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ORIGINAL ARTICLE

The oogonial aperture of *Oedogonium decipiens* var. *decipiens* (Oedogoniales, Chlorophyta) and its systematic significance

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Abstract According to traditional taxonomical criteria, the fertilization aperture in the genus *Oedogonium* has been classified into pore or circumcision type. To contribute with information useful to clarify the systematics of the Oedogoniales, we have studied the oogonial aperture in *O. decipiens* var. *decipiens* showing a circumcision in median position. The aperture mechanism and associated structures (line of weakness and thin layer) are similar to those previously described in *O. cardiacum*. The type and position of the aperture in mature oogonia was also studied in other seven *Oedogonium* species. In all the analyzed species, the fertilization aperture occurs along the line of weakness, but they would differ only in the opening degree of this line. Therefore, the types of oogonial aperture would not be so different as to discriminate groups of species belonging to the genus *Oedogonium*, in agreement with a previous molecular phylogenetic study of this group of algae.

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Introduction

The aperture that allows the passage of anterozoids exhibits a great interspecific variation in the genus *Oedogonium*. According to traditional taxonomical criteria, the aperture has been classified into pore or circumcision (operculate) type in the following positions: supreme, upper, supramedian, median, inframedian, lower and basal (Hirn 1900; Tiffany 1937; Gemeinhardt 1939; Gauthier-Lièvre 1964; Mrozinska 1985). The type of aperture can be clearly differentiated by light microscopy. In *Oedogonium cardiacum* (Hassall) Wittr., with a pore in upper position, studies using scanning electron and transmission microscopy (Hoffman 1971; Coss and Pickett-Heaps 1973; Pickett-Heaps 1975) show that the pore is placed on a line of weakness circumferential to the oogonium, suggesting high similarity with the circumcision type.

To contribute with information that can be used to clarify the systematics of the Oedogoniales, the objectives of this work were to: (a) study the ultrastructure of the fertilization aperture in *O. decipiens* Wittr. var. *decipiens*, which, contrarily to *O. cardiacum*, shows a circumcision in median position; (b) compare the ultrastructure of the oogonial aperture in *O. decipiens* and *O. cardiacum,* for establishing similarities and differences in both the mechanisms and structures involved in the opening; (c) compare the fertilization aperture of the mature oogonia in *O. decipiens* and *O. cardiacum* with those in other *Oedogonium* species showing circumcision or pore in different positions and (d) analyze the hypothesis that the type and position of the oogonial aperture do not define natural groups in the Oedogoniales (Alberghina et al. 2006).

Materials and methods

The strains of *O. decipiens* used in this study were isolated from filaments collected from a puddle in the Province of Entre Ríos, Argentina, (31°S 59°W) in June 1992. The strains were maintained in ad hoc glass flasks containing soil–water biphasic medium, under a continuous light regime at 25°C (Machlis 1962, 1973; Machlis et al. 1974; Starr and Zeikus 1993). The samples were deposited in the culture collection at the Laboratory of Phycology and Experimental Culture of Microalgae (BAFC-FyCE), Department of Biodiversity and Experimental Biology (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires), under the number BAFC-FyCE No 51.

To induce the formation of sexual reproduction structures, the filaments were placed on Petri dishes containing medium A (Hill 1980) with 95% lower nitrogen content (Vélez 1997), and kept under a 12:12 h light:dark regime at 25°C (Singh and Chaudary 1990). The mature oogonia of other *Oedogonium* species, which were collected from freshwater bodies in Argentina, were studied following the methodology described by Alberghina (2004). The species analyzed, their reproductive type and strain reference number of the culture collection BAFC-FyCE are: *Oedogonium acrosporum* De Bary (nannandrous gynandrosporous, 48), *O. nodulosum* Wittr. (macrandrous monoecious, 52), *O. pampeanum* Vélez (nannandrous idioandrosporous, ♀ 53; ♂ 54), *O. pluviale* Nordst. (nannandrous idioandrosporous, ♀ 55; ♂ 56), *O. pringsheimii* Cramer (macrandrous dioecious, 57), *O. rugulosum* f. *rotundatum* Hirn, (nannandrous gynandrosporous, 58), and *O. stellatum* Wittr. (nannandrous gynandrosporous, 59).

The oogonia thus obtained were observed and photographed with a Carl Zeiss "Axioplan" microscope equipped with a MC80 DX camera.

Scanning electron microscopy

Filaments with oogonia were fixed in 2% glutaraldehyde in culture medium at 5°C. Then, they were dehydrated in an acetone series, and dried in a critical-point dryer. Samples were coated with gold prior to examination in a Phillips 515 scanning electron microscope.

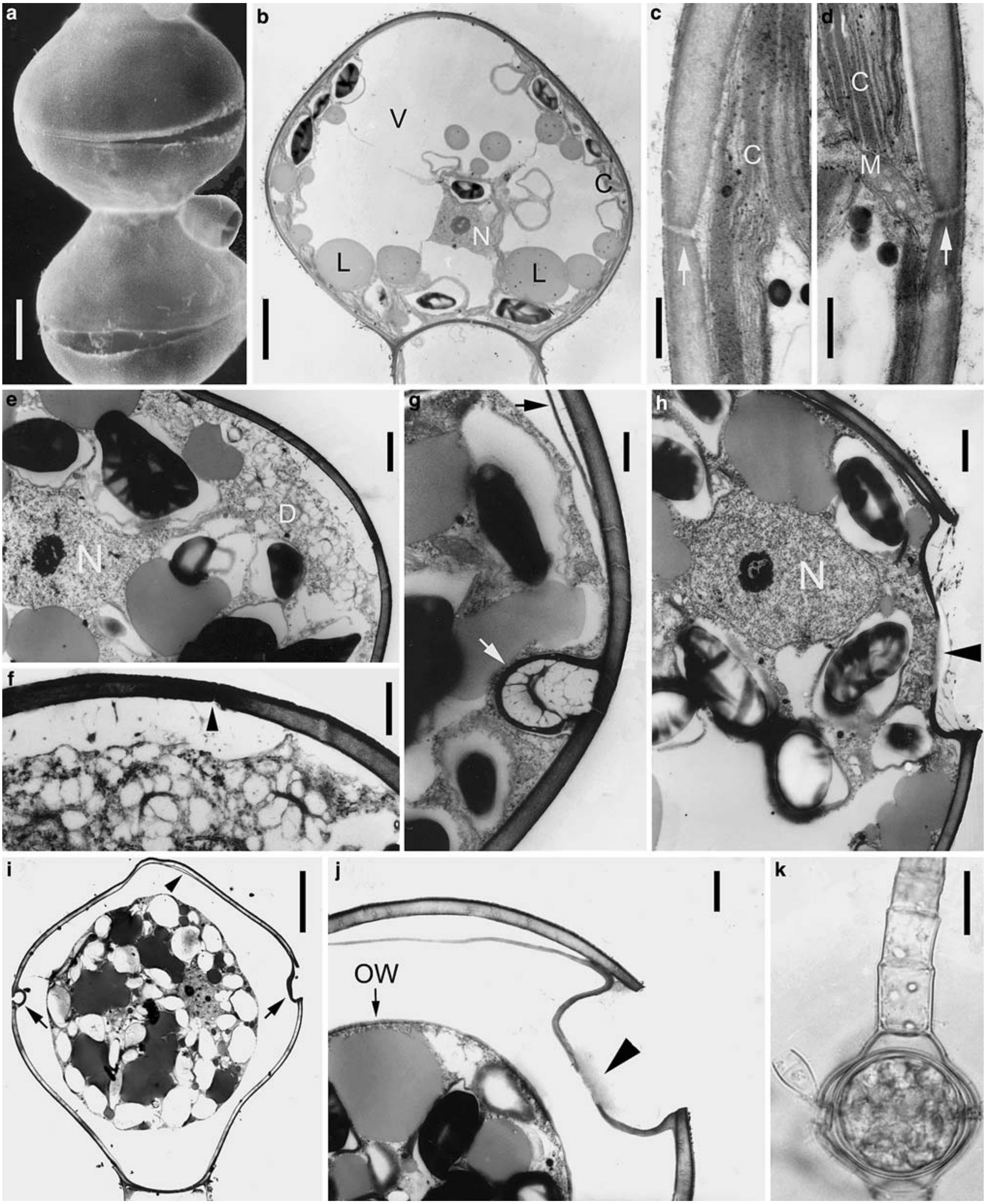
Transmission electron microscopy

Filaments with oogonia showing different degrees of maturation were fixed in 1.5% glutaraldehyde in culture medium at 5°C, postfixed in 1% OsO_4, dehydrated through a graded acetone series, and embedded with Spurr's low-viscosity resin using the flat-embedding method (Reymond and Pickett-Heaps 1983). Ultrathin sections were cut with a diamond knife (Diatome Ltd, Bienne, Switzerland) using 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, Tokyo, Japan) at the Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca (CRIBABB).

Fig. 1 Oogonial aperture of *Oedogonium decipiens* var. *decipiens*. **a** SEM micrograph of two oogonia in series with a nannandrous male. Note the circumcision in median position. *Scale bar* 5 µm. **b–j** TEM micrographs. **b** General view of young oogonium. *Scale bar* 4 µm. **c, d** Details of the line of weakness recently formed (*arrows*). In **d** the mitochondrial profile becomes close to one side of the line. *Scale bar* 0.5 µm. **e** General view of the wall weakening region. Numerous dictyosomes appear next to this region. The nucleus is located laterally at the level of this line. *Scale bar* 1 µm. **f** Detail of **e**. Dictyosomes are secreting hyaline material at the thinnest part of the wall, under the future rupture site (*arrowhead*). *Scale bar* 0.5 µm. **g** Detail of a doughnut–shaped ring (*white arrow*). The *black arrow* shows the thin layer surrounding the oogamete. *Scale bar* 0.5 µm. **h** Oogamete with the nucleus next to the circular foramen of the thin layer (*arrowhead*). *Scale bar* 1 µm. **i** General view of a zygote. The *arrows* indicate the circumcision, which is widened on the fertilization site. The thin layer is adjacent to the oogonial wall (*arrowhead*). *Scale bar* 4 µm. **j** Detail of an oospore under development. The *arrowhead* shows the foramen of the thin wall. *Scale bar* 1 µm. **k** Light micrograph of an oogonium with a mature oospore surrounded by the smooth wall. *Scale bar* 10 µm. *C* chloroplast, *D* dictyosome, *L* lipid droplet, *M* mitochondrium, *N* nucleus, *OW* oospore wall ▸

Results

In *Oedogonium decipiens* var. *decipiens*, a nannandrous and gynandrosporous species, the oogonium is arranged as a single structure or in series of 3 (Fig. 1a). The young oogonium is strongly vacuolated and has a central nucleus, a parietal chloroplast and abundant reserves (Fig. 1b). As the oogonium matures, a line of weakness resembling a delicate circumferential fracture can be distinguished in the median portion of the wall (Fig. 1c, d). The chloroplast retains its morphology and mitochondrial profiles become close to the line of weakness (Fig. 1d). The nucleus is located laterally at the level of this line (Fig. 1e), and numerous dictyosomes, adjacent to the wall weakening, produce hyaline material (Fig. 1e, f). The secretion process continues steadily, while building up a substantial doughnut-shaped ring attached to the cell wall at its outer edge (Fig. 1g). The resulting structure is called thin layer (sensu Pickett-Heaps 1975), which, in the studied species, surrounds the oogamete entirely. The thus formed aperture



is widened on one side (Fig. 1i), where the thin layer becomes exposed and the opening of a circular foramen allows the entrance of the antherozoid (Fig. 1j). After fertilization, the thin layer is adjacent to the oogonial wall (Fig. 1i, j) and remains this way throughout oospore development (Fig. 1k). The spores, which have a subdepressed or depressed-globose shape, exhibit a smooth wall (Fig. 1k).

Oogonial aperture (type and position) in mature oogonia of other *Oedogonium* species

The oogonial aperture (type and position) in mature oogonia of other *Oedogonium* species was also analyzed with light and electron microscopy (Fig. 2a–r).

Oedogonium acrosporum De Bary var. *acrosporum* shows a circumcision type of aperture in supreme position (Fig. 2a–c). The oogonium is solitary, terminal and ellipsoidal; the oospore is ellipsoidal, its cell wall has longitudinal ridges anastomosed at different sites and occupies almost the entire oogonium (Fig. 2a). There is a deciduous and small operculum in the aperture region [scanning electron microscopy (SEM); Fig. 2b]. The operculum is opened by the expansion of a semi-spherical dense mass of mucilage lying over the electron-dense thin layer, which is adjacent to the oogonial wall [transmission electron microscopy (TEM); Fig. 2c].

Oedogonium nodulosum Wittr. var. *nodulosum* shows a circumcision type of aperture in upper position (Fig. 2d–f). The oogonium is solitary or in pairs, somewhat obovoid-globose, rarely obovoid-ellipsoidal; the oospore is globose or subglobose, rarely globose-ellipsoidal, has a smooth cell wall and occupies almost the entire oogonium (Fig. 2d). The thin layer can be observed through the circumcision of the oogonium (SEM; Fig. 2e); it forms a tube-like structure (TEM; Fig. 2f).

Oedogonium pluviale Nordst. shows a circumcision type of aperture in upper position (Fig. 2g). The oogonium is solitary, rarely in series of 2–3, subellipsoidal, subobovoid-globose or subglobose; the oospore is sub-globose or subellipsoidal-globose, has a smooth cell wall and occupies almost the entire oogonium (Fig. 2g). The circumcision in the oogonium is longer than that of *O. nodulosum* Wittr. var. *nodulosum* (SEM; Fig. 2h). The thin layer becomes exposed and the opening of a circular foramen allows the entrance of the antherozoid. The foramen is occluded by a mucilaginous plug after fertilization (SEM; Fig. 2h).

Oedogonium pringsheimii Cramer var. *pringsheimii* shows a circumcision type of aperture in upper position (Fig. 2i, j). The oogonium is solitary, rarely in series of 2-6, subobovoid-globose or subpyriform; the oospore is globose, has a smooth cell wall and occupies almost the entire oogonium (Fig. 2i). The thin layer can be observed through the circumcision of the oogonium (SEM, Fig. 2j); it forms a tube-like structure (not shown) as in *Oedogonium nodulosum* Wittr. var. *nodulosum*.

Oedogonium rugulosum f. *rotundatum* Hirn shows a circumcision type of aperture in upper position (Fig. 2k–m). The oogonium is solitary, obovoid or ellipsoidal; the oospore is subglobose, globose or ellipsoid-globose, has a smooth cell wall and occupies almost the entire oogonium (Fig. 2k). The thin layer, exposed through the circumcision, undergoes the opening of a circular foramen which allows the entrance of the antherozoid (SEM; Fig. 2l). In anterior view, a line of weakness circumferential to the oogonium is observed prior to the opening of the aperture (SEM; Fig. 2m).

Oedogonium pampeanum Vélez shows a circumcision type of aperture in lower position, as opposed to the species mentioned above (Fig. 2n, o). The oogonium is solitary, rarely in pairs, subglobose or globose; the oospore is globose, has a smooth cell wall and occupies almost the entire oogonium (Fig. 2n). The wall of the oogonium forms a slight protrusion located under the circumcision (SEM; Fig. 2o). The thin layer thus exposed, undergoes the opening of a circular foramen which allows the entrance of the antherozoid (SEM; Fig. 2o).

Oedogonium stellatum Wittr. shows a pore type of aperture in upper position (Fig. 2p–r). The oogonium is solitary or in series of 2–3, subobovoid or subpyriform-globose; the oospore is globose, has a cell wall with four to seven spiral ridges and occupies nearly the entire or the entire oogonium (Fig. 2p). A pore placed on a line of weakness circumferential to the oogonium is observed in anterior view (SEM; Fig. 2q). In cross-section, the cell wall of the mature oogonium shows a pore type of aperture; the thin layer has a circular foramen allowing the entrance of the antherozoid (TEM; Fig. 2r).

Discussion

The ultrastructural observations reported here reveal that in *O. decipiens* var. *decipiens*, like in *O. cardiacum*, a line of weakness occurs in the form of a delicate circumferential fracture during the oogonial development.

The dictyosomes participate in the synthesis of fibrous hyaline material placed adjacent to the wall weakening. Hoffman (1971, 1973) stated that in *O. cardiacum* this material is related to the chemical rupture of the oogonial wall during the process of opening, and that the pore formation is related to alterations in the chemical composition of the oogonial wall. Instead, Pickett-Heaps (1975) claimed that in *O. cardiacum*, the oogonial wall has a predetermined rupture site (wall weakening), with polysaccharides being secreted at the site of pore formation. The description made by Retallack and Butler (1973) of the pore formation mechanism during the oogenesis of *Bulbochaete hiloensis* (Nordst.) Tiffany was consistent with that of Pickett-Heaps (1975), and according to our results, this mechanism also seems to work for *O. decipiens*.

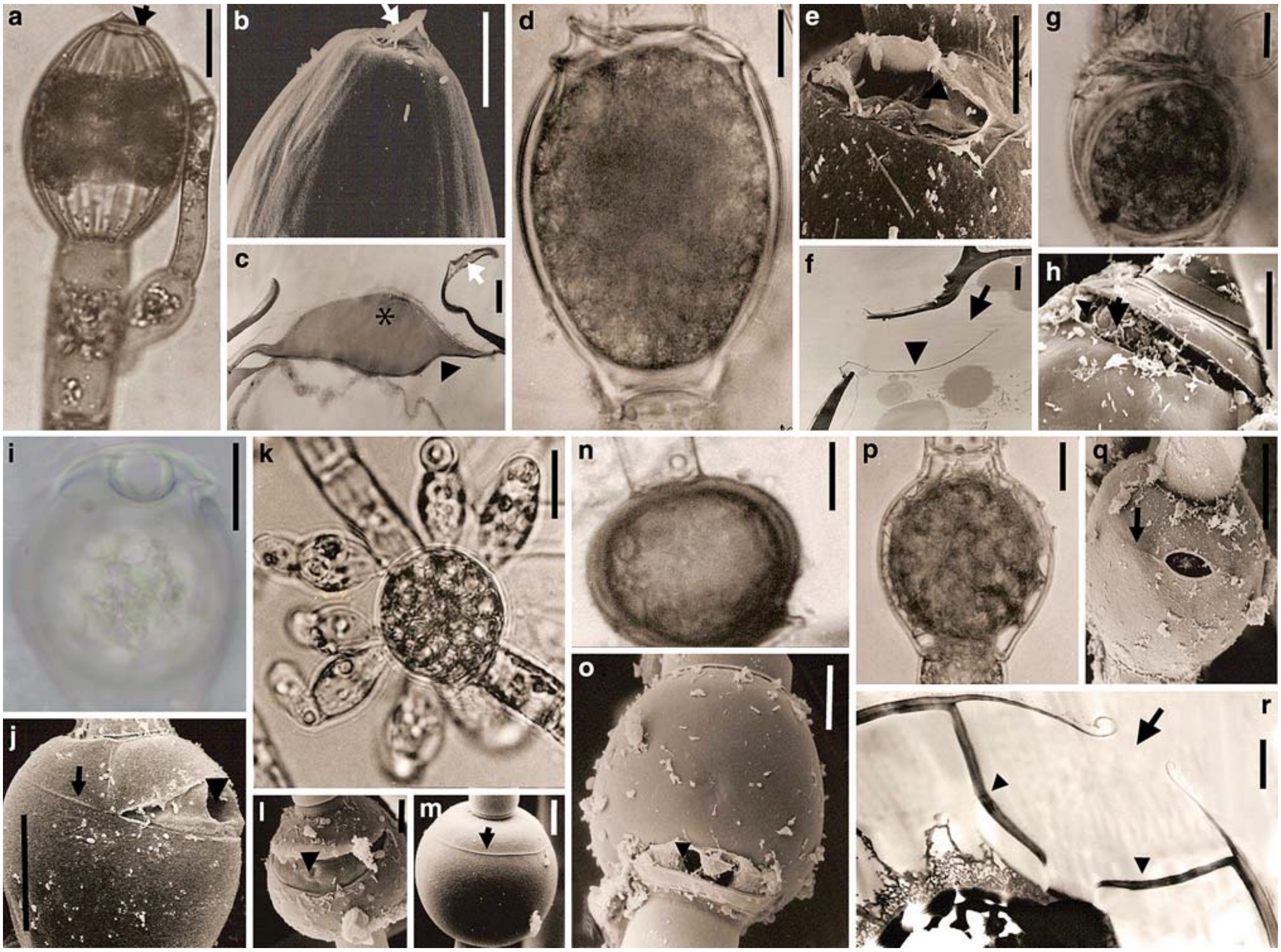


Fig. 2 *Oedogonium* species with circumcision type of oogonial aperture in different positions and with pore in upper position. **a–c** *Oedogonium acrosporum* De Bary var. *acrosporum*. **a** Light micrograph of a mature oogonium and nannandrous male filament. The operculum is still closed (*arrow*). **b** SEM micrograph showing detail of the aperture in supreme position. The operculum is indicated by *white arrow*. **c** TEM micrograph showing detail of the aperture in a mature oogonium. The thin layer is indicated by *arrowhead*, the operculum by *white arrow*, and the mucilage by *asterisk*. *Scale bar* 10 µm. **d–f** *Oedogonium nodulosum* Wittr. var. *nodulosum*. **d** Light micrograph of a mature oogonium. **e** SEM micrograph showing detail of the upper aperture. The entrance to the tube-like structure formed by the thin layer is indicated by *arrowhead*. *Scale bar* 10 µm. **f** TEM micrograph showing the tube-like structure. The thin layer is indicated by *arrowhead*, and the foramen at the end of the tube-like structure by *arrow*. *Scale bar* 2 µm. **g, h** *O. pluviale* Nordst. **g** Light micrograph of a mature oogonium. **h** SEM micrograph showing detail of the aperture. The thin layer (*arrowhead*) has a mucilaginous plug (*arrow*). *Scale bar* 10 µm. **i, j** *O. pringsheimii* Cramer var. *pringsheimii*. **i** Light micrograph of a mature oogonium. **j** SEM micrograph of a mature oogonium. The entrance to the tube-like structure formed by the thin layer is indicated by *arrowhead* and the line of weakness by *arrow*. *Scale bar* 10 µm. **k–m** *O. rugulosum f. rotundatum* Hirn. **k** Light micrograph of a mature oogonium. The oogonium aperture is not visible due to the presence of the nannandrous male. **l** SEM micrograph of a mature oogonium. The thin layer (*arrowhead*) has a circular foramen. **m** SEM micrograph, anterior view, a line of weakness circumferential to the oogonium is observed prior to the opening of the aperture (*arrow*). *Scale bar* 10 µm. **n, o** *Oedogonium pampeanum* Vélez. **n** Light micrograph of a mature oogonium with lower circumcision. **o** SEM micrograph of a mature oogonium. The thin layer (*arrowhead*) has a circular foramen. *Scale bar* 10 µm. **p–r** *Oedogonium stellatum* Wittr. **p** Light micrograph of a mature oogonium. **q** SEM micrograph of a mature oogonium with pore in *upper* position. The line of weakness is indicated by *arrow*. *Scale bar* 10 µm. **r** TEM micrograph showing detail of the aperture in a mature oogonium. The oogonium cell wall shows a pore (*arrow*). The thin layer is indicated by *arrowheads*. *Scale bar* 2 µm

In *O. decipiens*, with a circumcision type of oogonial aperture in a median position, we observed the presence of a thin layer that has already been mentioned for *O. cardiacum.* However, in *O. decipiens* the thin layer surrounds the oogamete, while in *O. cardiacum* it is attached to either side of the rupture site in the oogonial wall (Pickett-Heaps 1975). In *O. decipiens*, the aperture mechanism and associated structures (line of weakness and thin layer) are similar to those described by Pickett-Heaps for *O. cardiacum*. In both species the fertilization aperture occurs along the line of weakness, but they may only differ in the opening degree of the line of weakness. Thus, *O. decipiens* shows a circumferential aperture (circumcision type) and *O. cardiacum* a reduced aperture (pore type).

Only mature oogonia were considered for the other *Oedogonium* species studied in the present paper. *O. stellatum* Wittr shares an upper pore with *O. cardiacum* but differs in the position of the thin layer. In *O. cardiacum* the thin layer is attached to either side of the rupture site in the oogonial wall (Pickett-Heaps 1975), whereas in *O. stellatum* Wittr. it is adjacent to the oogonial wall and surrounds the oogamete. In the species with the circumcision type of aperture, namely *O. acrosporum* De Bary (supreme position), *O. nodulosum* Wittr., *O. pluviale* Nordst., *O. pringsheimii* Cramer, *O. rugulosum f. rotundatum* Hirn (upper position), and *O. pampeanum* Vélez (lower position), the thin layer is likely to be adjacent to the oogonial wall and to surround the oogamete. In addition, the thin layer shows a circular foramen allowing the entrance of the anterozoid. *Oedogonium nodulosum* and *O. pringsheimii* Cramer differ slightly from the other species in that the thin layer forms a tube-like structure with the circular foramen located at its end.

As in *O. decipiens* and *O. cardiacum*, the fertilization aperture occurs along the line of weakness, but there may be differences in the opening degree of this line. On this basis, *Oedogonium acrosporum* De Bary, *O. nodulosum* Wittr., *O. pampeanum* Vélez, *O. pluviale* Nordst., *O. pringsheimii* Cramer, and *O. rugulosum* f. *rotundatum* Hirn show a circumferential aperture (circumcision type) and *O. stellatum* a reduced aperture (pore type).

In a molecular phylogenetic study addressing the position of the Oedogoniales within the green algae, Alberghina et al. 2006 performed the mapping of morphological characters, including type and position of the oogonial aperture, for different *Oedogonium* species. The authors found that *Oedogonium* species were not clearly grouped according to type and position of the oogonial aperture, suggesting that these characters do not define natural groups. The present study compared these characters among several species of *Oedogonium* from an ultrastructural point of view. As a result, it became clear that the aperture mechanism and the associated morphological structures are highly similar in species having both pore and circumcision types of aperture. Consequently, the division of *Oedogonium* species into the pore and circumcision types recognized by classical taxonomy (Hirn 1900; Tiffany 1937; Gemeinhardt 1939; Gauthier-Lièvre 1964; Mrozinska 1985), would not be supported by molecular and ultrastructural data. However, it is necessary to study a large number of species using these approaches to perform a comprehensive revision of the systematics of the Oedogoniales group.

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