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Amitosis, including nucleolar behaviour during fragmentation, in both axial and corticating cells of *Chara contraria* (Charales, Charophyta)

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The results from the present study indicate that in *Chara contraria* the morphology of amitotic resting nuclei and amitosis depend essentially on the cell type as well to on some extent on the age and development of the cells. Young axial cells of principal axes and branches exhibited C-shaped resting nuclei. During amitosis nuclei initially stretched out and became helical shaped, and they then divided themselves into two similar C-shaped nuclei that remained close to each other. The nucleolar material gradually merged together to eventually form a central nucleolus, which adopted the curving of the nucleus. Spindle-shaped resting nuclei with numerous and irregularly distributed ovoid nucleolar structures were also observed. They duplicated their length during amitosis, underwent a constriction in the middle portion, and finally broke obliquely giving rise to two daughter nuclei, both with similar size. Young corticating cells exhibited initially ovoid nuclei. Then, they gradually stretched out concomitantly with the extension of the cells, giving rise to worm-shaped nuclei that bent irregularly and eventually divided themselves by constriction in portions of different sizes. The ultrastructure of amitotic nuclei in corticating cells was studied. The nuclear envelope remained intact in dividing nuclei. In section, resting nuclei exhibited numerous, small nucleolar profiles homogeneously distributed. In dividing nuclei, in contrast, few, large nucleolar profiles occupied the middle portion of the nucleus. Bundles of 3–66 tubular elements *c.* 20 nm in diameter ran approximately parallel to the long axis of the nucleus. Tubules were made of circular subunits *c.* 2 nm in diameter in cross section, and frequently they contacted nucleolar profiles and the inner membrane of the nuclear envelope.

KEY WORDS: Amitosis, *Chara contraria,* Charales, Corticating cells, Nucleolar behaviour, Ultrastructure

INTRODUCTION

Amitosis has been defined as a mechanism of nuclear division that occurs via constriction without chromosome condensation and spindle differentiation, as well as without breakdown of the nuclear membrane (Lawrence 2000). This mechanism occurs in very phylogenetically distant groups, such as amoebae (Gicquaud & Tremblay 1991), ciliates (Tucker 1967; Wolfe 1967; Tamura *et al.* 1969; Ammermann 1971; Williams & Williams 1976; Tucker *et al.* 1980; Orias 1991), sponges (Moorkejee & Bhaduri 1972), different tissues of vascular plants (Jjnno 1968; Guervin *et al.* 1976; Acatrinei & Lazar 1978; Miller 1980; Zheng *et al.* 1981; Appezzato-da-Glória & Machado 2004), arthropoda (Pessacq 1969; Francke 1979), mammals (Bast 1921; Cleland 1961; Palacios *et al.* 1976; Sugisaki & Sagaguchi 1977; Magalhães *et al.* 1991; Isakova & Shilova 2003; Isakova & Skvortsova 2003; Isakova & Mead 2004), and dinoflagellates (Tippit & Pickett-Heaps 1976). In all of them, some of the common traits that typify amitosis are observed, namely, that (1) this mechanism takes place in nuclei with high ploidy level, (2) it occurs in the nuclei of differentiated cells giving rise to multinucleate cells, and (3) it is irreversible and it never gives rise to reproductive cells.

Shen (1967a) was the first to demonstrate that in the Charophycean *Chara zeylanica,* amitosis occurred under a definite and synchronized pattern, and he concluded that amitosis was not a degenerative process because it was a regular method of nuclear replication in which each daughter nucleus maintained its replication capacity. Later, in a spectrophotometric study conducted on amitotic nuclei of *Ch. zeylanica,* Shen (1967b) indicated that the amitotic daughter nuclei exhibited equivalent DNA amounts. Maszewski (1991) also demonstrated that in internodal cells of *Chara* spp., amitotic nuclei divided symmetrically and DNA was equally distributed in both daughter nuclei.

Pickett-Heaps (1967) made the first observations of the ultrastructure of amitotic nuclei in Charales in vegetative cells

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Figs 1–11. Amitotic nuclei of *Chara contraria* from central internodal cells of principal axes, branches and branchlets.

Fig. 1. Bright-field image of a resting C-shaped nucleus stained with propiocarmin to show the nucleolar component as numerous spherical or ovoid tiny portions (arrows).

Figs 2–4. Nuclei stained with Feulgen and illuminated with green light of 546 nm.

Fig. 2. C-shaped nucleus at the beginning of the amitotic process. Epifluorescence shows DNA localization. Nucleolar material did not fluoresce, so it was possible to observe this material merging in a long, cylindrical nucleolus adopting the curving typical of the nucleus (arrows).

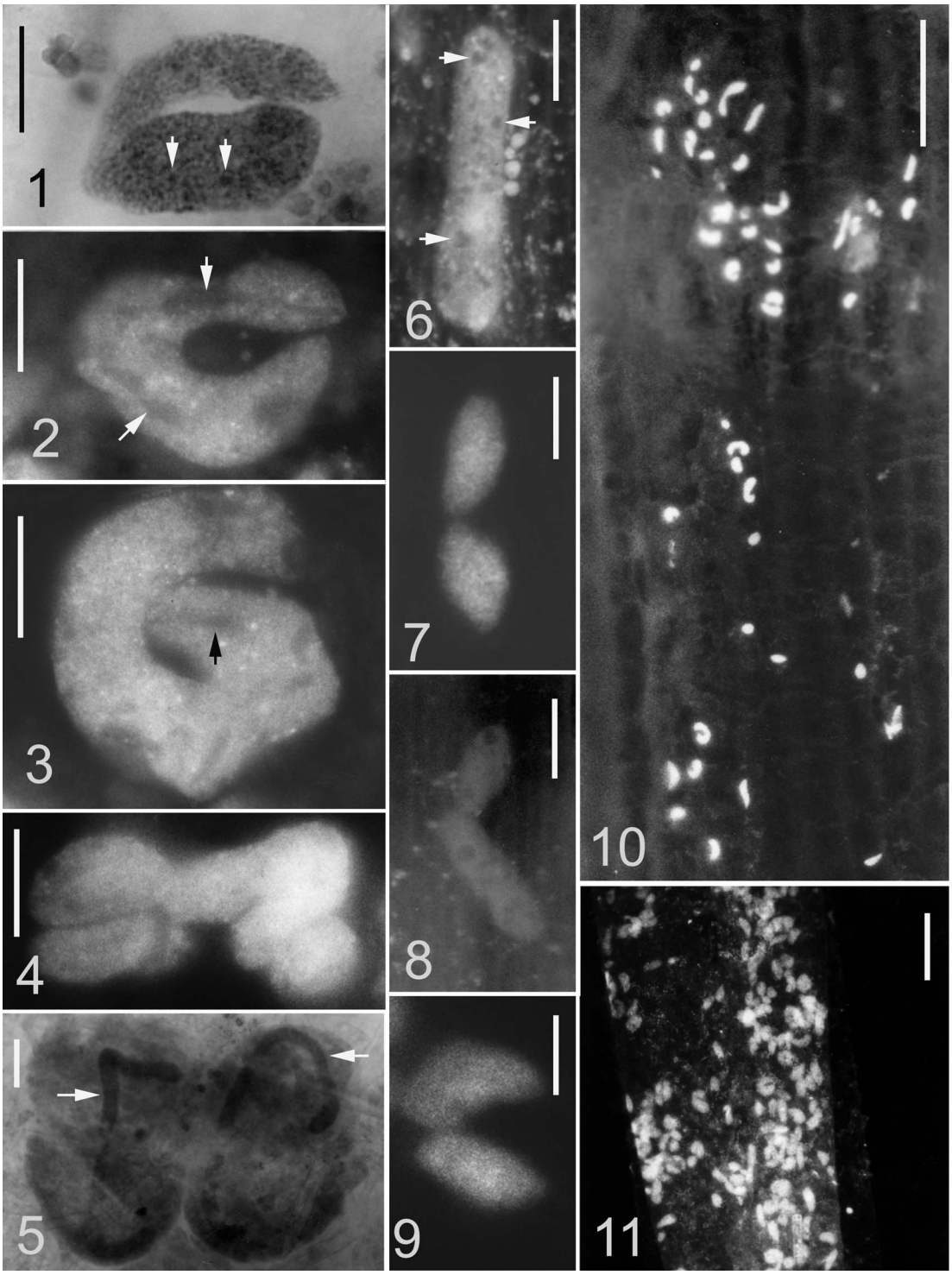


Fig. 3. Helical-shaped nucleus in a more advanced stage of amitosis. The cylindrical nucleolus is visible (arrow).

Fig. 4. Recently divided helical-shaped daughter nuclei close to each other.

Fig. 5. Bright-field image of two recently divided nuclei stained with propiocarmin to show a cylindrical nucleolus in each daughter nucleus (arrows).

Fig. 6. Spindle-shaped resting nucleus stained with Feulgen and illuminated with green light of 546 nm. The nucleolar material did not fluoresce, so it was possible to see this material formed by numerous small spherical or ovoid tiny portions (arrows).

Figs 7–9. Recently divided spindle-shaped daughter nuclei close to each other.

Fig. 7. DAPI stained. The dividing nucleus has not bent before separation.

Figs 8–9. Stained with Feulgen and illuminated with green light of 546 nm and stained with DAPI, respectively. The dividing nucleus has bent before separation.

Figs 10–11. Group of DAPI-stained amitotic nuclei of the principal axis (Fig. 10) and branchlet (Fig. 11) of central cells. Cylindrical, C-shaped, and helical-shaped nuclei are observed in both cell types. Scale bars: Figs 1–9 = 25 μm; Figs 10–11 = 150 μm.

of *C. fibrosa,* and Barton (1967) reported the occurrence of intranuclear crystals in amitotic nuclei of *Chara* sp. cells. In the genus *Nitella,* Roberts & Chen (1975) studied amitotic divisions in internodal cells of *N. axillaris,* and Cáceres & Parodi (1985) described different steps in the amitosis of internodal cells of *N. clavata.* Parodi & Cáceres (1991) studied, for the first time in this genus *Nitella,* the ultrastructure of amitotic nuclei and the amitosis in internodal cells. Foissner & Wasteneys (2000) have made a complete analysis of the amitotic nuclei of internodal cells in different species of *Chara, Nitella,* and *Nitellopsis.* They recognized chromosome-like structures in nuclei of *Nitella flexilis* and *Nitellopsis obtusa* putatively involved in the distribution of genetic material during nuclear fragmentation and demonstrated by statistical analysis that amitosis is a nonsynchronous process independent of the dark–light cycle.

In the genus *Chara,* the thallus has—with a few exceptions (Wood & Imahori 1964, 1965; Cáceres 1978)—a corticating system around the internodal, axial cells in principal axes, branches, and branchlets. The main goal of the present study in *C. contraria* A. Braun ex Kütz (see Cáceres *et al.* 1990) is to compare the amitotic nuclei and the amitotic sequences in both types of cells, that is, axial cells and corticating cells, and to describe the morphological similitudes and differences between them by light and electron microscopy.

MATERIAL AND METHODS

Thalli of *Chara contraria* were collected in the pond Lago Lido, Parque de Mayo, Bahía Blanca, and in channels of CORFO, Colorado River, Province of Buenos Aires, Argentina, from June 1996 to September 2000.

Light microscopy

Entire apical nodes (2–5) and erect internodes from principal axes and branches of thalli of *C. contraria* were fixed in Carnoy II and were stained with Feulgen (Johansen 1940) and DAPI (Coleman 1985) to reveal DNA and with propiocarmin (Wells & Hoshaw 1971) to evidence nucleolar material. Cells were examined with a Zeiss Axiolab microscope using ×40 objectives, or ×63 and ×100 oil immersion objectives (NA 1.4). Feulgen stain epifluorescence was obtained with green light (546 nm).

Electron microscopy

Entire apical nodes (2–5) and erect internodes from principal axes and branches of thalli of *C. contraria* were mechanically removed from field-collected thalli and were fixed for 2 h at 5°C in 1% glutaraldehyde in 0.05 M Na-cacodylate buffer, postfixed for 2 h in 1% OsO_4, dehydrated through a graded acetone series, embedded with Spurr's low-viscosity resin (Spurr 1969), and included using the flat method (Reymond & Pickett-Heaps 1983). Thin sections were cut with a diamond knife (Diatome Ltd, Bienne, Switzerland) in a Reichert-Jung Ultracut ultramicrotome (C. Reichert Optische Werke, Wien, Austria), mounted on Formvar-coated grids, and stained with uranyl acetate and lead citrate. Sections were observed with a Jeol 100 CX-II electron microscope (Jeol Ltd, Akishima, Tokio, Japan).

RESULTS

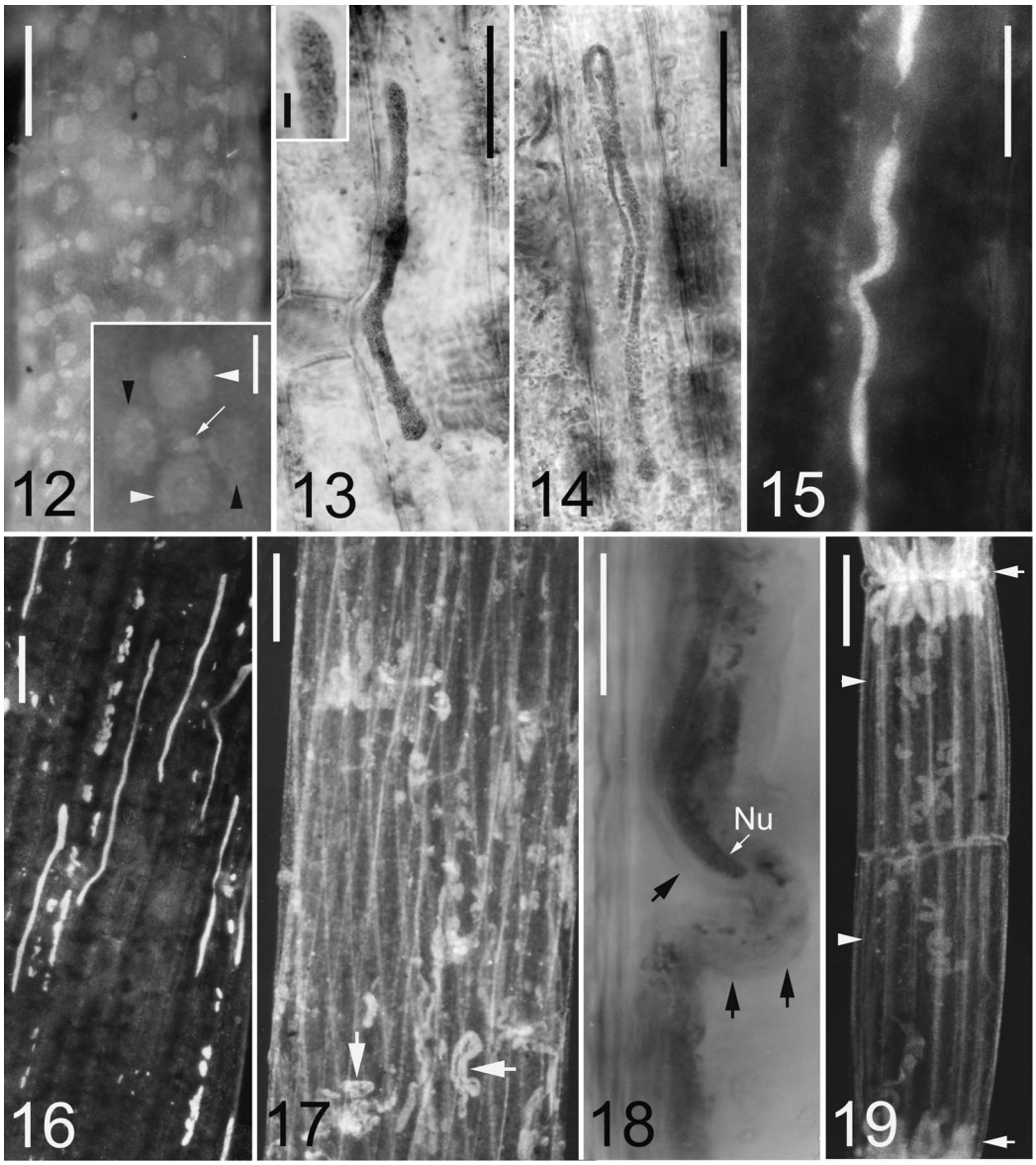
Amitosis in axial cells of principal axes, branches, and branchlets

Young axial cells of principal axes and branches exhibited initially only C-shaped resting nuclei (Fig. 1). Nuclei showed homogeneous chromatin and numerous ovoid nucleolar structures irregularly distributed (Fig. 1, arrows). During amitosis, the dividing nucleus initially stretched out and became helical shaped (Figs 2, 3), and then it divided itself into two similar daughter nuclei that initially remained close to each other (Figs 4, 5). During the process, the nucleolar material gradually merged together (Figs 2, 3, arrows) and formed a single central nucleolus that adopted the curving of the nucleus (Fig. 5, arrows).

In young, elongating axial cells, spindle-shaped resting nuclei with numerous ovoid nucleolar structures randomly distributed could also be observed (Fig. 6, arrows). During amitosis, they initially duplicated its length, then they constricted in the middle portion, and finally they broke obliquely giving rise to two daughter nuclei (Fig. 7). Not fully mature axial cells also showed dividing nuclei drawing out in the constriction region. The latter could be subsequently seen gradually bending (Fig. 8) and finally dividing themselves (Fig. 9). On occasions, constriction in the middle portion of the dividing nuclei originated a thread-like isthmus that then broke down to give rise to two daughter nuclei (not illustrated). Successive amitosis with the above-mentioned sequences gave rise to large, dense groups of resting nuclei in axial cells of both principal axes and branches (Fig. 10). In axial cells of the branchlets, whether corticated or noncorticated, the same patterns of amitotic stages could be observed (Fig. 11).

Amitosis in corticating cells of principal axes, branches, and branchlets

Very young corticating cells of principal axes and branches exhibited initially ovoid nuclei (Fig. 12); they were nuclei derived of the mitosis and citoquinesis that occurred during the initial growth of the diplostichous cortication; it could be distinguish small nuclei in the short cells (Fig. 12 insert, arrow) and large nuclei in the elongating cells, that is, those from the primary series (Fig. 12 insert, white arrowheads) and those from the secondary series (Fig. 12 insert, black arrowheads). The large nuclei elongated concomitantly with the extension of the cells. They gradually stretched out becoming worm shaped (Fig. 13), then bending irregularly (Fig. 14), and repeatedly divided themselves by constrictions in portions of different sizes (Fig. 15). Nuclei augmented several times the length (Fig. 16), twisting and bending repeatedly and finally exhibiting very irregular shapes (Fig. 17, arrows). Chromatin was homogeneous (Figs 13, 14). The nucleolar material was initially divided in tiny portions (Fig. 13, insert): they united gradually in a single, worm-shaped nucleolus that in due course divided itself simultaneously with the nucleus (Fig. 18). In corticating cells of the branchlets, nuclei remained located mainly at their bases near the branchlet nodes (Fig. 19, arrows).



Figs 12–19. Nuclei of *Chara contraria* from corticating cells of principal axes, branches, and branchlets.

Fig. 12. Corticating cells of a young axial internode exhibiting nuclei stained with Feulgen and illuminated with green light of 546 nm. A detail (insert) shows a small nucleus in a short cell (arrow) and large nuclei from the primary series (white arrowheads) and from the secondary series (black arrowheads).

Figs 13–14. Resting amitotic nuclei of corticating cells of elongate internodes stained with propiocarmin. They are worm shaped, and as shown in Fig. 14, they can bend irregularly. Nucleolar material can be observed as tiny granules (insert Fig. 13).

Fig. 15. Dividing nuclei stained with Feulgen and illuminated with green light of 546 nm. They are worm shaped and irregularly bent and fragmented.

Fig. 16. Corticating cells of a fully mature principal axis internode exhibit C-shaped and worm-shaped resting nuclei distributed at different levels. Nuclei were stained with Feulgen and illuminated with green light of 546 nm.

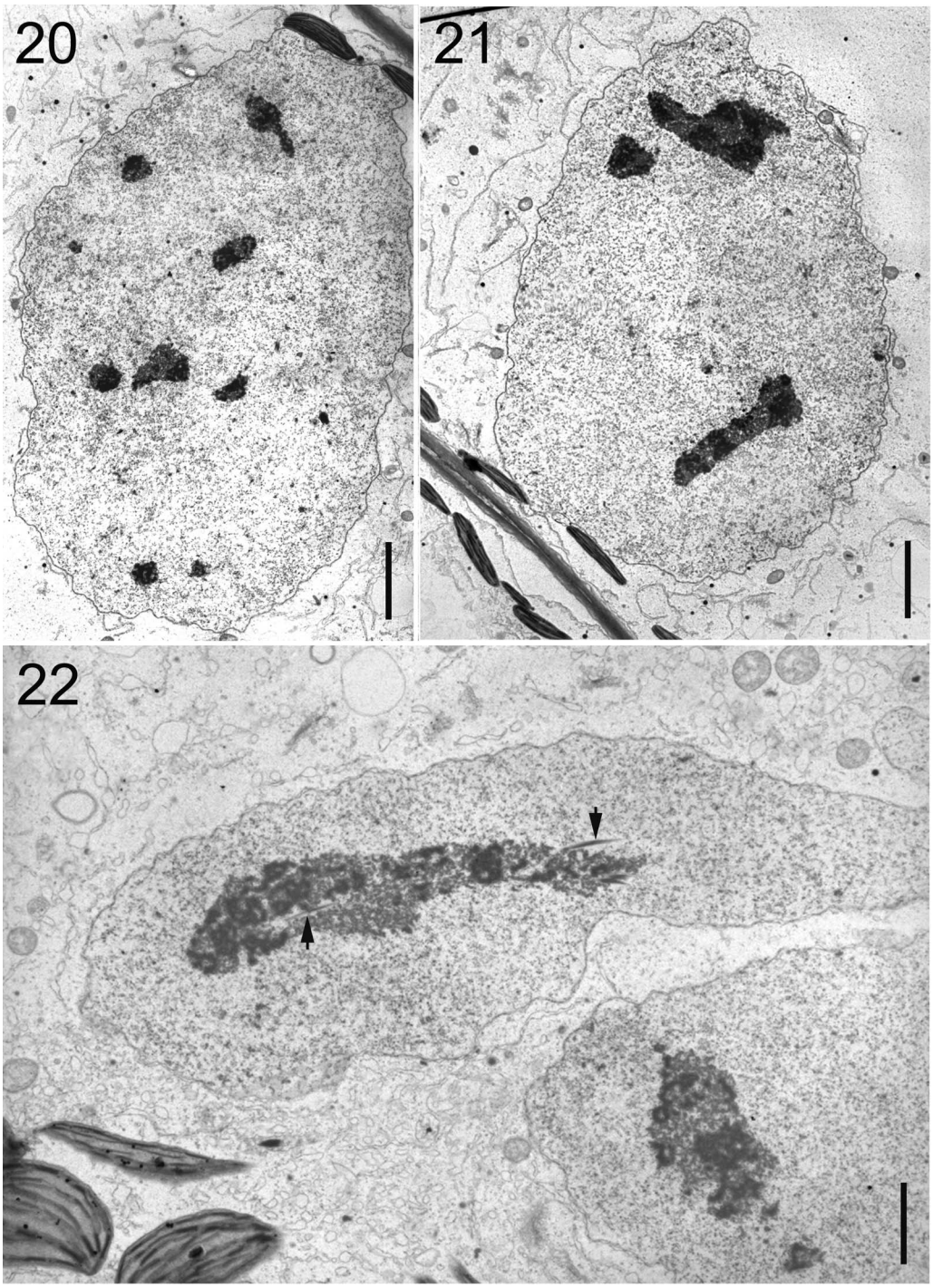
Fig. 17. Corticating cells of a young principal axis with amitotic nuclei stained with Feulgen and illuminated with green light of 546 nm. The extremely irregular shapes they adopted are due to numerous bendings and twistings (arrows).

Fig. 18. Detail of a dividing nucleus stained with propiocarmin to show the cylindrical nucleolus (Nu) that has adopted the curving of the nucleus. The arrows pinpoint the nuclear envelope.

Fig. 19. Young corticated branchlet internode with amitotic nuclei stained with Feulgen and illuminated with green light of 546 nm. Nuclei are located at their bases, near the branchlet nodes (arrows). Nuclei of the central internodal cell are seen by transparency (arrowheads). Scale bars: Figs 12, 16, 18–19 = 150 μm; Fig. 12, insert = 40 μm; Figs 13–15 = 50 μm; Fig. 13, insert = 5 μm; Fig. 17 = 25 μm.

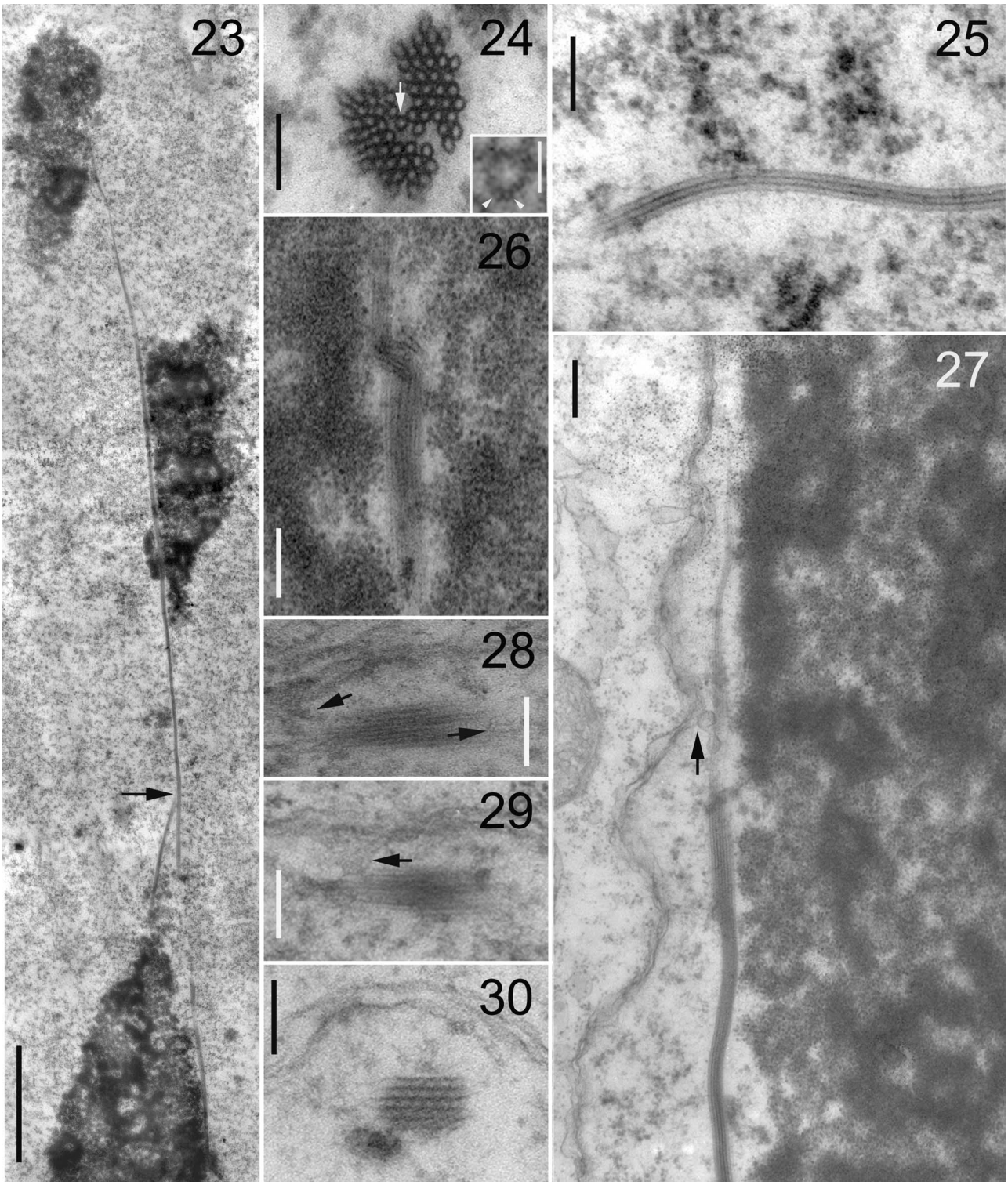
Ultrastructure of amitotic nuclei of corticating cells

The ultrastructure of amitotic nuclei of corticating cells is identical to those occurring in nuclei of axial cells (the latter not illustrated). In corticating cells, young resting nuclei showed initially numerous, small nucleolar profiles homogeneously distributed (Fig. 20). In dividing, worm-shaped nuclei, nucleolar profiles became gradually fewer (Fig. 21), and finally they unified into a single nucleolar profile that occupied the central portion of the nucleus (Fig. 22). Bundles of 3–66, hollow tubules *c.* 20 nm in diameter (Figs 22–30) appeared.



Figs 20–22. Transmission electron micrographs of sections of amitotic nuclei in corticating cells of *Chara contraria.*
Fig. 20. Resting nucleus. Note that the nucleolar material is segregated in numerous tiny portions homogeneously distributed. Chromatin is homogeneous, without heterochromatin condensations.
Fig. 21. Nucleus at the beginning of amitotic division. Note that the nucleolar profiles became less and larger.
Fig. 22. Dividing, worm-shaped nucleus. The section is longitudinal in the upper profile and transversal in the lower one. Note the nucleolar profiles are central and that they are traversed by bundles of tubules (arrows). Scale bars: Figs 20, 21 = 5 μm; Fig. 22 = 3 μm.

Tubules were made of circular subumits *c.* 2 nm in diameter in cross section (Fig. 24 insert, arrowheads). Tubules ran longitudinally approximately parallel to the long axis of the nucleus (Figs 22–23, arrows) and were, in general, immersed in a diffuse, median electron-dense material (Fig. 24). Bundles could be seen either straight (Fig. 23) or winding (Fig. 25), sometimes crossing (Fig. 23, arrow) or zigzagged (Fig. 26); they made frequently contact with large nucleolar profiles (Figs. 22 23) and with the inner membrane of the nuclear envelope (Figs 27–29, arrows).



Figs 23–30. Transmission electron micrographs of dividing amitotic nuclei of *Chara contraria* showing details of the longitudinal, paracrystalline bundles of tubular elements.

Fig. 23. Longitudinal section. Note that the nucleolar portions are in contact with the tubules. The arrow pinpoints a site in which two bundles cross.

Fig. 24. Cross section of a bundle of *c.* 66 tubules. Note that the tubules are very close to each other and that they are circular in transversal section. The insert shows a detail of the tubule pinpointed with an arrow to show that tubules are hollow and that they are made of subunits *c.* 2 nm in diameter in cross section (arrowheads).

Fig. 25. Details of a curved segment of a bundle, longitudinally sectioned.

Fig. 26. Details of a zigzagged portion of a bundle, longitudinally sectioned.

Fig. 27. Details of a sector of a bundle that passes close to the nuclear membrane. The arrow shows the point in which the bundle gets in contact with the inner membrane of the nuclear membrane.

Figs 28–29. Details of bundles longitudinally sectioned in contact with the inner membrane of the nuclear membrane (arrows).

Fig. 30. Detail of a bundle obliquely sectioned. Scale bars: Fig. 23 = 2 μm; Figs 24, 25, 30 = 0.2 μm; Fig. 24, insert = 0.02 μm, Figs 26–29 = 0.5 μm.

DISCUSSION

The results indicate that in *C. contraria* the morphology of amitotic resting nuclei and amitosis depend essentially on the cell type as well as on some extent on the age and development of the cells.

In all young axial cells, from principal axes, branches, and branchlets, the pattern of nuclei morphology and amitosis was similar and in general terms in agreement with the patterns that were known for all the studied species of *Chara* (Sundaralingam 1947; Pickett-Heaps 1967; Shen 1967a). The C-shaped and kidney-shaped nuclei observed in internodal cells in the present study were very similar to those found by Shen (1967a) in young internodal cells of *Chara zeylanica* and Foissner & Wasteneys (2000) in *C. braunii* and *C. corallina,* whereas the spindle-shaped nuclei found in elongating and mature cells were similar to those found in senecent cells of *C. braunii* and *C. corallina* (Foissner & Wasteneys, 2000). Interestingly, spindle-shaped nuclei are distinctive of members of *Nitella* as *N. axillaris* (Roberts & Chen, 1975), *N. clavata* (Parodi & Cáceres 1991) and several more species of the genus (Foissner & Wasteneys 2000).

Amitosis in elongating, corticating cells from principal axes, branches, and branchlets, in turn, exhibited a remarkable characteristic: the amitotic division did not separate equivalent portions of nuclei since nuclear fragmentation segregated unequal portions of chromatin. The derived elongated, worm-shaped nuclei divided themselves repeatedly, giving rise to polymorphic nuclei, with a maximum degree of polymorphism in those of corticating cells of principal axes and branches.

In studies of amitosis in organisms not related to Charales (Tamura *et al.* 1969; Williams & Williams 1976; Magalhães *et al.* 1991), the distribution of the nucleolar material of resting nuclei in small ovoid portions typical in Charales was never observed. Barton (1967), Pickett-Heaps (1967), and Parodi & Cáceres (1991) assumed that these numerous electron-dense bodies of amitotic nuclei were in fact of nucleolar nature, but Foissner & Wasteneys (2000) were the first to confirm their nucleolar nature visualizing them with the fluorescent stain acridine orange in several species. Propiocarmin stains allowed us now to register for the first time the behaviour of the nucleolar material of amitotic nuclei, that is, the unique mechanism of nucleolar condensation into a single nucleolus during amitosis and prior to fragmentation. This phenomenon also seems to occur in *N. clavata* (see Parodi & Cáceres 1991, fig. 6).

The present is the first report of the ultrastructure of amitotic nuclei in corticating cells in Charales. The ultrastructure of amitotic nuclei in corticating cells is similar to those occurring in axial cells of *Chara* and *Nitella* (Barton 1967; Pickett-Heaps 1967; Roberts & Chen 1975; Cáceres & Parodi 1985; Parodi & Cáceres 1991; Foissner & Wasteneys 2000; the present study not illustrated).

The intranuclear tubular components observed in *Chara contraria* were identical to those found in *C. fibrosa* (Pickett-Heaps 1967), *C. vulgaris* (Barton 1967), *C. braunii* and *C. corallina* (Foissner & Wasteneys 2000), *N. clavata* (Parodi & Cáceres 1991), *N. cristata, N. flexilis, N. furcata, N. hyalina, N. pseudoflabellata, N. translucens,* and *Nitellospsis obtusa* (Foissner & Wasteneys 2000). Similarly, in our study the tubules were seen also running longitudinally inside nuclei. Nevertheless, an interesting difference with the tubules of all these previous species is that in *C. contraria* tubules also made frequent contact with the inner membrane of the nuclear envelope besides the normal contact with nucleolar material.

At present, there is no direct evidence about the nature and function of these enigmatic tubules in Charales. Pickett-Heaps (1967) assumed that they are microtubular structures, and Barton (1967) inferred their proteinaceaus nature taking into account that they have a storage function and that they are synthesized in the nucleoli; still, he reports no conclusive confirmation of their function. Barton also suggested a virus nature of the tubules in view of their crystalline arrangement. Delay (1958) clearly demonstrated that the amitotic divisions of growing internodal cells were not affected by colchicine, thus suggesting that these intranuclear tubuli are not constituted by tubuline. Parodi & Cáceres (1991) claimed that the paracrystalline arrangement of these unique tubular components is not characteristic of microtubules, although they observed that in *N. clavata* tubules ended in electron-dense, intranuclear, pyramidal structures located at the poles of the spindle-shaped nuclei recalling the MTOCs (microtubule organizing centres) of land plants (Hepler & Palevitz 1974) and certain fungi (Kubai 1978). On the contrary, although Foissner & Wasteneys (2000) suggested tubules resembled microtubules in shape, they admitted the attempts to stain them with tubulin antibodies were unsuccessful. Also, Foissner & Wasteneys (2000) claimed that tubules contained neither DNA nor RNA and that they are probably proteinaceaous. Finally, the present study shows that the tubules, like microtubules, are hollow and are made of subunits with a diameter very similar to the diameter of tubulin, although the number of they in cross section is *c.* 10–11 and not 13 as is typical in cytoplasmic microtubules of most of the cells.

There are characteristics that support the assumption that the function of these tubules in *C. contraria* is mechanical. The reasons for such conclusion are that (1) they are parallel to the long axis of nuclei, (2) they can adopt the curvings of the nucleus for long distances, and (3) they contact the nuclear envelope in several points. Foissner & Wasteneys (2000) were also aware that the tubules of several species run consistently longitudinally considering that they may play a capital mechanical role during amitosis since they assumed that tubules may determine the longitudinal axes of daughter nuclei and also seemed to be responsible for the tapered ends of spindle-shaped nuclei.

In all the studied species hitherto, tubules are moreover consistently related with the nucleolar material. Considering that nucleoli suffer a dramatic rearrangement during amitosis, as is demonstrated in the present study, we predict a role of these tubules in this process. The question is if tubuli play an active role in this dynamics or if they play only a structural role supporting nucleolar portions. There are many examples in which tubules (i.e. microtubules) join up in specific patterns to play structural roles, for instance, the microtubular roots of flagellate cells (see Mattox & Stewart 1984). In Ciliates, in which the microtubular nature of the tubules of the macronucleous has already been demonstrated (Tamura *et al.* 1969; Williams & Williams 1976), it is clear that they have participation in the nuclear elongation and that they are associated to the nuclear material and the nuclear membrane (Tucker *et*

al. 1980). Paradoxically, Williams & Williams (1976) also found that in *Tetrahymena* macronuclear elongation occurred even under the action of colchicine. Consequently, further studies are necessary to clarify both the nature and the function of these nuclear inclusions although they have some evident characteristics, namely, that (1) they are exclusively intranuclear, (2) they appear in dividing nuclei, (3) they run longitudinally, (4) they are proteins (Barton 1967; Foissner & Wasteneys 2000), and (5) they are clearly associated with nucleoli and the nuclear membrane.

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