Plant Syst. Evol. 251: 89–105 (2005)
DOI 10.1007/s00606-004-0237-9

Plant Systematics and Evolution
Printed in Austria

Changes in the absolute configuration of the basal/flagellar apparatus and evidence of centrin during male gametogenesis in *Chara contraria* var. *nitelloides* (Charales, Charophyta)

A. A. Vouilloud, E. J. Cáceres, and **P. I. Leonardi**

Laboratorio de Ficología y Micología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina

Received April 15, 2003; accepted August 28, 2004
Published online: February 24, 2005

Abstract. The absolute configurations of the basal/flagellar apparatus during male gametogenesis of *Chara contraria* var. *nitelloides* (Charales, Charophyta) were carefully analysed. Emphasis was placed on the changes in the angles and lengths of the basal bodies, the microtubular root angles and the development of the distal as well the proximal connecting fibers. Six principal stages were recognized: a) *parallel, non-axonemal, developing basal bodies connected by a non-striated, proximal fiber*; b) *non-parallel, non-axonemal, mature basal bodies connected by a developing, striated, distal fiber;* c) *non-parallel, axonemal basal bodies connected by a fully developed, striated, distal fiber;* d) *opposite, axonemal basal bodies not connected by fibers,* e) *axonemal basal bodies not connected by fibers and directed backwards and* f) *parallel, axonemal basal bodies not connected by fibers*. A headpiece, a 3-membered root and a reduced multilayered structure developed during ontogeny. The initial parallel disposition of the basal bodies, the initial lack of MLS and the presence of only two microtubular roots from the very inception of the basal apparatus development, suggest a *Mamiella*-like ancestor for Charales. Ontogenetic evidence supports previous ideas in the sense that similarities of sperm morphology of charalean and bryophytan gametes are likely due to convergent evolution. In addition, the present study clearly reveals the presence of centrin in Charales.

Key words: Absolute configuration, basal apparatus, centrin, *Chara*, *Chara contraria* var. *nitelloides*, Charales, Charophyta, flagellar apparatus, MLS, male gametes, ontogeny, sperm, spermatozoids.

Male gametes of the Charales are highly differentiated cells with a spirally shaped cell body and two similar subapical flagella directed backwards. Rhombic scales cover the cell body and flagella (van den Hoek et al. 1995, Graham and Wilcox 2000).

According to Moestrup (1970), the head, the median region and the tail are the three cell body regions. The head runs from the anterior end to the level at which flagella emerge and it contains mitochondria and the basal apparatus (Duncan et al. 1997). A root of about 25 microtubules runs between the parallel basal bodies and the mitochondria. The median region posseses the nucleus and the microtubular root, and the tail contains the plastids. Sluiman (1983) discovered the existence of a second short, dorsal, 4-membered microtubular root in mature gametes in *Nitella* (Turner

1968) and *Chara* (Pickett-Heaps 1968). Male gametogenesis in the Charales involves a gradual withdrawal of the spermatocyte cytoplasm until it reaches the dimensions, shape and structure of mature gametes (Pickett-Heaps 1968; Moestrup 1970; Turner 1968; Coccuci and Cáceres 1976; Kwiatkowska and Maszewski 1978, 1985, 1986).

McCourt et al. (1996) and Duncan et al. (1997) stressed the need to obtain precise data on the morphology of extant and fossil charophytes in order to complement molecular studies to elucidate the relationships of the charophycean green algae and land plants - relationships that Bhattacharya and Medlin (1998) considered to be still in question. Apparently, Huss and Kranz (1997) considered the rRNA data to be unable to resolve the question. They argued that there is no indication that any of the charophytes investigated up to that point is significantly more closely related to the bryophytes than are the others. In this sense, Ragan et al. (1994) had indicated a monophyletic origin of the Charophyceae whereas Friedl (1997), in turn, referred to the ambiguity of the phylogenetic position of the Charales because some analyses suggested either a para- or a polyphyletic origin of the Charophyceae.

More recently, Karol et al. (2001), using data from four genes, found with strong statistical support the order Charales as a sister to the land plants. Chapman and Waters (2002) concluded that molecular and morphological data favor the Coleochaetales and the Charales as the groups closest to the land plants, but with respect to the question of what are the relationships among the charophyte orders, that every conceivable topography has been supported in the literature. They also stressed the necessity of not overlooking the importance of morphological characters in resolving the question of land plant ancestry.

The contributions on the fine structure of motile cells in Charophyta have focused on the study of male gametes of the Charales (Pickett-Heaps 1968, Turner 1968, *Chara corallina*, Moestrup 1970, Cocucci and Cáceres 1976, Duncan et al. 1997) and the Coleochaetales (Graham and McBride 1979, Graham and Wedemeyer 1984, Grahman and Repavich 1989). In addition, there are also studies on zoospores of the Klebsormidiales (Marchant et al. 1973), the Chlorokybales (Rogers et al. 1980), and the Coleochaetales (Moestrup 1974, Sluiman 1983, Graham and Taylor 1986).

Comparisons of the characteristics of motile cells have proven to be more reliable in the inference of phylogenetic relationships, than comparisons of the characteristics of vegetative cells (Friedl and Zeltner 1994). In this sense, the study of the absolute configurations of flagellar apparatuses is a tool largely used in phylogenetic speculations (O'Kelly and Floyd 1984, Melkonian 1989). In the charophytes, analyses of mature flagellar apparatuses are done (Stewart and Mattox 1978, Mattox and Stewart 1984, Melkonian 1989, Sluiman 1983). Nevertheless, O'Kelly and Floyd (1984) in a critical revision of more than a hundred fully developed algal flagellar apparatuses did not include *Chara* and *Nitella* in the general scheme, because it was not customary to reconstruct the absolute configuration of the mature flagellar apparatuses of these genera. However, O'Kelly and Floyd (1984) inferred the counterclockwise orientation of the *Chara* and *Nitella* apparatuses by the analysis of individual images of developmental stages of the flagellar apparatuses previously published by Turner (1968) and Pickett-Heaps (1968). They noted that the developmental stages of the flagellar apparatuses of *Chara* and *Nitella* were very similar to the mature flagellar apparatuses of extant charophytes such as *Coleochaete* and *Klebsormidium* (Pickett-Heaps 1968). O'Kelly and Floyd (1984) also noted that during the ontogeny of the flagellar apparatus of the gametes of *Chara*, basal bodies moved from an initial position to a mature position although they admitted that the precise sequence of these changes had not been followed in detail. The purpose of the present report is to reconstruct the absolute configuration of all developmental stages of the flagellar apparatus of *Chara contraria* var. *nitelloides*. No comprehensive

study on the ontogeny of the flagellar apparatus of the Charophyceae exists to date. Turner (1968) and Pickett-Heaps (1968) only elucidated some developmental stages of the basal apparatuses of *Nitella* and *Chara*. Duncan et al. (1997) and Sluiman (1983) have analyzed in detail only mature basal apparatuses in *Chara vulgaris* gametes and *Coleochaete pulvinata* zoospores, respectively.

The present report also shows clear evidence of the presence of centrin during the spermatogenesis in *Chara contraria*. The presence of centrin has clearly been established in green algae (Bhattacharya et al. 1993), bryophytes and pteridophytes (Salisbury 1992, Vaughn and Renzaglia 1993, Vaughn et al. 1993, Klink and Wolniak 2001, Renzaglia and Garbary 2001). It is also present in the lamellar strips of the MLSs of male gametes of archegoniates (Vaughn et al. 1993) and in Phaeophyceae (Katsaros et al. 1993) and Dinophyceae (Höhfeld et al. 1994). Centrin is a normal protein in the striated fibers of algal flagellar apparatuses (Salisbury 1989, Höhfeld et al. 1994). It is necessary for the basal body assembly (Marshall et al. 2001, Klink and Wolniak 2001) in association with procentrioles (Lechtreck and Grunow 1999), as well as for the regulatory control over the positioning of centrioles and basal bodies during the development of motile apparatuses (McFadden et al. 1987, Hart and Wolniak 1998, Wolniak et al. 2000, Renzaglia et al. 2002, Koblenz et al. 2003) and mitosis (Motomura et al. 2001). Marshall et al. (2001) found that a mutation in the centrin gene reduced the rate of *de novo* centriole assembly. Therefore, the occurrence of centrin in Charophyta was expected (Duncan et al. 1997).

Materials and methods

Electron microscopy. Immature and mature antheridia of *Chara contraria* var. *nitelloides* were mechanically removed from field collected thalli and were fixed for 2h at 5°C in 1% glutaraldehyde in 0.05 M cacodylate buffer, postfixed for 2h in

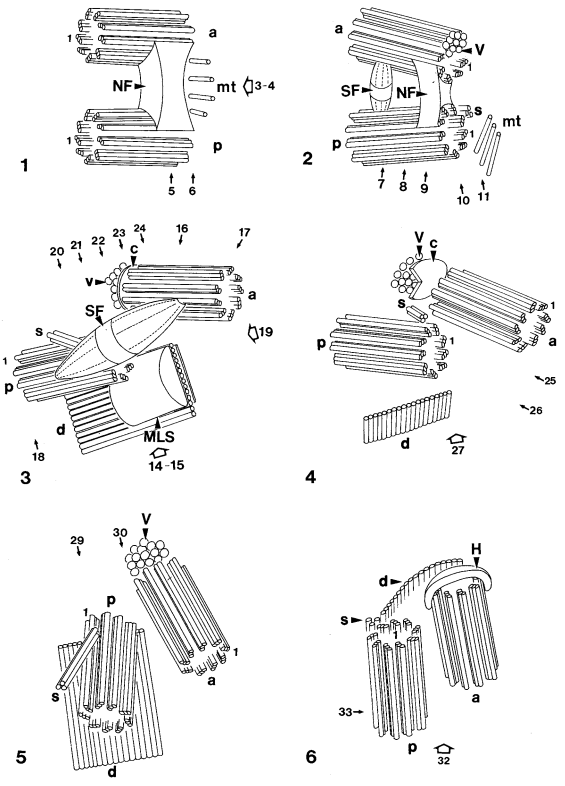


Fig. 1. Male gametogenesis in *Chara contraria* var. *nitelloides*. Diagrammatic depictions of the different developmental stages of the male gamete basal apparatus, with an indication of various planes of sections and the corresponding numbers of the figures in which these planes of section are illustrated. Linear arrows (→) indicate that the section passes perpendicular to the plane of the page; square arrows (⇒) indicate that the section passes approximately parallel to the plane of the page

1% OsO_4, dehydrated through a graded acetone series and embedded with Spurr's low viscosity resin (Spurr 1969) using flat embedding (Reymond and 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) at the Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca (CRIBABB). 109 basal/flagellar apparatuses were serially sectioned and analyzed.

Triplets were numerated clockwise during the first stage of non-axonemal, parallel basal bodies (Fig. 5), following Sluiman (1983). We considered triplet number 1 in both basal bodies to be the triplet located on the opposite side to the side contiguous to the lateral microtubules.

Fluorescence microscopy. Antheridia mechanically removed from the thalli were dissected under a microscope. Manubria, capitular cells, and spermatangial filaments were incubated in MT buffer pH 7.0 (HEPES 15 mM, EGTA 15 mM, $MgSO_4$ 5 mM, DTT 1 mM) for 1 h. Cells were fixed for 15 min in 1.6% paraformaldehyde in MT buffer and then washed three times in PBS buffer pH 7.4 (NaCl 150 mM, Na_2HPO_4 8.1 mM, NaH_2PO_4 1.5 mM). Cells were treated with 1% Igepal (Sigma Chemical Co. Mo, USA) in PBS for 1 h, followed by three washes in PBS. Cells were subsequently incubated during 3 h in a mixture of 1% cellulase-0.5% pectinase (Sigma Chemical Co. Mo, USA) and after three washes in PBS, cells were allowed to adhere to 1% Poly-L-lysin (Sigma Chemical Co. Mo, USA) coated coverslips and were allowed to dry. After three washes in PBS, the cells were blocked overnight in 3% fish gelatin (Sigma) in PBS. The permeabilized cells were incubated for 1.5 h at 37°C in a polyclonal rabbit anti-centrin IgG (directed against centrin of *Spermatozopsis similis)* diluted 1:100 in PBS/3% fish gelatin. They were subsequently washed three times in PBS, blocked for 1h in 3% fish gelatin/PBS, and incubated for 1.5 h at 37°C in FITC-conjugated goat-anti-rabbit IgG (Sigma Chemical Co. Mo, USA), diluted 1:100 in 3% fish gelatin/PBS. Cells were carefully washed in PBS and the coverslips were mounted on slides on a drop of 0.1% 1,4-phenylenediamine (in 1:2 PBS/glycerin). Controls were carried out omitting the first antibody. Cells were examined with a Zeiss Axiolab microscope using a 100x oil immersion objective (NA 1.4).

Results

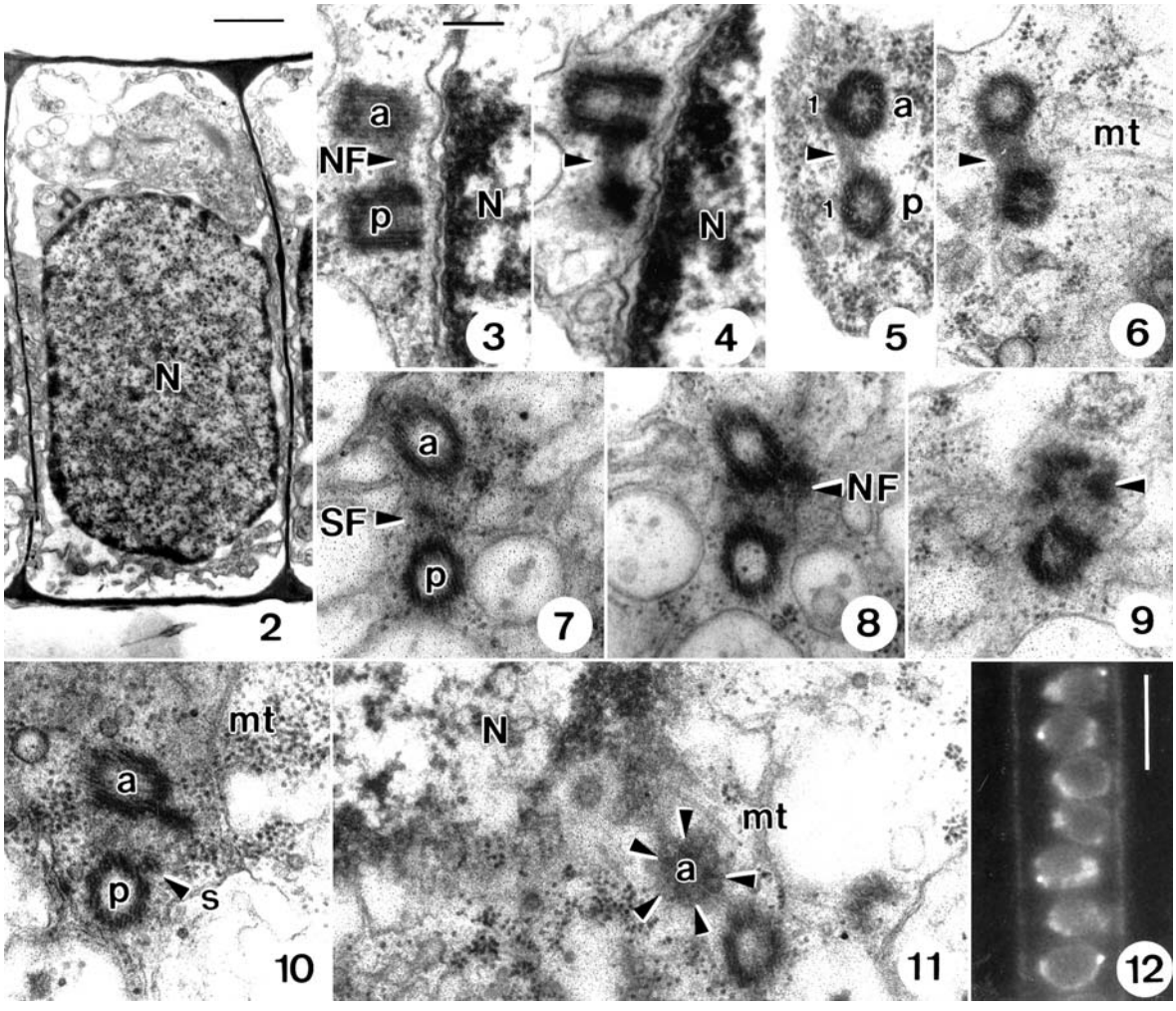
Figure 1 diagramatically represents the sequence of the absolute configurations adopted by the basal/flagellar apparatus, during male gametogenesis in *Chara contraria* var. *nitelloides,* reconstructed by the analysis of the different sections showed in Figs. 2-33. We have identified the following six developmental stages during gametogenesis:

Stage 1. Parallel, non-axonemal, developing basal bodies connected by a non-striated, proximal fiber (Fig. 1.1). Spermatids displayed an

Figs. 2–12. Male gametogenesis in *Chara contraria* var. *nitelloides*. **2–6**. Basal apparatus with non-axonemal, parallel basal bodies connected by a non-striated proximal fiber. **2**. The gametocyte displays an ovoidal nucleus. Parallel basal bodies are close to the nuclear envelop and perpendicular to it. Scale bar = 1.2 μm. **3–4**. Two contiguous sections of the basal apparatus in which developing basal bodies '**a**'' and ''**p**'' are longitudinally sectioned. The '' cart-wheel'' region is well developed in both basal bodies and a non-striated proximal fiber (**NF**) connects them at this level. Scale bar = 0.5 μm, also valid for Figs. 5–11. **5–6**. Basal bodies are transverselly sectioned and seen from above (note clockwise imbrication of the triplets). **5**. The non-striated proximal fiber is seen (arrowhead). The position of triplet 1 is indicated in both basal bodies. **6**. The proximal fiber has been fully sectioned (arrowhead) and is seen to connect both basal bodies. Microtubules (mt), perpendicular to the long axis of basal bodies appear. **7–11**. Non-axonemal, non-parallel basal bodies are connected by both a mature proximal non-striated fiber and a developing striated distal fiber. **7–9**. Serial sections of the basal apparatus, in which basal bodies ''**a**'' and ''**p**'' are lightly obliquely sectioned. The angle formed by the basal bodies is approximately 60 degrees (deduced by the different separation of the profiles in each section). In Fig. 7 the striated, distal fiber (**SF**) is observed connecting the zone of triplets 3–4 of basal body ''p'' and the zone of triplets 6–7 of basal body ''a''. In Figs. 8 and 9 the proximal fiber (NF) is partially sectioned. **10–11**. Sections of the basal apparatus in which basal bodies ''**a**'' and ''**p**'' are lightly obliquely sectioned and seen from below. **10**. The 3-membered microtubular root ''**s**'' appears aproximately opposed to triplet 2 of basal body ''**p**''. **11**. Section at the level of the proximal end of basal bodies. Vesicles are gathered in a circle with the same diameter as basal body ''**a**'' (arrowheads). Unilateral microtubules (mt) remain close and parallel to the plane of the nuclear envelope. **12**. Immunofluorescence reveals centrin in this stage of the gametogenesis. Centrin labelling is concentrated in one apical spot in all gametocytes. Scale bar = 9 μm

ovoidal nucleus with some heterochromatin located against the nuclear envelope (Fig. 2). Developing basal bodies "a" and "p" were in this stage 250 nm long, and were separated by a distance of 125 nm each other and by a distance of 5 nm from the nuclear envelope, flattened in this region. They were normally parallel to each other although sometimes they were positioned at an angle of 5 degrees (Figs. 3–4) to each other. A straight, non-striated, medium electron-opaque proximal fiber (NF), 75 nm in width, and 175 nm in length (Figs. 3–6) connected triplets 2–5 of the posterior basal body "p" and triplets 6–9 of the anterior basal body "a" (Fig. 5, numbered after Sluiman 1983) at the level of the "cart-wheel" region (Fig. 4). Undulated, parallel microtubules (mt) ran parallel to the nuclear envelope, ending unilaterally and close to the proximal end of both basal bodies (Fig. 6).

Stage 2. Non-parallel, non-axonemal, mature basal bodies connected by a developing, striated, distal fiber (Fig. 1.2). Non-parallel basal bodies formed an angle of about 60 degrees (angle ascertained from cross sections, Figs. 7–9). A developing distal striated fiber (SF), 220–260 nm in length and 150 nm in width, was connected in the region of triplets 3–4 of basal body "p" and triples 6–7 of basal body "a" (Fig. 7). At the level of triplet 2 of basal body "p", a developing 3-membered microtubular root "s" occurred (Fig. 10). Numerous lateral microtubules (mt) remained

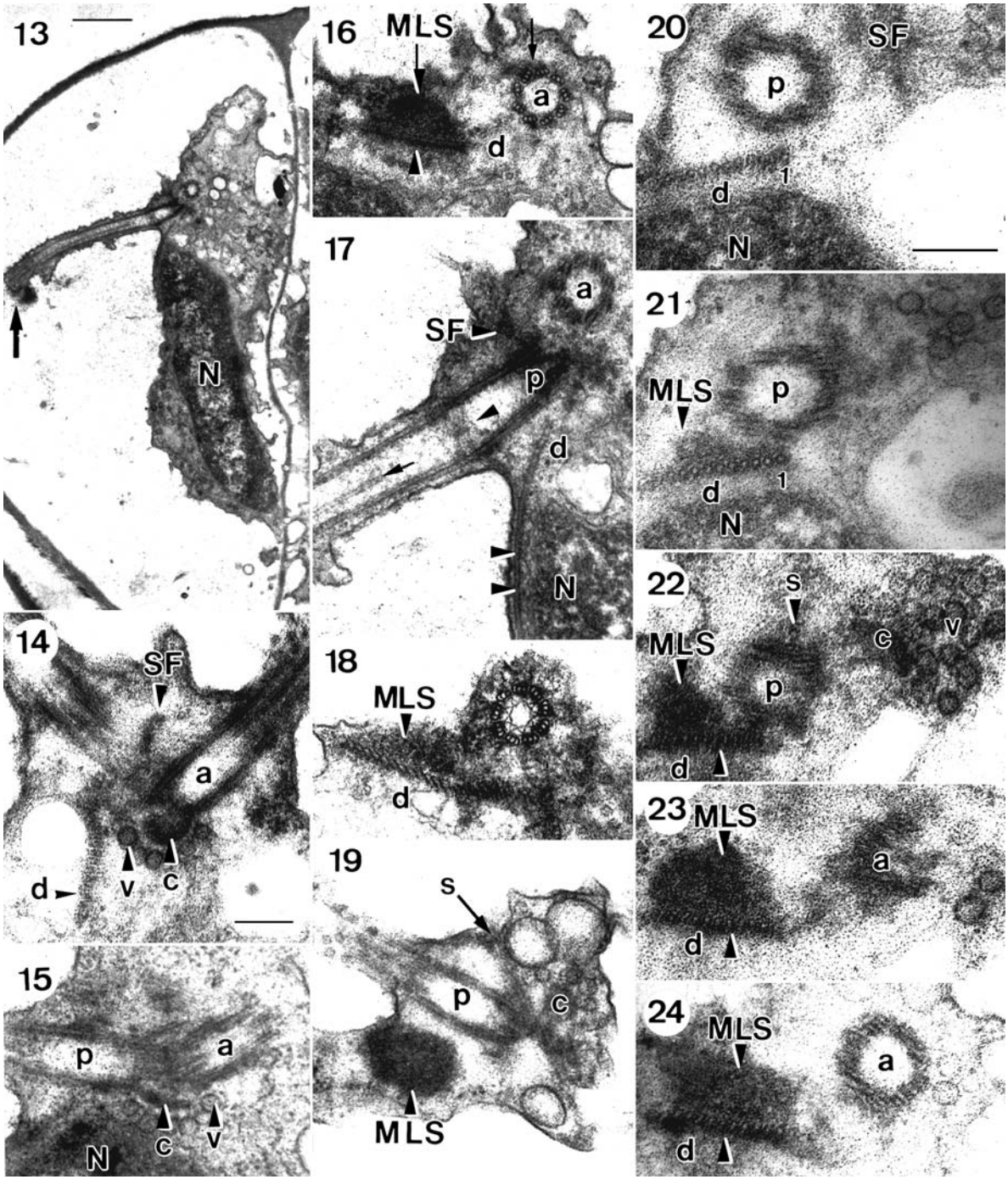


close and parallel to the plane of the nuclear envelope, adopting a less undulated appearance and not running parallel to each other in frontal view (Fig. 11). Under basal body "a" an electronically opaque cap appeared (c), that partially covered its proximal end (Figs. 8–10); several vesicles, 35–40 nm in diameter, appeared gathered in a circle of about the same diameter as that of the basal body (Fig. 11, arrowheads in circle). Centrin was labelled only in these initial developmental stages of gametogenesis, concentrated on one manifest apical spot in all developing spermatid (Fig. 12).

Stage 3. Non-parallel, axonemal basal bodies connected by a fully developed, striated, distal fiber (Fig. 1.3). Developing flagella emerged laterally (Fig. 13, arrow). Basal bodies "a" and "p", 550 nm long and 250 nm in diameter, underwent a gradual change of position until becoming approximately parallel to the nuclear envelope but forming an angle of 90–160 degrees with one another (Figs. 14–15). A fully developed, striated distal fiber (Figs. 14, 17, SF) connected both basal bodies. The site of attachment of the distal fiber had high electron opacity (Fig. 16, arrow). The transitional zone of basal bodies, extended up to the triplets zone (Fig. 17, arrowhead, Fig. 18). The central pair of microtubules of the axonemes ended at about 600 nm of the transitional zone (Fig. 17, arrow). Under basal body "a" and partially covering its proximal end, an electronically opaque cap (c) appeared. It was sheltered by vesicles 30–40 nm in diameter with median electronic opacity (v) (Figs. 14, 15). A large microtubular root "d" formed by 10–14 microtubules (Figs. 18, 20–24) ended proximally at the level of the proximal end of basal body "p" (Fig. 17). This root ran initially at an angle of 45° from basal body "p" and then ran

Figs. 13–24. Male gametogenesis in *Chara contraria* var. *nitelloides*. Basal apparatus with axonemal, non-parallel basal bodies only connected by an apparent striated, distal fiber. **13**. Gametocyte in which spiralization has begun. Basal bodies are approximately parallel to the nuclear envelope and form an angle of about 90 degrees to each other. Axonemes have begun to grow (arrow) and the developing flagella emerge laterally. Scale bar = 1.3 μm. **14–15**. Basal apparatus in which both basal bodies are longitudinally sectioned. An electron opaque cap (**c**) partially covers the proximal end of the basal body "**a**" and it is sheltered itself by vesicles (**v**). **14**. Section passes tangentially through basal body "**p**" and root "**d**". Basal bodies form an angle of 90 degrees to each other and a fully developed striated distal fiber (**SF**) appears. Scale bar = 0.5 μm, also valid for Figs. 15–19. **15.** Basal bodies form an angle of about 160 degrees to each other. **16**. Basal apparatus in which basal body "**a**" is transversely sectioned and seen from above. The distal fiber attachs to the triplets 6–7 (arrow). Root "**d**" is longitudinally sectioned and its proximal end is sandwiched between a dense, dome shaped body (MLS) and a thin layer of a similar dense material (arrowhead). **17**. Basal apparatus in which basal body "**a**" is obliquely sectioned and seen from above, whereas basal body "**p**" and root "**d**" are longitudinally sectioned. The section has passed through the first microtubule of root "**d**" (see Figs. 20–21). Note that root "**d**" diverges from basal body "**p**" and then passes between the nuclear envelop and the plasmalemma (pair of arrowheads). The arrowhead in "**p**" pinpoints the fully developed transition region and the small arrow indicates the proximal end of the axoneme central microtubules. **18**. Basal apparatus in which basal body "**p**" is transverselly sectioned at the level of the transition region and is seen from below, and root "**d**" is somewhat obliquely sectioned. Note that the arms of the stellate structure are connected to the A microtubules of the triplets. **19**. Basal apparatus in which basal body "**p**" and root "s" are longitudinally sectioned. The section also passes through the dense body of the MLS. **20–24.** A series of contiguous sections through the basal apparatus in which basal bodies "**a**" and "**p**" are slightly obliquely sectioned and roots "**d**" and "**s**" are transversely sectioned. Scale bar = 0.5 μm. **20**. The striated, distal fiber (**SF**) is transversally sectioned. The first microtubule of root "**d**" is indicated as "1". **21**. The dense body of the MLS appears on root "**d**". **22**. The proximal cap (**c**) and the vesicles (**v**) covering the proximal end of basal body "**a**" are sectioned. **23–24**. The sections pass through the middle part of the MLS (arrowheads) along with basal body "**a**"

between the plasmalemma and the nuclear envelope and very close to them (Fig. 17, two arrowheads). Microtubule "1" of root "d" ran under triplets 6–7 of basal body "p" (Figs. 20–21). The most proximal end of root "d", displayed a reduced multilayered structure or MLS (Figs. 16, 18). The root appeared in a "sandwich-like" configuration between a dome-shaped, electron-opaque body (Figs. 16, 18, 21–24) and a thin layer of a similar electron-opaque material, which was contiguous to the plasmalemma (Figs. 16, 22–24,



arrowhead). The three-membered microtubular root "s" ran at an angle of about 30 degrees from basal body "p" (Fig. 19) and close to its triplet 2 (Fig. 22).

Stage 4. Opposite, axonemal basal bodies not connected by fibers (Fig. 1.4). Flagella were positioned subapically and perpendicular to the long axis of the gamete (Figs. 25–27). The distal fiber totally disappeared and basal bodies moved up, to be positioned almost parallel to each other and counterclockwise overlapped (Figs. 25–27). The proximal cap under basal body "a" remained covered by vesicles (Figs. 25–26, c, v). Root "d" folded down and ran backwards at an angle of 90 degrees from the basal bodies (Figs. 25–27). Both the electron-opaque body and the thin layer of the same electron-opaque material of the MLS disappeared. Root "s" remained with both the same position and the same angle of divergence from basal body "p" (Fig. 27).

Stage 5. Axonemal basal bodies not connected by fibers and directed backwards (Fig. 1.5). The disappearance of the proximal cap under basal body "a" occurred and only the vesicles (v) remained (Fig. 28). Initially, basal body "p" folded down, following the previous folding down of root (d), until both were almost parallel each other (Figs. 29–30). The proximal region of axoneme "a" began its absorption into the gamete body (Figs. 29–30, two arrowheads).

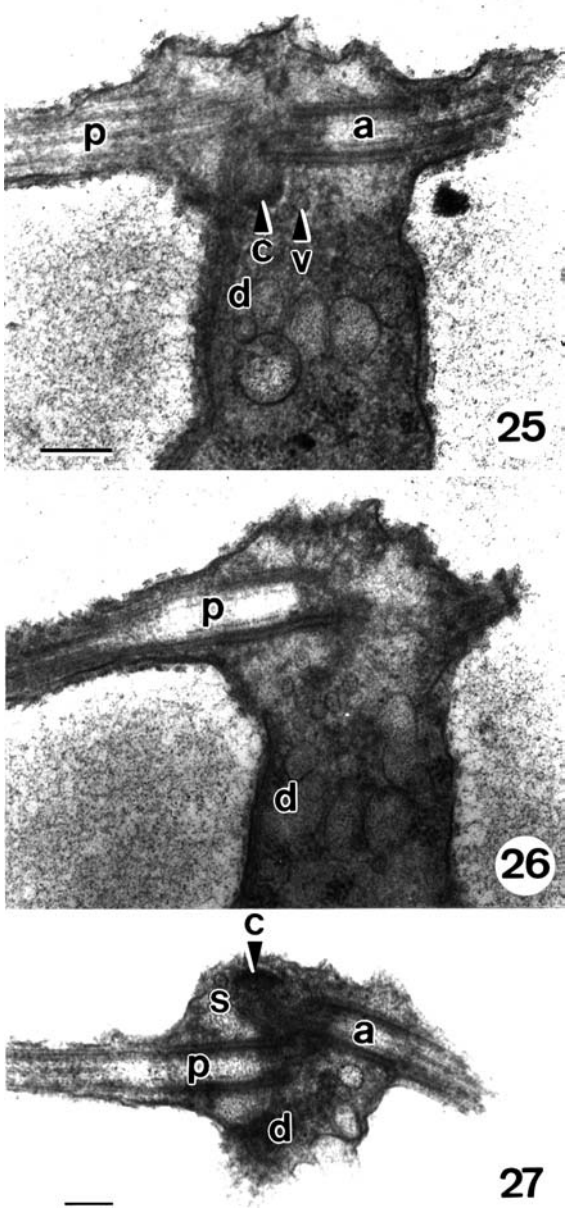
Stage 6. Parallel, axonemal basal bodies not connected by fibers (Fig. 1.6). Basal bodies and roots moved gradually (Fig. 31) until they were parallel each other, with their basal ends directed forwards (Figs. 32–33). In this final position, basal bodies were set off, i.e. basal body "a" was located about 200 nm forwards with respect to basal body "p" (Fig. 32). Both basal bodies were 500 nm long. A dense headpiece, "c"- shaped in section, covered now the proximal end of basal body "a" (Fig. 32, H). The basal portion of both axonemes was now fully immersed into the gamete body (Figs. 32–33). In the apical portion of mature gametes, the multimembered root (d) showed 27–29 microtubules while root (s) exhibited 3 microtubules (Fig. 33).

Discussion

Ultrastructure

Basal bodies. Gametogenesis in Charales involves dramatic changes in the appearance and structure of the cytoplasm, including the disappearance of organelles (Ge-Hong et al. 1988). Likewise, spermatangial filaments also produce *de novo* a new generation of organelles, i.e. basal bodies from procentrioles, since vegetative cells are acentric (Pickett-Heaps 1968). This phenomenon is well known in bryophytes (Moser and Kreitner 1970, Duckett et al. 1983, Graham and Kaneko 1991), *Equisetum* (Duckett 1973) and *Marsilea* (Klink and Wolniak 2001). Interestingly, that is not the case in the charophycean *Coleochaete* (Graham and Repavich 1989, Graham and Kaneko 1991), since in this genus the second basal body develops from a parental one, as in unicellular green algae (Melkonian et al. 1987, Moestrup and Hori 1989, see Beech et al. 1991, for review).

During the initial stages of the *Chara contraria* gametogenesis, elongating basal bodies were parallel to each other and perpendicular to the nuclear envelope. This position was similar to that observed by Turner (1968) in *Nitella missouriensis* and by Pickett-Heaps (1968, see Figs. 11–13) in *Chara fibrosa*. In bryophytes such as *Polytrichum* (Paolillo 1974), *Anthoceros* and *Marchantia* (Moser and Kreitner 1970), this feature is clearly apparent, since both nascent basal bodies lie initially side-by-side after a very short planar rotation (see Renzaglia and Garbary 2001). Carothers (1975) also observed young gametes with parallel basal bodies oriented perpendicular to plasmalemma and nuclear envelope in the hornwort *Phaeoceros*. On the contrary, this is not the case of gametes of the charophyte *Coleochaete* (Graham and Wedemayer 1984), in which no a similar stage occurs during gametogenesis.



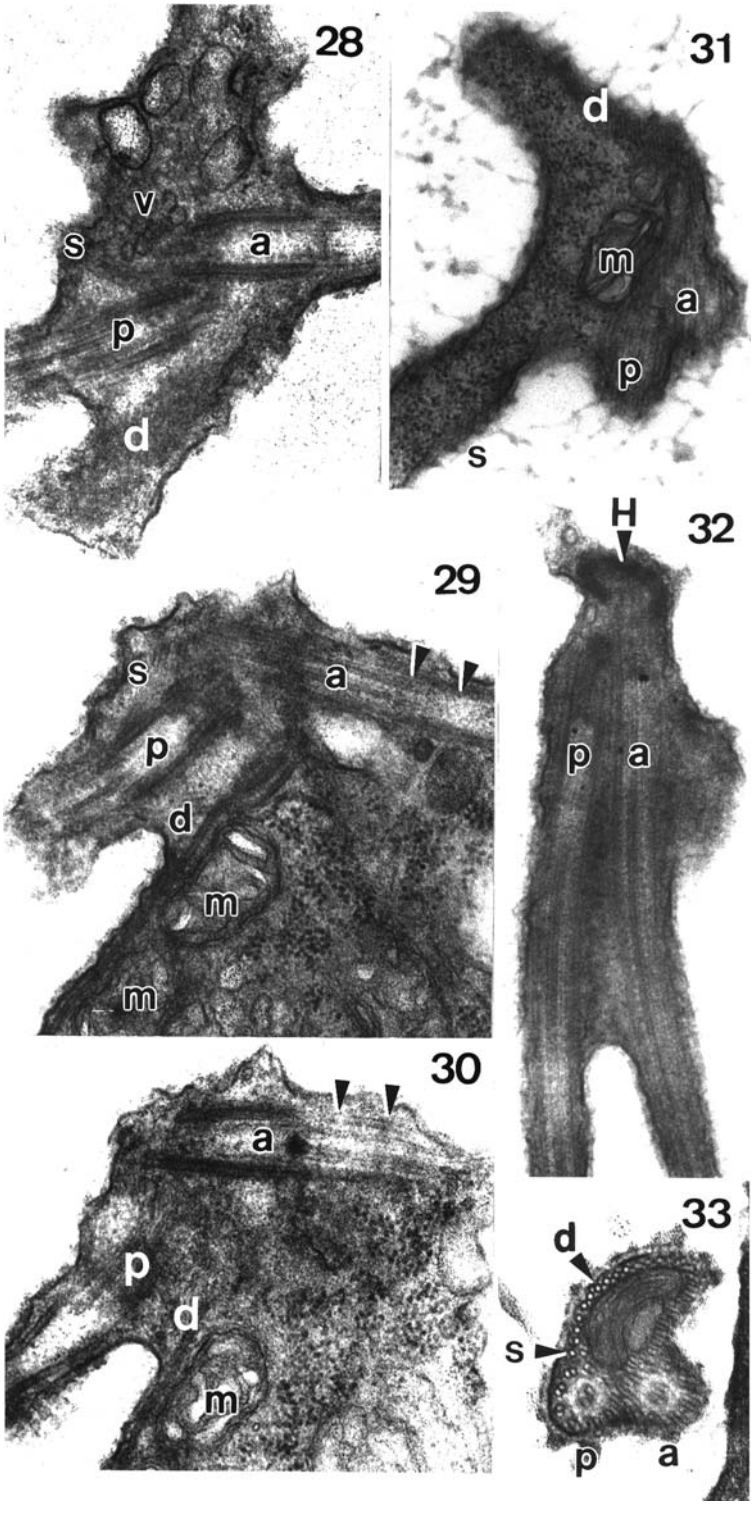
Figs. 25–27. Male gametogenesis in *Chara contraria* var. *nitelloides*. Basal apparatus with axonemal basal bodies aligned at 180 degrees to each other, not connected by fibers. **25–26.** Longitudinal serial sections through a gamete in which basal bodies are longitudinally sectioned. The distal fiber has disappeared and basal bodies lie almost parallel to each other with their proximal ends facing and with the axonemes directed in opposite directions. Root "**d**" forms an angle of 90 degrees with basal bodies. The fully developed proximal cap (**c**) and vesicles (**v**) are seen under basal body "**a**". Scale bar = 0.5 μm. **27.** Transversal section through a gamete in which basal bodies are longitudinally sectioned to show their counterclockwise overlapping. Proximal cap (**c**) is seen under basal body "**a**". Root "**s**" diverges of about 30 degrees from basal body "**p**" and root "**d**" is obliquely sectioned. Scale bar = 0.5 μm

Non-striated fiber. A similar non-striated proximal fiber connecting both parallel basal bodies also appeared clearly in developing gametes of *Nitella missouriensis* (Turner 1968, Figs. 7 and 11) although no description of it was included in that paper. In the hornwort *Phaeoceros* a non-striated proximal fiber is apparent at the beginning of the spermatid maturation (Carothers 1975). Non-striated connecting fibers are present in different green algae (Melkonian 1980). Particulary, in the prasinophycean *Pyramimonas* (Melkonian 1981, Moestrup and Hori 1989) they also connect parallel basal bodies. Remarkably, non-striated proximal fibers are totally absent in flagellated cells of all the non-charalean charophytes, i.e. *Chaetosphaeridium* (Moestrup 1974), *Chlorokybus* (Rogers et al. 1980), *Coleochaete* (Sluiman 1983, Graham and Wedemayer 1984), and *Klebsormidium* (Marchant et al. 1973).

Striated fiber. The distal striated fiber eventually disappears in mature gametes of Charales (Turner 1968, Pickett-Heaps 1968). In all the other studied genera of the division, i.e. *Chaetosphaeridium* (Moestrup 1974), *Chlorokybus* (Rogers et al. 1980), *Coleochaete* (Sluiman 1983, Graham and Wedemayer 1984), and *Klebsormidium* (Marchant et al. 1973) the distal striated fiber remains intact in mature flagellar apparatuses. Mature basal apparatuses without any type of connecting fiber are also characteristic of bryophytes (Paolillo 1965; Carothers 1973, 1975; Duckett et al. 1982, 1983), pteridophytes and gymnosperms (Renzaglia and Garbary 2001), although striated fibers are totally absent from the inception of gametogenesis.

Microtubular roots. The 3-membered root "s" related to basal body "p" was manifest

from the very beginning of the *Chara contraria* basal apparatus development. Sluiman (1983) also noted that a similar 3-membered microtubular root was indeed present in mature gametes of *Ch. fibrosa* (Pickett-Heaps 1968, Figs. 70–72) and *N. missouriensis* (Turner



1968, Fig. 28e), running parallel to the multimembered root "d". Nevertheless, its early presence during the ontogeny was not clearly demonstrated. Also Duncan et al. (1997) observed this 3-membered microtubular root in mature gametes of *Ch. vulgaris* but they interpreted it as remnants of fibrous MLS-like structures. The present study clearly indicates the microtubular nature of the root and its "s" position, and thus that it has no relation with the MLS.

Multilayered structure or MLS. No multilayered structure or MLS has been reported in mature gametes of the genus *Chara* (Pickett-Heaps 1968, Moestrup 1970, Graham and McBride 1979, Duncan et al. 1997). Nevertheless, we agree with the interpretation of Moestrup (1974), Graham and McBride (1975) and Duckett et al. (1982), in the sense that the dense body that appeared in the proximal end of the multistranded root "d" in developing spermatids of *Chara* -the "manchette adjunt" according to Pickett-Heaps (1975) terminology is part of an incomplete MLS with no lamellar strata. In fact MLS is a relatively variable structure in which the only element always present is the microtubular component named S1. Zoospores of *Chaetosphaeridium* (Moestrup 1974), *Klebsormidium* (Marchant et al. 1973), and *Coleochaete* (Sluiman 1983) exhibit a complete MLS, i.e. all the complementary strata, that is the S2–S4 lamellar layers and the S5–S6 electronically opaque layers, are present. In gametes of *Coleochaete* (Graham and Wedemayer 1984), only one lamellar stratum S2 and the opaque S6 are present. In the prasinophycean *Mesostigma* (Rogers et al. 1981), strata S3, S4 and S6 are absent. In other organisms in the higher plant line, cases either of an incomplete MLS at maturity or of an entirely absent MLS have been registered. In both the hepatic *Phaeoceros* and the pteridophyte *Selaginella*, lamellar strata disappear during gamete maturation (Duckett et al. 1982). In the water fern *Marsilea* (Rice and Laetsch 1967) no MLS appears, and in certain musci a derived maturational loss of the MLS occurs (Duckett et al. 1982).

Phylogeny

Mattox and Stewart (1984) indicated that many extant Prasinophyceae have their flagella positioned inside a spacious apical depression and that this trait has been used to separate them from the Chlorophyceae, which have their flagella positioned inside a conspicuous apical papilla. Nevertheless, they suggested

Figs. 28–33. Male gametogenesis in *Chara contraria* var. *nitelloides*. **Figs. 28–30.** Flagellar apparatuses in which the axonemal basal bodies, not connected by fibers gradually adopt a backwards orientation. **28.** Transverasal section through a gamete in which basal bodies are longitudinally sectioned. Roots "**s**" and "**d**" maintain the same counterclockwise overlapping, the proximal cap has disappeared and only vesicles (**v**) now cover the proximal end of the basal body "**a**". Scale bar = 0.5 μm, also valid for Fig. 29–33. **29–30.** Series of two oblique sections through a gamete in which basal bodies are longitudinally sectioned. Basal bodies and roots have changed their positions: basal body "**p**" is now almost parallel to root "**d**", which in turn runs close to the plasmalemma and anterior mitochondria (**m**). In Fig. 29, root "**s**" diverges from basal body "**p**". The basal portion of the axoneme of basal body "**a**" is now included into the gamete body (pairs of arrowheads), whereas that of basal body "**p**" is still free. Figs. 31–33. Flagellar apparatuses in which the axonemal basal bodies, not connected by fibers with a final parallel orientation. 31. Oblique section through a gamete in which basal bodies and roots are also obliquely sectioned. Basal bodies and root "**d**" are already almost parallel to each other. 32. Longitudinal section through a gamete in which basal bodies are also longitudinally sectioned. Basal bodies direct their proximal ends forwards. Basal body "**a**" is staggered about 200 nm forward of basal body "**p**". The basal portion of both axonemes are now immersed into the gamete body. A dense "**c**" -shaped headpiece has developed and covers the proximal end of basal body "**a**". 33. A transverse section through gamete seen from above. Axonemes are seen from their basal ends (indicated by the counterclockwise imbrication of the doublets). The multimembered root "**d**" exhibits c. 27–29 microtubules and root "s" exhibits 3 microtubules

that in the evolution of the flagellar apparatuses from *Tetraselmis* to *Carteria*, the basal bodies could have undergone a folding down with an intermediate state similar to *Hafniomonas*. The ontogeny of the basal/flagellar apparatus of *Chara* gives support to the idea that a similar folding down process took place during the evolution of Charales: i.e. basal bodies change from an initial or primary parallel position, passing through a counterclockwise position to eventually end in an inverted final or secondary, parallel position with their basal ends directed forwards.

Rogers et al. (1980) claimed that the flagellar apparatus of the Charales derives from a *Pyramimonas*-like flagellar apparatus in which the gametes exhibit a half-cell configuration. Melkonian (1984, 1990) on the contrary, suggested that the Mamiellales (see Moestrup 1984, Moestrup and Throndsen 1988) could be the ancestral flagellate more closely related to Charales. However, recent molecular studies (Melkonian and Surek 1995, Marin and Melkonian 1999) suggest that the Mamiellales are not close to the origin of the streptophyte (''charophyte'') green algae, and that *Mesostigma* could be the predecessor of the unilateral system in the streptophyte green algae, by reduction of the microtubular roots. According to Marin and Melkonian (1999), *Mesostigma* integrates a monophyletic group with *Chaetosphaeridium*, although the precise phylogenetic relationships with the streptophyte green algae have not so far been elucidated by SSU rRNA sequence comparisons. Conversely, Karol et al. (2001) claim that analyses of amino acid data from both the plastid and mitochondrial genomes of *Mesostigma* strongly support the categorization of this genus as a sister of all green algae rather than as a basal charophyte lineage. The ontogenetic evidence of the present study shows that from its initial developmental stages, the basal apparatus of *Chara contraria* has consistently only two microtubular roots related to basal body ''p'', and only one MLS, i.e. the basal apparatus did not show an initial 180° rotational symmetry. These traits do not therefore support the idea of a *Mesostigma*-like, cruciform flagellar apparatus (Rogers et al. 1981; Melkonian 1984, 1989; Nakayama et al. 1998; Marin and Melkonian 1999) in the origin of the Charales. In fact, the developmental traits of the flagellar apparatus of male gametes of *Chara contraria* var. *nitelloides*, specially the initial parallel disposition of the basal bodies, the presence of only two microtubular roots, and the absence of MLS-like structures at the very beginning of the basal apparatus development could be indicative of a *Mamiella*-like ancestor for Charales.

Molecular studies (Huss and Kranz 1997) also suggest that Charales constitute a distinct and ancient lineage of the class originated from the Mamiellales, previously to the divergence from the prasinophyceae and the rest of the charophyceae. As the Mamiellales have been reported to belong to a reduction series characterized by apomorphic traits (Nakayama et al. 1998, Marin and Melkonian 1999), the resulting question is whether or not the divergence of the Charales is independent from the divergence of the streptophytes. The majority of molecular studies carried out to date indicate that the Charales forms normally isolated clades, as an order separated even from the rest of the charophytes (Friedl 1997, Karol et al. 2001). In this sense, the different ultrastructural characteristics of the ontogenies of flagellar apparatuses of non-charalean charophytes (i.e. absence of *de novo* basal bodies replications, non-parallel initial position of basal bodies, absence of non-striated proximal fibers, striated distal fiber remaining intact in mature basal apparatuses) support that molecular evidence.

The characteristics of male gametes of the Charales were normally considered homologous to those of flagellated male gametes of bryophytes and vascular cryptogams (Mattox and Stewart 1984, Renzaglia and Garbary 2001). Nevertheless, we found important ontogenetic differences between basal apparatuses of Charales and Bryophytes that give support to the concept of Graham and Kaneko (1991) and Graham and Wilcox (2000), who proposed that charalean algae

produce spermatozoids that only superficially resemble bryophyte sperm solely by exhibiting flagella directed backwards and by being coiled. These differences can be summarized as follows: 1) In Charales, roots and basal bodies undergo gradual changes in position to end parallel to each other only in mature gametes, whereas in bryophytes they are parallel to each other throughout gamete development (Carothers 1975, Renzaglia and Garbary 2001). In consequence, the final parallel position of the basal bodies in Charales is in fact a result of a total inversion in their direction as they eventually direct their basal ends towards the anterior part of the gamete. On the contrary this orientation in bryophytes is unchanged from the very inception of basal apparatus development (Carothers 1975, Duckett et al. 1983, Renzaglia and Garbary 2001). For this reason, Moestrup's (1984) and Melkonian's (1984) interpretation of the parallel disposition of basal bodies in Charales as primitive is open to discussion, since they did not take into account this inversion. 2) In Charales, roots "d" and "s" initially have a different angular divergence and end parallel to each other and to the basal bodies due to the gradual retraction of the gamete cytoplasm. In the bryophytes, on the contrary, the root is parallel to the basal bodies from the start of the process (Carothers 1975, Duckett et al. 1983, Renzaglia and Garbary 2001). 3) The staggering or offset of the basal bodies in charalean and bryophyte spermatozoids has an entirely different origin in both groups. The present study makes it clear that this characteristic in Charales is due to the different, final, relative position of basal bodies "a" and "p". In contrast, in bryophytes, this attribute results from an early rearward migration of the posterior basal body coupled to differential triplets and cartwheel growths (Duckett et al. 1983, Renzaglia and Garbary 2001). 4) The typical adsorption of the basal portion of both axonemes into the cytoplasm of mature charalean and bryophyte male gametes are presumably not homologous peculiarities in view of their different ontogenies. In *Chara*, axonemes are entirely independent from the gamete body during almost all the developmental stages until their basal portions enter into the gamete body by the end of gametogenesis. In contrast, in Bryophytes, the basal portions of axonemes are immersed into the gamete body from the start of gametogenesis (Brown et al. 1983).

Centrin

Renzaglia and Garbary (2001) include presence/absence of centrin in the list of characters used in analysis of charophycean and land plant male gametogenesis and mention *Chara fibrosa, Ch. vulgaris*, *Nitella missouriensis* and *Coleochaete pulvinata* as possesing centrin between basal bodies but without mentioning the stage of the gametogenesis in which it appeared. In *Chara contraria* var *nitelloides* centrin was present only in immature gametes, coinciding with the presence of both the striated distal fiber and the MLS, but it was totally absent in mature gametes in which both structures are absent. Duncan et al. (1997), considered it necessary to exactly show the localization of centrin to infer MLS homologies. We, nevertheless, rule out an MLS localization, since the reduced MLS in Charales devoids of layered structures.

This study was started during a visit of EJC to the laboratory of Dr Michael M. Melkonian, at the Botanisches Institut, Köln, Federal Republic of Germany with Fellowships of the Alexander von Humboldt Foundation and the Research Council of the Province of Nordhein-Westfallen, Germany. Subsequent funds have been provided by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, Grants PIP 0949/98 to EJC and PEI 0433/97 to PIL), CIC (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Grants 1454/97 and 1895/99 to EJC) and by the Universidad Nacional del Sur (Grants PGI SGCyT 1462/99 and 2119/00 to EJC). The Alexander von Humboldt Foundation donated scientific material to EJC. AAV is a research fellow of the CONICET. EJC is a research member of the CIC. PIL is a research member of

CONICET. The authors gratefully acknowledge Dr. Michael Melkonian for providing the primary anti-centrin antibody and for reading the manuscript. We also thank Dr. Russell L. Chapman for reading the manuscript.

References

Beech P.L., Heimann K., Melkonian M. (1991) Development of the flagellar apparatus during the cell cycle of unicellullar algae. Protoplasma 164: 23–37.

Bhattacharya D., Medlin L. (1998) Algal phylogeny and the origin of land plants. Plant Physiol. 116: 9–15.

Bhattacharya D., Steinkötter J., Melkonian M. (1993) Molecular cloning and evolutionary analysis of the calcium- modulated contractile protein, centrin, in green algae and land plant. Plant Mol. Biol. 23: 1243–1254.

Brown R.C., Carothers Z.B., Duckett J.G. (1983) Comparative spermatogenesis in the Sphaerocarpales. II. Blepharoplast structure in *Geothallus tuberosus* Campb. The Bryologist 83: 234–243.

Carothers Z.B. (1973) Studies of spermatogenesis in the Hepaticae. IV. On the blepharoplast in *Blasia*. Amer. J. Bot. 60: 819–828.

Carothers Z.B. (1975) Comparative studies on spermatogenesis in bryophytes. In: Duckett J.E., Racey P.A. (eds.) The biology of the male gametes. Biol. J. Linn. Soc. 7: 71–84.

Chapman R.L., Waters D.A. (2002) Green algae and land plants-An answer at last? J. Phycol. 38: 237–240.

Cocucci A.E., Cáceres E.J. (1976) The ultrastructure of the male gametogenesis in *Chara contraria* var. *nitelloides* (Charophyta). Phytomorphology 30: 5–16.

Duckett J.D. (1973) An ultrastructural study of the differentiation of the spermatozoids of *Equisetum*. J. Cell Sci. 12: 95–129.

Duckett J.D., Carothers Z.B., Miller C.C.J. (1982) Comparative spermatology and bryophyte phylogeny. J. Hattori Bot. Lab. 53: 107–125.

Duckett J.D., Carothers Z.B., Miller C.C.J. (1983) Gametogenesis. In: Schuster R.M. (ed.) Manual of bryology. Hattori Bot. Lab. Nichinan, pp. 232–275.

Duncan T.M., Renzaglia K.S., Garbary D.J. (1997) Ultrastructure and phylogeny of the spermatozoid of *Chara vulgaris* (Charophyceae). Plant Syst. Evol. 204: 125–140.

Friedl T. (1997) The evolution of the green algae. In: Bhattacharya D. (ed.) Origin of Algae and their plastids. Springer, Wien New York, pp. 88–101.

Friedl T., Zeltner C. (1994) Assesing the relationships of some coccoid green lichen algae and the Microthamniales (Chlorophyta) with 18S ribosomal RNA gene sequence comparisions. J. Phycol. 30: 500–506.

Ge-Hong S., Taro Q.P., Kuroiwa T. (1988) Destruction of organelle during spermatogenesis in *Chara corallina* examined by staining with DAPI and anti DNA antibody. Protoplasma 144: 185–188.

Graham L.E., Kaneko Y. (1991) Subcellular structures of relevance to the origin of land plants (Embryophytes) from green algae. CRC Critical Reviews in Plant Sciences 10: 323–342.

Graham L.E., McBride G.E. (1975) The ultrastructure of multilayered structures associated with flagellar bases in motile cells of *Trentepohlia aurea*. J. Phycol. 11: 86–96.

Graham L.E., McBride G.E. (1979) The occurrence and phylogenetic significance of a multilayered structure in *Coleochaete* spermatozoids. Amer. J. Bot. 66: 887–894.

Graham L.E., Repavich W.M. (1989) Spermatogenesis in *Coleochaete pulvinata* (Charophyceae): Early blepharoplast development. Amer. J. Bot. 76: 1266.

Graham L.E., Taylor C.III (1986) The ultrastructure of meiospores of *Coleochaete pulvinata* (Charophyceae) J. Phycol. 22: 299–307.

Graham L.E., Wedemayer G.J. (1984) Spermatogenesis in *Coleochaete pulvinata* (Charophyceae) sperm maturation. J. Phycol. 20: 302–309.

Graham L.E., Wilcox L.W. (2000) Algae. Prentice Hall, Upper Saddle River, 640 pp.

Hart P., Wolniak S.M. (1998) Spermiogenesis in *Marsilea vestita*: a temporal correlation between centrin expression and blepharoplast differentiation. Cell Motil. Cytoskelet. 41: 39–48.

Höhfeld I., Beech P.L., Melkonian M. (1994) Immunolocalization of centrin in *Oxyrrhis marina* (Dinophyceae). J. Phycol. 30: 474–488.

Huss V.A.R., Kranz H.D. (1997) Charophyte evolution and the origin of land plants. In: Bhattacharya D. (ed.) Origin of Algae and their plastids. Springer, Wien New York, pp. 104–114.

Karol K.G., McCourt R.M., Cimino M.T., Delwiche C.F. (2001) The closest living relatives of land plants. Science 294: 2351–2353.

Katsaros C.I., Maier I., Melkonian M. (1993) Immunolocalization of centrin in the flagellar apparatus of male gametes of *Ectocarpus siliculosus* (Phaeophyceae) and other brown algal motile cells. J. Phycol. 29: 787–797.

Klink V.P., Wolniak S.M. (2001) Centrin is necessary for the formation of the motile apparatus in spermatids of *Marsilea*. Mol. Biol. Cell 12: 761–776.

Koblenz B., Schoppmeier J., Grunow A., Lechtreck K.-F. (2003) Centrin deficiency in *Chlamydomonas* causes defects in basal body replication, segregation and maturation. J. Cell Sci. 116: 2635–2646.

Kwiatkowska M., Maszewski J. (1978) Ultrastructure of the nuclei during different phases of the cell cycle in antheridial filaments of *Chara vulgaris*. Protoplasma 96: 59–74.

Kwiatkowska M., Maszewski J. (1985) Changes in the ultrastructure of plasmodesmata during spermatogenesis in *Chara vulgaris*. Planta (Berl.) 166: 46–50.

Kwiatkowska M., Maszewski J. (1986) Changes in the occurrence and ultrastructure of plasmodesmata in antheridia of *Chara vulgaris* L., during different stages of spermatogenesis. Protoplasma 132: 179–188.

Lechtreck K. F., Grunow A. (1999) Evidence for a direct role of nascent basal bodies during spindle pole initiation in the green alga *Spermatozopsis similis*. Protist 150: 163–181.

Marchant H.J., Pickett-Heaps J.D., Jacobs K. (1973) An ultrastructural study of zoosporogenesis and the mature zoospore of *Klebsormidium flaccidum*. Cytobios 8: 95–107.

Marin B., Melkonian M. (1999) Mesostigmatophyceae, a new class of streptophyte green algae revealed by SSU rRNA sequence comparisons. Protist 150: 399–417.

Marshall W.F., Vucica Y., Rosenbaum J.L. (2001) Kinetics and regulation of de novo centriole assembly. Implications for the mechanism of centriole duplication. Curr. Biol. 11: 308–317.

Mattox K.R., Stewart K.D. (1984) Classification of the green algae: A concept based on comparative cytology. In: Irvine D.E.G., John D.M. (eds.) Systematics of the green Algae. Academic Press, London Orlando, pp. 29–72.

McCourt R.M., Karol K.G., Guerlesquin M., Feist M. (1996) Phylogeny of extant genera in the family Characeae (Charales, Charophyceae) based on *rbc*L sequences and morphology. Amer. J. Bot. 83: 125–131.

McFadden G.I., Schulze D., Surek B., Salisbury J.L., Melkonian M. (1987) Basal body reorientation mediated by a C^{2+} - modulated contractile protein. J. Cell Biol. 105: 903–912.

Melkonian M. (1980) Ultrastructural aspects of basal body associated fibrous structures in green algae: A critical review. BioSystems 12: 85–104.

Melkonian M. (1981) The flagellar apparatus of the scaly green flagellate *Pyramimonas abovata*: Absolute configuration. Protoplasma 108: 341–355.

Melkonian M. (1984) Flagellar apparatus ultrastructure in relation to green algal classification. In: Irvine D.E.G., John D.M. (eds.) Systematics of the green Algae. Academic Press, London Orlando, pp. 73–120.

Melkonian M. (1989) Flagellar apparatus ultrastructure in *Mesostigma viride* (Prasinophyceae). Plant Syst. Evol. 164: 93–122.

Melkonian M. (1990) Phylum Chlorophyta, Class Prasinophyceae. In: Margulis L., Corliss J.O., Melkonian M., Chapman D.J. (eds.) Handbook of Protoctista, Vol 1. Jones and Barlett, Boston, pp. 600–607.

Melkonian M., Surek B. (1995) Phylogeny of the Chlorophyta: congruence between ultrastructural and molecular evidence. Bull. Soc. Zool. France 120: 191–208.

Melkonian M., Reize I.B., Preisig H.R. (1987) Maturation of a flagellum/basal body requires more than one cell cycle in algal flagellates. Studies on *Nephroselmis olivacea* (Prasinophyceae). In: Wiessner W., Robinson D.G., Starr R.C. (eds.) Algal development, molecular and cellular aspects. Springer, Berlin Heidelberg New York Tokyo, pp. 102–113.

Moestrup Ø. (1970) The fine structure of mature spermatozoids of *Chara corallina*, with special reference to microtubules and scales. Planta (Berl.) 93: 295–308.

Moestrup Ø. (1974) Ultrastructure of the scale-covered zoospores of the green alga *Chaetosphaeridium*, a possible ancestor of the higher plants and bryophytes. Biol. J. Linn. Soc. 6:111–125.

Moestrup Ø. (1984) Further studies on *Nephroselmis* and its allies (Prasinophyceae). II. *Mamiella* gen. nov. (Mamiellaceae fam. nov.). Nord. J. Bot. 4: 109–121.

Moestrup Ø., Hori T. (1989) Ultrastructure of the flagellar apparatus in *Pyramimonas octopus* (Prasinophyceae) II. Flagellar roots, connecting fibers, and numbering of individual flagella in green algae. Protoplasma 148: 41–56.

Moestrup Ø., Throndsen J. (1988) Light and electron microscopical studies on *Pseudoscourfieldia marina*, a primitive scaly green flagellate (Prasinophyceae) with posterior flagella. Canad. J. Bot. 66: 1415–1434.

Moser J. W., Kreitner G.L. (1970) Centrosome structure in *Anthoceros laevis* and *Marchantia polymorpha*. J. Cell Biol. 44: 454–458.

Motomura T., Nagasato C., Komeda Y., Okuda K. (2001) Transient localization of Y-tubulin around the centrioles in the nuclear division of *Boergenia forbesii* (Siphonocladales, Chlorophyta). J. Phycol. 37: 783–792.

Nakayama T., Marin B., Kranz H.D., Surek B., Huss V.A.R., Inouye I., Melkonian M. (1998) The basal position of scaly green flagellates among the green algae (Chlorophyta) is revealed by analyses of nuclear-encoded SSU rRNA sequences. Protist 149: 367–380.

O'Kelly C.J., Floyd G.L. (1984) Flagellar apparatus absolute orientations and the phylogeny of the green algae. BioSystems 16: 227–251.

Paolillo D.J. Jr. (1965) On the androcyte of *Polytrichum*, with special reference to the Dreiergruppe and the limosphere (Nebenkern). Canad. J. Bot. 43: 669–676.

Paolillo D.J. Jr. (1974) Motile male gametes in plants. In: Robards A.W. (ed.) Dynamic aspects of plant ultrastructure. McGraw Hill, London, pp. 504–531.

Pickett-Heaps J.D. (1968) Ultrastructure and differentiation in *Chara (fibrosa)* IV. Spermatogenesis. Austral. J. Biol. Sci. 21: 655–690.

Pickett-Heaps J.D. (1975) Green Algae. Structure, reproduction and evolution in selected genera. Sinauer Assoc, Sunderland, Massachussets, 606 pp.

Ragan M.A., Parsons T.J., Sawa T., Strauss N.A. (1994) 18S ribosomal sequences indicate a monophyletic origin of Charophyceae. J. Phycol. 30: 490–500.

Renzaglia K.S., Garbary D.J. (2001) Motile gametes of land plants: Diversity, development, and evolution. Crit. Rev. Plant Sci. 20: 107–213.

Renzaglia K.S., Dengate S.B., Schmitt S.J., Duckett J.G. (2002) Novel features of *Equisetum arvense* spermatozoids: insights into pteridophyte evolution. New Phytol. 154: 159–174.

Reymond L.O., Pickett-Heaps J.D. (1983) A routine flat embedding method for electron microscopy of microorganisms allowing selection and precisely oriented sectioning of single cells by light microscopy. J. Microscopy 130: 79–84.

Rice H.V., Laetsch W.M. (1967) Observations on the morphology and physiology of *Marsilea* sperms. Amer. J. Bot. 54: 856–866.

Rogers C.E., Domozych D.S., Stewart K.D., Mattox K.R. (1981) The flagellar apparatus of *Mesostigma viride* (Prasinophyceae): Multilayered structures in a scaly green flagellate. Plant Syst. Evol. 138: 247–258.

Rogers C.E., Mattox K.R., Stewart K.D. (1980) The zoospore of *Chlorokybus atmorphyticus*, a charophyte with sarcinoid growth habit. Amer. J. Bot. 67: 774–783.

Salisbury J.L. (1989) Centrin and the algal flagellar apparatus. J. Phycol. 25: 201–206.

Salisbury J.L. (1992) Centrin-based calcium sensitive contractile fibers of the green algae. In: Menzel D. (ed.) The Cytoskeleton of the Algae. CRC Press, Boca Raton, pp. 393–410.

Sluiman H.J. (1983) The flagellar apparatus of the zoospore of the filamentous green alga *Coleochaete pulvinata:* Absolute configuration and phylogenetic significance. Protoplasma 115: 160–175.

Spurr A.M. (1969) A low-viscosity epoxy embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43.

Stewart K.D., Mattox K.R. (1978) Structural evolution in the flagellated cells of green algae and land plants. BioSystems 10: 145–152.

Turner F.R. (1968) An ultrastructural study of plant spermatogenesis. J. Cell Biol. 37: 370–393.

Vaughn K.C., Renzaglia K.S. (1993) Centrin in spermatogenesis of archegoniate. Amer. J. Bot. 80: 20.

Vaughn K.C., Sherman T.D., Renzaglia K.S. (1993) A centrin homologue is a component of

the multilayered structure in bryophytes and pteridophytes. Protoplasma 175: 58–66.
van den Hoek C., Mann D.G., Jahns H.M. (1995) Algae. An introduction to phycology. Cambridge University Press, Cambridge, 623 pp.
Wolniak M.W., Klink V.P., Hart P.E., Chia-Wei T. (2000) Control of development and motility in the spermatozoids of lower plants. Gravitational and Space Biology Bulletin 13: 85–93.

Address of the authors: Amelia A. Vouilloud, Eduardo J. Cáceres (e-mail: uscacere@criba.edu.ar), Patricia I. Leonardi, Laboratorio de Micología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. 8000 Bahía Blanca, Argentina.