

Ultrastructure of multicellular dwarf males with external gametangium in *Oedogonium macrandrium* (Oedogoniales, Chlorophyta)

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Key words: androspores; Chlorophyta; dwarf males; Oedogoniales; *Oedogonium macrandrium*; ultrastructure

ABSTRACT: This is the first comprehensive ultrastructural study on dwarf males with external gametangia in the genus *Oedogonium*, from androspore germination to the liberation of mature male gametes. The ultrastructure of the process in *O. macrandrium* Wittrok is similar to that of *Bulbochaete hiloensis* (Nordstedt) Tiffany, but with two remarkable differences. In *O. macrandrium*: 1) instead of a true transverse wall, only condensed mucilage appears between the gametes of each antheridial cell, and 2) the cell wall between the basal cell and the basal most antheridial cell has simple plasmodesmata similar to those present in the transverse walls of vegetative cells, which are absent in *B. hiloensis*.

Introduction

The genus *Oedogonium* Link ex Hirn was established by Link (1820) and it has been reviewed worldwide several times (Tiffany, 1937; Gemeinhardt, 1939; Gauthier-Lievre, 1963; 1964; Mrozinska, 1985). There are dioecious species where dwarf male gametophytes develop from a special type of zoospores called androspores, after attachment on a female gametophyte (Ohashi, 1930; Pickett-Heaps, 1975; Mrozinska, 1985; Bold and Wynne, 1985; Van den Hoek, 1995). Two types of dwarf males are known: 1) with internal antheridium and 2) with external antheridium (Mrozinska, 1985; 1991). In the first type the cell wall of the basal, vegetative cell derived from the attached androspore becomes the antheridium

wall. In the second case, (1- n) vegetative, strongly asymmetrical, apical mitoses and cytokineses occurred in the basal, vegetative cell, resulting in an external (1-n)-celled antheridium whose own walls are outside the vegetative mother-cell wall.

Several aspects have been studied at ultrastructural level in the genus *Oedogonium*, such as morphology, cytology and reproduction (Hoffman, 1967; 1968a; 1968b; 1971; 1973), the process of cell division and formation of terminal caps (Pickett-Heaps and Fowke, 1969; Pickett-Heaps, 1975), the zoosporogenesis, with special emphasis on the zoospores' flagellar apparatus (Pickett-Heaps, 1971), the differentiation and development of germlings and the cell division (Pickett-Heaps, 1971; 1972a, b), the spermatogenesis process in macrandric species (Hoffman and Manton, 1963; Coss and Pickett-Heaps, 1974), the oogenesis and fertilization (Hoffman, 1973), the oogonial aperture (Alberghina et al., 2009) and the reproduction by zoospores (Pickett-Heaps, 1971; 1973a, b).

At present there are no ultrastructural studies on dwarf males with external gametangium in the genus *Oedogonium*. The only contribution for this genus is the study of the development of pseudo single-celled dwarf males with internal gametangium in *O. pluviale* (Leonardi et al., 1998). In that work the authors claimed that additional ultrastructural knowledge in different species of the genus were needed in order to understand their evolution. Therefore, we are now presenting the first comprehensive ultrastructural study on dwarf males with external gametangium in the genus *Oedogonium*, from androspore germination to the formation of mature male gametes. The only ultrastructural study in dwarf males with external gametangium has been performed in *Bulbochaete hiloensis* (Pickett-Heaps, 1975), which is a species of a closely related genus.

Material and methods

Material came from a pond located in a recreational city park ("Parque de Mayo", 38° 41' S, 62° 16' W, Bahía Blanca, Argentina). For light microscope observations, thalli were fixed with glacial acetic acid/alcohol absolute (1:3), for 24 hours at room temperature. Samples were stained with a solution of 45% propionic acid, 2% iron hematoxylin and 1% iron citrate (Núñez, 1968). For observations with transmission electron microscope, samples were fixed for 2h at 5°C in 1% glutaraldehyde in 0.05 M Na-cacodylate buffer; postfixed for 2h in 1% OsO₄; dehydrated through a graded acetone series; and embedded with low viscosity Spurr's resin by using flat embedding (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) at the Centro Científico y Tecnológico (CCT CONICET), Bahía Blanca.

Results

Light microscopy

Newly settled androspores had a primary nucleus located in a middle-lateral position and developed a finger-like holdfast (Fig. 1). After settlement, the development of an apical division ring took place and the nucleus migrated to the ring's level (Figs. 2-3). Then, the nucleus underwent mitosis (Fig. 3-4) and only one of the daughter nuclei remained at the apical ring's level, i.e. the future antheridial nucleus (Fig. 4). The other daughter nucleus remained in the centre of the mother cell. Afterwards, the androspore underwent a markedly asymmetric cytokinesis with the first expansion of a division ring (Figs 5-6). As a result, the dwarf male became composed of two uninucleate cells: a basal vegetative, mother cell and an apical antheridial cell (Fig. 7) Then, the antheridial primary nucleus suffered a gametic mitosis and two gametes differentiated inside (Fig. 8). Successive similar, asymmetrical, apical mitoses, expansions of the division ring and cytokineses in the basal cell generated the basipetal development of 3 (4) new antheridial cells (Figs 9-11) with two gametes per antheridial

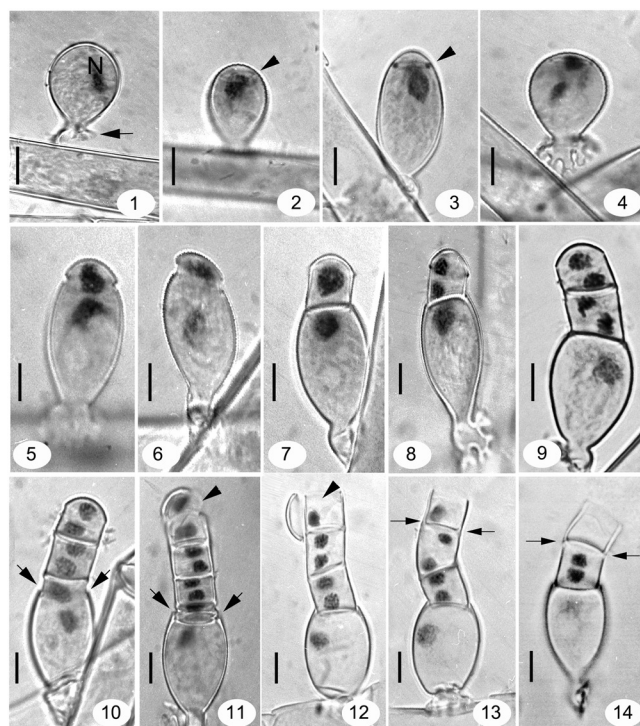


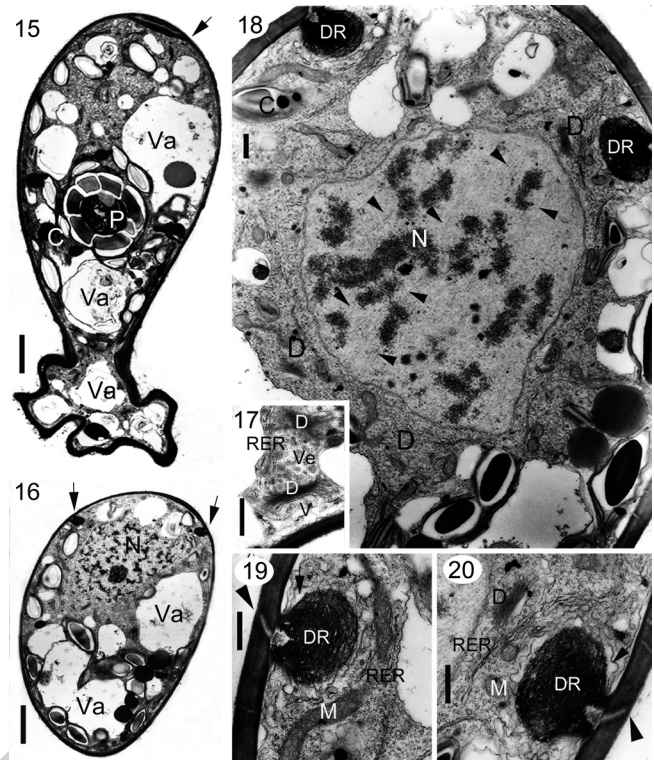
FIGURE 1-14: Light micrographs of *O. macrandrium*'s dwarf males. 1. Newly settled androspore with a primary nucleus located in a middle-lateral position, and a finger-like holdfast. 2-3. Cells with an apical division ring (arrowheads), which is incipient in Fig. 2. Note that the nucleus has migrated to the ring's level. 4. Mitosis has already occurred. Note that only one of the daughter nuclei remained at the ring's level. 5-6. Germinating androspores that are undergoing a markedly asymmetrical cytokinesis. The division rings are partially expanded. 7. The first asymmetrical cytokinesis is completed. The young dwarf male is composed by two uninucleated cells: a basal, vegetative mother cell and an apical antheridial cell. 8. The nucleus of the antheridial cell has undergone mitosis. 9. Dwarf male with two antheridial cells. In the apical one the antheridial mitosis is completed and in the basal one the antheridial mitosis is underway. 10-11. Dwarf males in which a third (10) and a fourth (11) antheridia are forming (arrows). In Fig. 11 the operculum has already opened and one of the gametes of the first antheridium is being released (arrowheads). 12. Dwarf male in which the first mature gamete has been released from the most apical antheridium, after the opening of the operculum (arrowheads). 13-14. Dwarf males where the remaining basal antheridial cells open by the rupture of each dehiscence line (arrows). Scale bars = 5 μ m.

cell. Gametes maturation was basipetal, so that the first mature gametes were those located at the first, most apical antheridial cell. They were released after the opening of an operculum through a dehiscence line (Figs 11-12). The subsequent antheridial cells (2 to 4) successively opened via its own dehiscence line (Figs 13-14).

Transmission electron microscopy

Early germinating androspores showed a nucleus with a nucleolus, large vacuoles and a reticulate chloroplast with a typical pyrenoid (Figs 15-16). The cytoplasm also displayed abundant rough endoplasmic reticulum (RER) and dictyosomes, especially in the holdfast where numerous transition vesicles between the RER and the *cis* face of the dictyosomes were observed. Besides, vesicles with translucent content at the *trans* face of the dictyosomes appeared near the plasmalemma (Fig. 17). The thick, electron-dense cell wall was covered by mucilage (Fig. 15).

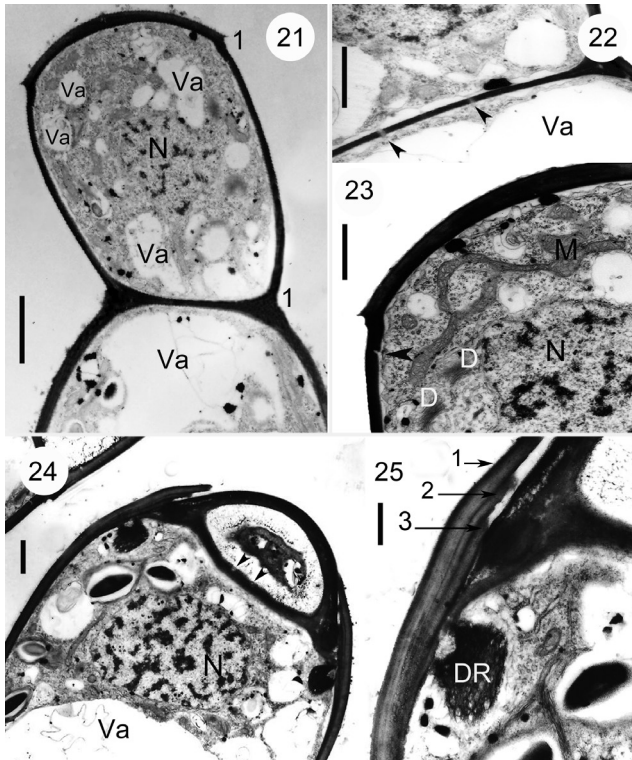
Before the first vegetative mitosis had started, the apical division ring began its development (Figs 15-16). During the first vegetative mitosis the nucleolus disappeared and numerous perinuclear dictyosomes became visible (Fig. 18). At this stage the apical division ring became mature and an apparent cell wall fracture zone appeared in front of it (Figs 19-20). Undulated plasmalemma was seen around the division ring as well as numerous mitochondrial profiles, abundant RER and dictyosomes (Figs 19-20). After the first expansion of the division ring the first cell wall caps were formed (Fig. 21)



FIGURES 15-20: Transmission electron micrographs of *O. macrandrium*. Recently attached dwarf males. 15. General view in longitudinal section. Note the holdfast with numerous vacuoles with concentric membranes. The cell body shows a parietal, reticulate chloroplast with a central pyrenoid, and large vacuoles. The section has not passed through the nucleus. The arrow indicates the developing division ring. Scale bar = 2 μ m. 16. Oblique section not passing through the holdfast. The nucleus has already migrated to the level of the incipient division ring (arrows). Note its abundant heterochromatin and the nucleolus. Scale bar = 2 μ m. 17. Detail of a holdfast's basal portion; numerous transition vesicles between RER and the dictyosomic *cis* face are seen. Note the translucent vesicles near the plasmalemma, at the dictyosomic *trans* face. Scale bar = 0.5 μ m. 18. Longitudinal section. The first mitosis is underway in prometaphase. Arrowheads indicate spindle microtubules. Note perinuclear dictyosomes. The division ring is already fully developed. Scale bar = 0.5 μ m. 19-20. Details of the cross sections of the same fully developed division ring of Fig. 18. Abundant RER, mitochondrion profiles and dictyosomes are close to the ring. Note the undulated plasmalemma around the ring (arrows). The cell wall has already split at the level of the division ring (arrowheads). Scale bars = 0.5 μ m. C, chloroplast; D, dictyosome; DR, division ring; M, mitochondrion; N, nucleus; P, pyrenoid; RER, rough endoplasmic reticulum; V, translucent vesicles; Va, vacuole; Ve, transition vesicles

and after the first cytokinesis the first antheridial cell was delimited (Fig. 21). The mother cell cytoplasm became heavily vacuolated (Fig. 21) and the transverse wall showed plasmodesmata (Fig. 22). The apical transverse wall of the first antheridial cell was the operculum (Figs 21, 23). The dehiscence line of this first antheridial cell was already apparent at the level of a less perceptible vestigial ring before antheridial mitosis had taken place (Fig. 23). The antheridial nucleus lacked nucleolus (Figs 21, 23) and the antheridial cytoplasm showed abundant perinuclear dictyosomes and an anterior reticulate mitochondrion (Fig. 23). Successive similar

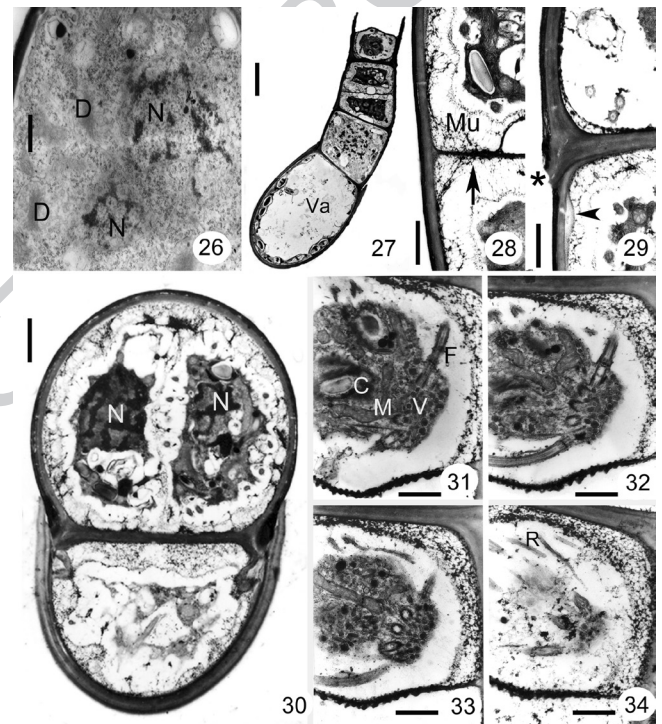
mitoses and cytokines lead to the formation of 2-3 proximal antheridial cells. The basal, lateral cell wall caps resulting from each expansion of the division ring accumulated at the apical end of the mother cell (Figs 24-25).



FIGURES 21- 25: Transmission electron micrographs of *O. macrandrium*'s dwarf males. 21. Dwarf male with one antheridial cell. The antheridial nucleus is in middle position and the cytoplasm shows numerous round vacuoles. The basal, mother cell is conspicuously vacuolated. The numbers indicate the first cell wall caps. Scale bar = 1 μm . 22. Detail of the traverse cell wall between the antheridial cell and the basal cell. The arrowheads pinpoint two plasmodesmata. Scale bar = 0.5 μm . 23. Detail of the anterior portion of a first antheridium that shows the nucleus, a reticulate mitochondrion and dictyosomes. Note that the operculum dehiscence line is already formed (arrowhead) at the level of a vestigial division ring. Scale bar = 0.5 μm . 24-25. Dwarf male where three mitoses and three cytokines have taken place. 24. Note plasmodesmata in the transversal cell wall (arrowheads). Scale bar = 1.5 μm . 25. Detail of 24 that shows (1) that the basal, upward-facing cell wall caps (1-3) are accumulated at the mother cell's apical end after successive division ring expansions and (2) that the division ring is functional meaning that a fourth expansion is underway. Scale bar = 0.5 μm . D, dictyosome; DR, division ring; M, mitochondrion; N, nucleus; Va, vacuole.

During the division of each antheridial nucleus numerous dictyosomes appeared nearby (Fig. 26). After gamete delimitation, mucilage appeared and became more condensed and electron dense between gametes (Figs 27-28). The dehiscence line in each antheridial cell was visible at the level of vestigial division rings (Fig. 29). The gamete long axis was normally parallel to the transverse antheridial walls (Fig. 27) and exceptionally perpendicular to them (Fig. 30).

Mature gametes showed a cytoplasm with a large nucleus without nucleolus (Fig. 30), an anterior reticulate mitochondrion and a reduced chloroplast without pyrenoid (Fig. 31). An apical dome showed numerous spherical, electron-dense vesicles and the flagellar apparatus with 8-10 axonemic basal bodies (Fig. 31-34). Figure 35 illustrates schematically the successive formation of antheridial cells and the successive antheridial cell wall dehiscence.



FIGURES 26-34: Transmission electron micrographs of *O. macrandrium*'s mature dwarf males. 26. Detail of an antheridial cell where recently divided nuclei are seen. Note numerous dictyosomes nearby. Scale bar = 1 μm . 27. Dwarf male with three antheridial cells in different stages of development: in the first apical antheridial cell one gamete has been already released, in the second one both gametes are already mature and in the third one the nucleus has not yet completed the antheridial mitosis. Note the strong vacuolation of the mother cell. Scale bar = 3 μm . 28. Detail of an antheridial cell. The arrow indicates the condensed mucilage between the two gametes. Scale bar = 1 μm . 29. Detail of two contiguous antheridial cells. Note the downward-facing cap in the lateral wall (asterisk) and the vestigial division ring at the level of the wall's dehiscence split (arrowhead). Scale bar = 1 μm . 30. Dwarf male where the basal antheridial cell has the gametes located with their own longitudinal axes parallel to the dwarf male's longitudinal axis. Scale bar = 1 μm . 31-34. Serial, oblique sections of a mature, non-liberated male gamete. The flagellar apparatus is well developed thus, longitudinal cross and oblique sections of basal bodies and axonemes are clearly seen. The apical dome has a great number of electron-dense vesicles; below mitochondrial profiles and a small chloroplast appear. Scale bars = 1 μm . C, chloroplast; D, dictyosome; F, flagellum; M, mitochondrion; Mu, mucilage; N, nucleus; R, microtubular root; Va, vacuole.

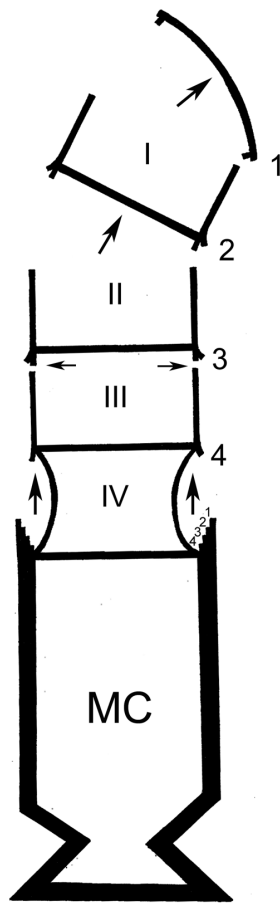


FIGURE 35: Schematic illustration of successive antheridial cell formation and antheridial cell wall dehiscence. Roman numerals (I-IV) pinpoint the successive antheridial cells; large Arabic numerals (1-4) indicate the downward-facing cell wall caps and small ones (1-4) show the accumulated upward-facing cell wall caps. MC, mother cell.

Discussion

This is the first ultrastructural work carried out on the development of multicellular dwarf males with external antheridia in the genus *Oedogonium*. For that reason, the only source of comparison to our results is the ultrastructural work done for similar dwarf males in *Bulbochaete hiloensis* (Pickett-Heaps, 1975).

Ultrastructurally, the developmental process is almost identical to that of dwarf males in *Bulbochaete hiloensis* (Pickett-Heaps, 1975), but with two remarkable differences: in *O. macrandrium*, (1) only condensed mucilage instead of a true transverse wall appeared between both gametes of each antheridial cell. This infrequent characteristic has only been reported in the pseudo-single-celled dwarf males with

in the pseudo-single-celled dwarf males with internal gametangium of *O. pluviale* (Leonardi et al., 1998), and (2) the cell wall between the mother cell and the basal antheridial cell showed simple, vegetative plasmodesmata. In *B. hiloensis*' dwarf males such plasmodesmata are absent (Pickett-Heaps, 1975).

Ultrastructural similarities can also be recognized in dwarf males of both genera: (1) antheridial cell opening for gamete liberation is produced through a dehiscence line formed along a vestigial ring in the antheridial cell lateral cell walls and not along a full developed division ring, and (2) numerous highly active dictyosomes in association with RER cisternae were observed in the basal portion of the holdfast of germinating androspores, near the plasmalemma, producing vesicles with a translucent content. Their localized basal position suggests their involvement in the holdfast development and thus, in the androspore adhesion.

The mitotic plane in antheridial cells in *O. macrandrium* was in most cases transverse to the dwarf-male longitudinal axis, and only exceptionally parallel to it. According to Ohashi (1930), this predominant condition may arise because there is more room for the gametes in nanandrous species, since the antheridial cells are longer than those in species with macrandry. An exception is *O. grande* Kützing, where the vertical plane of division normally occurs, probably due to the space available in the antheridia (Ohashi, 1930; Pickett-Heaps, 1973; 1975).

The mature sperms' dome had numerous small electron-dense vesicles whose function is so far unclear (Pickett-Heaps, 1975). Different functions have been proposed for them (Hoffman 1973), e.g.: (1) the vesicles would contain an adhesive that allows the sperm to adhere to the oogonium or to cells close to it, and (2) the vesicles would contain digestive substances that facilitate pore opening during fertilization, since the oogonial pore is usually occluded by a hyaline material, which disappears when the sperm makes contact with the oogonium. Numerous anterior mitochondrial profiles were also seen in mature gametes close to the basal apparatus. Associations of mitochondria with flagellar apparatuses have been also shown in gametes of *O. cardiacum* (Hassall) Wittrock and *B. hiloensis* (Hoffman,

1973; Pickett-Heaps, 1975) and in numerous flagellate cells of several algal genera (Salisbury et al., 1988; Watanabe and Floyd, 1989; Leonardi et al., 2000).

Acknowledgements

Funds were provided by the Universidad Nacional del Sur (UNS, SGCyT), Grant PGI 24/B151 to EJC. PIL is research member of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina). EJC is research member of CIC (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, República Argentina).

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