producing multinucleate cells with internal chloroplasts. One of the



Figs 37–39. Electron micrographs of chloroplasts in *Xanthonema*. Fig. 37. Showing chloroplast-mitochondrion association and structure of girdle lamella with external and internal arms and anastamosing lamellae. Fig. 38. Bent zone of girdle lamella consisting of only one thylakoid (arrow) at early interphase. Fig. 39. Showing abnormal internal chloroplast–mitochondrion association, structure of girdle lamella and presence of plastoglobules. Scale bars: 0.75 μm (Figs 37, 38); 1.5 μm (Fig. 39). Abbreviations: Ch: chloroplast; m: mitochondrion; N: nucleus.

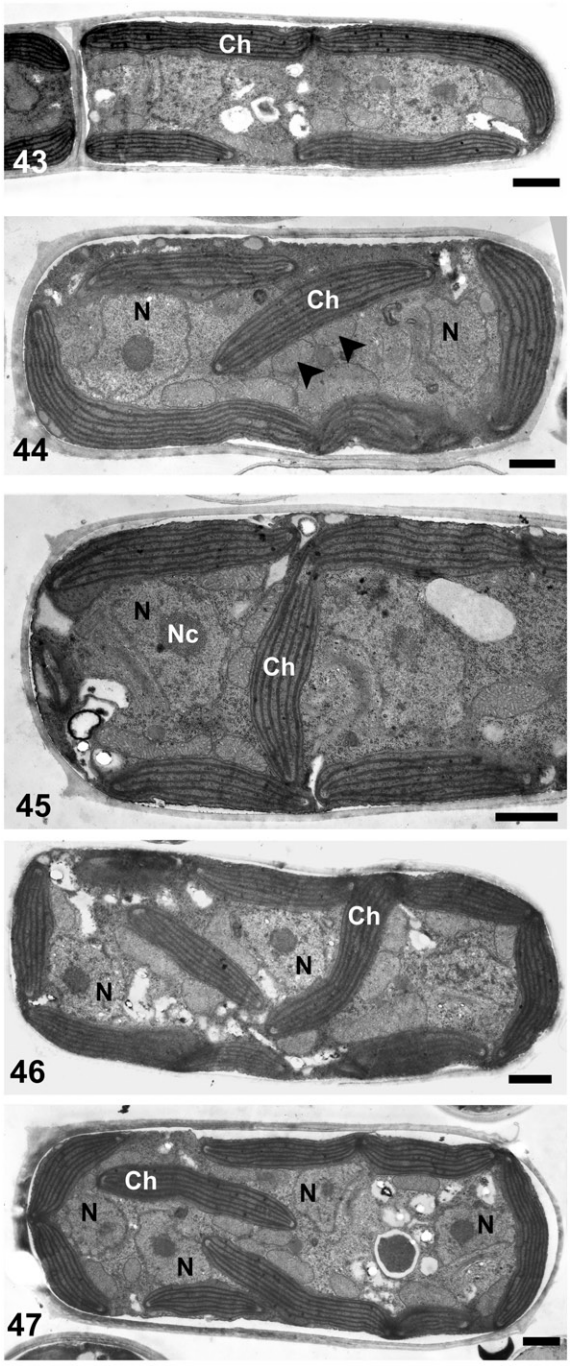
chloroplasts does not divide but continues to grow in late interphase (Fig. 41), shifting a progeny chloroplast from a parietal position to the central area of the cell. When mitosis is finished the chloroplast moves to the cell centre (we term such chloroplasts 'internal'), disturbs the translocation of vesicles to the equatorial plane of the cell, and cytokinesis fails (Fig. 44). Further nuclear divisions lead to the formation of cells with three (Fig. 46) or four nuclei (Fig. 47).



Figs 40–42. Variation in multinuclear cell formation. Fig. 40 After chloroplast division the mitochondrion does not divide and vesicles cannot move to the plane of the future transverse cell wall. Fig. 41. One chloroplast does not divide and the other moves towards the plane of the future transverse cell wall. Fig. 42. One chloroplast moves to the centre of the cell and the mitochondrion adjacent to the neighbouring chloroplast makes contact with it; two chloroplast-mitochondrial complexes interfere with normal vesicle migration to the plane of the future transverse cell wall. Scale bar: 3 μm.

Variant C. Chloroplasts and mitochondrial profiles divide abnormally. After chloroplast division in late interphase or during mitosis, one of the chloroplasts elongates more rapidly than the other and, probably as a result of pressure from the neighbouring chloroplast, begins to bend towards the cell interior. The outer side of the chloroplast may touch the mitochondrion of the neighbouring chloroplast, forming the associated complex (Figs 39, 42, 45). Normal translocation of vesicles containing wall-building material for transverse cell wall formation is prevented by that part of the chloroplast near the cell centre. After rearrangement of the mitochondrial profiles, chloroplast division produces one parietal and one internal chloroplast. The internal chloroplast does not interfere with subsequent nuclear divisions, however, it does interfere with cytokinesis. As a result, all cells with internal chloroplasts are multinucleate and contain two, three or four nuclei.

Restitution of the mononucleate stage may occur as a result of equal or unequal division of



Figs 43–47. Electron micrographs of multinuclear cell formation. Fig. 43. Undivided mitochondrial profile prevents normal cytokinesis. Nucleus on the left-hand side approaching prophase of the next division. Fig. 44. Progeny chloroplast with two internal mitochondrial profiles (arrow heads) moving towards the central part of the cell prevents the next cell division. The nucleus on the right hand side is starting to divide; two cross-sectioned centrioles are visible near the interphase nucleus. Fig. 45. Abnormally elongated chloroplast still undivided and bent at almost right angles inside the cell; mitochondria associated with the external side of the bent chloroplast; centriole and terminal pyrenoid-like body are visible. Fig. 46. Tri-nucleate cell with two internal chloroplasts; nucleus on the right hand side appears to be in the early prophase. Fig. 47. Quadri-nucleate cell with parietal, one internal and one bent chloroplast. Scale bar: 1.5 μm. Abbreviations: Ch: chloroplast; N: nucleus; Nc: nucleolus.

multinucleate cells, or as a result of aplanospore formation. During division, cytokinesis normally proceeds only when the vesicles containing building material for the transverse primary cell wall are positioned in the transverse cell space, and when the space is not penetrated by chloroplast or mitochondrial profiles.

Discussion

Mitosis

Different types of mitosis have been reported for the Xanthophyta. Ott & Brown (1972) described closed mitosis with centrioles for *V. litorea*. In this species, the spindle was intranuclear and separated chromosomes were not visible; instead the chromatin formed a compact chromosome plate. Centriole replication took place during anaphase or early telophase, and the telophase nucleus was dumb-bell shaped. Subsequently, mitosis and cytokinesis were investigated in *T. regulare* (Lokhorst & Star, 1988). This species has semi-closed mitosis with centrioles, an intranuclear spindle and lacks the dumb-bell shaped nucleus in telophase.

In our *Xanthonema* strain, *Vaucheria* De Candolle and *Tribonema*, an intranuclear spindle is formed (Ott & Brown, 1972; Lokhorst & Star, 1988) centrioles participate in mitosis, and the chromosomes form a compact plate during metaphase. As in *Tribonema*, the nuclear envelope forms two large polar perforations and the nucleus does not acquire a dumb-bell shape in telophase. Progeny nuclei stay close to each other until the beginning of cytokinesis. A specific feature of the *Xanthonema* from Antarctica is that replication of centrioles occurs in early interphase, after the end of karyokinesis. Another peculiarity is the separation of progeny nuclei with the help of a CER invagination. The Golgi apparatus is associated with the polar nuclear perforation and the centrioles. Association of the Golgi apparatus with the interphase nucleus has been described and/or demonstrated in electron micrographs of vegetative cells and zoospores of unicellular (Andreoli *et al.*, 1999), filamentous (Falk & Kleinig, 1968; Massalski & Leedale, 1969; Broady *et al.*, 1997; Lokhorst, 2003; Lokhorst & Star, 2003) and heterotrichous (Massalski, 1969; Andersen *et al.*, 1998) species of Xanthophyta, but was never found in siphonous *Vaucheria*.

The similarity of the interphase nucleus of our *Xanthonema* strain with nuclei of other non-siphonous xanthophycean genera suggests that semi-closed mitosis is most probably more widespread than closed mitosis in the *Xanthophyta*.

Multinuclearity

In keys and floras of xanthophycean algae (Pascher, 1939; Ettl, 1978; Ettl & Gärtner, 1995), species of *Xanthonema* are referred to as uninucleate. However, Massalski (1969) demonstrated the presence of vegetative cells with several nuclei in some *Xanthonema* strains, but did not analyse the process of multinucleate cell formation. Our data show that multinuclearity occurs as often as the uninucleate state. Multinucleate cells can be easily recognized even under LM, being characterized by a higher number of chloroplasts, some of which are central or sub-central, in addition to the normal parietal position. The presence of cells with parietal and internal chloroplasts has been previously documented for several *Xanthonema* species, e.g. *X. solidum* (Vischer) Silva, *X. exile*, *X. debile* (Vischer) Silva, *X. montanum* (Vischer) Silva, *X. sessile* (Vinatzer) Ettl *et* Gärtner (Pascher, 1932, 1939; Vischer, 1936; Ettl, 1978; Broady *et al.*, 1997, and others). Thus it seems that multinuclearity is quite typical for the genus.

The origin of cells with several nuclei is connected to disturbances in the normal division pattern of parietal chloroplast-mitochondrion complexes during interphase. As a result of such irregularities, mitosis and cytokinesis are no longer coordinated. It is interesting to note that complexes with mitochondrial profiles associated with the internal face of the parietal chloroplast only are present in all published electron micrographs of these species (Massalski, 1969; Broady *et al.*, 1997; Olech *et al.*, 1998; Lokhorst, 2003), but are absent on micrographs of other filamentous and coccoid xanthophycean genera. i.e. *Tribonema*, *Bumilleria* Borzi and *Bumilleriopsis* Printz (Falk & Kleinig, 1968; Massalski & Leedale, 1969; Deason, 1971; Hibberd & Leedale, 1971; Böger & Kiermayer, 1974; Lokhorst, 2003). It is known that multinucleate vegetative cells of *Tribonema viride* Pascher can possess up to six nuclei (Falk & Kleinig, 1968), but the chloroplast-mitochondrion association has not been documented.

Cytokinesis

It is known that the cell wall in *Xanthonema* consists of two U-shaped segments (Massalski, 1969; Broady *et al.*, 1997; Lokhorst, 2003) and thus resembles the cell wall of *Tribonema* (Falk & Kleinig, 1968; Lokhorst, 2003), despite the absence of the 'zweispitzig' structure at the filament ends. When a filament of *Tribonema* ruptures, it breaks in the middle of a cell, in the zone of overlap of two cell-wall segments, hence the 'zweispitzig' structure at the filament ends. In contrast, in *Xanthonema*, fragmentation occurs between neighbouring

cells, where two U-shaped segments abut, resulting in fragmented filaments with rounded ends. Lokhorst (2003) suggested that the rupture of filaments without 'zweispitzig' structure formation in *Xanthonema* (and also in some species of *Tribonema*) is caused by the presence of transverse cell walls of an intercellular lumen, bounded distally by an outer layer of cell wall. Such lumina are absent in the majority of *Tribonema* species, and because of this, H-shaped ends are formed.

Our data fully support Lokhorst's model. The morphology of fragmented filament ends depends on the behaviour of the primary transverse cell wall that joins the two U-shaped segments of secondary wall into H-shaped structures. During secondary cell-wall formation (which proceeds centrifugally) in *Xanthonema*, the primary cell wall disintegrates centripetally and intercellular lumina appear in place of the disintegrated primary cell wall (Lokhorst, 2003). If the primary cell wall completely or almost completely disintegrates, the progeny cells separate to give rounded-ended filaments. If the primary cell wall does not disintegrate, or only partially disintegrates, the U-shaped segments of the progeny cells remain joined by the primary cell wall, and fragmentation occurs in the middle of the cell, where the U-shaped segments overlap. In this case the fragmented filament ends have the 'zweispitzig' structure.

It is interesting to note, that during cytokinesis aplanospore formation of our strain the primary cell wall is not formed, and aplanospores always lie singly within sporangia, without filament formation.

Intercellular lumina are present in published electron micrographs of other strains of *Xanthonema* (Broady *et al*., 1997, figs 33, 35). Intercellular plates (primary cell wall) are also present in micrographs of *Tribonema aequale* Pascher (Falk & Kleinig, 1968, fig. 1b), and traces are visible as electron-dense zones on electromicrographs of transverse cell walls of *T. minus* (Klebs) Hazen, *T. regulare*, *T. affine* (Kützing) G.S. West, *T. hormidioides* (Fischer) Lokhorst (Falk & Kleinig, 1968, fig. 1a; Lokhorst, 2003, figs 58–60). It is interesting to note that the intercellular lumina in *T. hormidioides* (Lokhorst, 2003), in which filaments can fragment, as in *Xanthonema*, are also situated in the zone of primary cell wall disintegration.

Behaviour of the primary cell wall and of vesicles with secondary wall building-material may be an important generic character in the Tribonematales s.l. In particular, differences between *Xanthonema* and *Tribonema* seem to be related to the behaviour of the primary cell wall during the cell cycle. Similarly, these genera differ from *Bumilleria* in their secondary cell wall formation. In *Tribonema* and *Xanthonema*, each progeny cell produces only one U-shaped segment *de novo* during cytokinesis, whereas in *Bumilleria* progeny cells seem to make both U-shaped segments, and thus the remnants of the parent cell wall form intercalary H-shaped structures (Hibberd & Leedale, 1971; Hibberd, 1980).

Organization of the photosynthetic apparatus

Chloroplast-mitochondrion association. An important feature of the organization of the photosynthetic apparatus in our strain is its association with mitochondrial profiles. In early and middle interphase uninucleate cells, mitochondrial profiles are adjacent to the internal side of the chloroplast poles. Prior to mitosis and cytokinesis, the chloroplast and mitochondrial profiles associated with the chloroplast divide. To our knowledge the presence of 'chloroplast-mitochondrion' complexes in *Xanthonema* has not been described before, although such complexes can be seen in all electron micrographs of previously investigated *Xanthonema* species (Massalski, 1969; Broady *et al*., 1997; Lokhorst, 2003).

Tribonema and *Bumilleria* present a different cellular arrangement of chloroplasts and mitochondria. For example, cells of *Bumilleria sicula* Borzí have mitochondrial profiles of two types: (i) peripheral profiles that are situated between chloroplast and plasmalemma, and (ii) internal profiles, that, as in *Xanthonema*, are adjacent to the internal side of the chloroplast (Massalski, 1969; Massalski & Leedale, 1969). In *T. viride* and *T. vulgare* mitochondrial profiles are numerous and small. Some of them are situated in free cytoplasm, whereas others are adjacent to the nuclear envelope (Falk & Kleinig, 1968; Massalski, 1969; Massalski & Leedale, 1969). Mitochondrial profiles are also present in a *Tribonema* sp., isolated from Antarctica (unpublished data). The absence of a chloroplast-mitochondrion association is also evident in TEM pictures of *T. affine* and *T. hormidioides* (Lokhorst, 2003).

Girdle lamellae. Like other *Xanthonema* and *Tribonema* species, the chloroplasts of our *Xanthonema* strain, possess a girdle lamella. The presence of a girdle lamella is regarded as a typical feature of the Xanthophyta (Lefort, 1962; Falk & Kleinig, 1968; Massalski & Leedale, 1969; Lohhorst & Star, 1988; Broady *et al*., 1997; Lokhorst, 2003), but details of its structure have not been discussed.

In our *Xanthonema*, the girdle is composed of branches of two different, U-shaped lamellae, each consisting of one peripheral and one

subcentral arm. The presence and organization of girdle lamellae may be an important taxonomical character for filamentous yellow-green algae. For example, *Bumilleria* and *Bumilleriopsis* lack the girdle lamella (Massalski & Leedale, 1969; Deason, 1971; Hibberd & Leedale, 1971).

If the girdle lamella is present, its structure can differ among species with respect to the number of girdle lamellae per chloroplast, and the mode of girdle lamella packing. In *T. vulgare* (Massalski & Leedale, 1969, figs 7, 13), *X. solidum* (Lokhorst, 2003, fig. 63), *Pseudopleurochloris antarctia* Andreoli *et al.* (Andreoli *et al.*, 1999, figs 7, 8), and the closely related genus, *Phaeothamnion* Lagerheim (Andersen *et al.*, 1998, figs 8, 9), a single-girdle lamella is present in the chloroplast. In *T. vulgare*, the girdle lamella has a circular appearance. In *X. solidum*, the two ends of the girdle lamella overlap for half the chloroplast length, resembling the single turn of a helix. In *Ph. confervicola* Lagerheim the girdle lamella forms two irregular helical loops, while in *Ps. antarctica*, the girdle lamella forms a regular helix of several turns.

Tribonema viride has two girdle lamellae per chloroplast (Falk & Kleinig, 1968, fig. 5), as do *Heterococcus caespitosus* Vischer (Massalski, 1969, fig. 47), *Xanthonema* sp. strain 395 (Broady *et al.*, 1997, fig. 35), *Xanthonema* sp. strain 601 (Broady *et al.*, 1997, fig. 38) and *Xanthonema* cf. *exile*. In *Xanthonema* and *Heterococcus* each girdle lamella is U-shaped, and consists of a peripheral arm, situated just under the chloroplast envelope, and an internal arm, separated from the chloroplast envelope by the peripheral arm of another girdle lamella or by the free lamella. In *H. caespitosus* and *Xanthonema* sp. strain 601, free lamellae are absent between the peripheral and internal arms of the first and second girdle lamellae, but in our *Xanthonema* and *Xanthonema* sp. strain 395, one or several free lamellae separate the peripheral and internal arms of the first and second girdle lamellae. *Tribonema viride* has two girdle lamellae, each of which forms a helix with two loops.

In conclusion, it appears that the presence or absence of girdle lamellae may be used as a taxonomic character at genus level in the Xanthophyta, whereas the number and type of girdle lamellae (when they are present) may be taxonomically informative at species level.

Taxonomic conclusions

Published data and our results show that, in addition to filament end morphology and the presence of intercalary cell wall segments, the presence of a girdle lamella, behaviour of the primary transverse cell wall during cytokinesis and association of mitochondria with other cell structures are important diagnostic features at genus level.

Thus, *Xanthonema* and *Tribonema* are characterized by the presence of girdle lamellae, and by the formation of only one segment per cell of secondary cell wall during cytokinesis. But *Xanthonema* clearly differs from *Tribonema* by complete disintegration of the primary transverse cell wall, and by the presence of a chloroplast–mitochondrion association. In *Tribonema* the primary transverse cell wall does not disintegrate at all, or disintegrates only slightly, resulting in solid junctions of U-shaped segments of secondary cell wall of progeny cells. Furthermore, mitochondria are not associated with the chloroplast, but sometimes with the nucleus. *Xanthonema*, *Bumilleria*, and *Bumilleriopsis* share the chloroplast-mitochondrion association, but during interphase, mitochondria of *Bumilleria* are not only associated with the internal side, but also with the external side of the chloroplast, whereas in *Xanthonema* mitochondria are associated only with the internal side of the chloroplast. In contrast to *Xanthonema* and *Tribonema*, *Bumilleria* and *Bumilleriopsis* lack the girdle lamella.

According to Broady *et al.* (1997), species delimitation in the genus *Xanthonema* is problematic. Based on LM features, our specimen resembles four species of *Xanthonema*: *X. debile*, *X. exile*, *X. montanum* and *X. solidum*, but differs from all of them except *X. exile* by the presence of a slightly protruding terminal pyrenoid. Our specimen is also almost identical with *Xanthonema* sp. strain 395 (Broady *et al.*, 1997), differing only in the absence of a stigma in the zoospores, in accordance with Pascher's (1939) diagnosis of the species.

In conclusion, our investigation demonstrates that ultrastructural data may significantly improve the classification system of filamentous yellow-green algae at the generic level, and also be relevant to species-level separations.

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