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1 **Ultrastructural observations of mitochondrial morphology through the life cycle of the**
2 **brown alga, *Mutiomo cylindricus* (Cutleriaceae, Tilopteridales)**

3

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27 **Abstract**

28 Mitochondrial morphology varies according to development and the physiological conditions of
29 the cell. Here, we performed electron tomography using serial sections to analyze the number,
30 individual volume, and morphological complexity of mitochondria in the cells across two
31 generations in the life cycle of the brown alga *Mutimo cylindricus*. This species shows a
32 heteromorphic alternation of generations between the macroscopic gametophyte and the
33 crustose sporophyte during its life cycle and displays anisogamous sexual reproduction. We
34 observed the mitochondria in the vegetative cells of gametophytes and sporophytes to mainly
35 show tubular or discoidal shapes with high morphological complexity. The morphology of the
36 mitochondria in the male and female gametes changed to a nearly spherical or oval shape from a
37 tubular or discoidal shape before release. In this species, degradation of the paternal
38 mitochondria was observed in the zygote 2 h after fertilization. Morphological changes in the
39 mitochondria were not observed until 6 h after fertilization. Twenty-four-hour-old zygotes
40 before and after cytokinesis showed a similar number of mitochondria as six-hour-old zygotes;
41 however, the volume and morphological complexity increased. The results indicated that the
42 maternal mitochondria did not undergo fission or fusion until this stage. Based on the analysis
43 results of the number and total volume of mitochondria before and after the release of the
44 gametes, it is possible that the mitochondria in the female gametes fuse immediately before
45 release.

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47

48 **Keywords**

49 Brown algae · electron microscopy · life cycle · mitochondria · serial section tomography

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51

52 **Introduction**

53 Mitochondria are membrane-bound organelles with their own DNA and supply
54 adenosine triphosphate (ATP), which is used as a source of chemical energy in cell activity. It is
55 believed that morphological characteristics of the mitochondria, such as shape, size, and number,
56 change throughout the life of a cell. This suggests that, morphologically, mitochondria vary
57 depending on development, growth, differentiation, apoptosis, maturation, and aging (Chan
58 2012).

59 In unicellular organisms, following changes in mitochondrial morphology throughout
60 the life cycle is comparatively easier, and in some cases, the life cycle may directly indicate the
61 cell cycle. *Cyanidioschyzon merolae* possesses a single mitochondrion whose division, along
62 with chloroplast division, precedes nuclear division. The mitochondria change their morphology
63 into spherical, ovoid, and dumbbell-shaped structures before division and return to a spherical
64 shape after division (Kuroiwa 2010; Yoshida et al. 2017). In the unicellular green alga
65 *Chlamydomonas reinhardtii*, mitochondria form a tubular network toward asexual reproduction,
66 and mitochondria in the liberated daughter cells exhibit a giant form and, subsequently, a thick-
67 and thin-corded form (Ehara et al. 1995). However, during meiosis after conjugation, the
68 mitochondrial morphology exhibits a continuous change into spherical, tubule-, branch-, and
69 mesh-like structures (Aoyama et al. 2009).

70 In multicellular organisms, almost all studies relating to mitochondrial morphology
71 have focused on a specific developmental period, such as gametogenesis, fertilization, or early
72 embryonic development. Mitochondria in animal sperm exhibit characteristic features and
73 maternal inheritance (Sato and Sato 2013). Morphological changes and the transition of mtDNA
74 content during spermatogenesis can serve as a key to understanding the mechanism of maternal
75 inheritance of this organelle. In mammals, to prevent the transmission of paternal mtDNA to the
76 progeny, the number of nucleoid mitochondria gradually decreases during sperm formation
77 (Rantanen and Larsson 2000). In addition, a drastic morphological transformation of the

78 mitochondria are observed during spermatogenesis. The mitochondria in spermatogonia,
79 leptotene, and zygotene spermatocytes are generally small and spherical. They elongate in
80 pachytene spermatocytes and early spermatids, and fragment again in late spermatids (De
81 Martino et al. 1979). In maturing spermatids, small mitochondrial spheres are regularly arranged
82 across the midpiece, and they elongate as they wrap around the midpiece (Ho and Wey 2007).
83 In the early stage of spermatogenesis in rabbits, mitochondrial cristae have been observed to
84 become highly dilated during the transition phase between spermatogonia and primary
85 spermatocytes (Nicander and Ploen 1969).

86 In a previous study, we clarified paternal inheritance of mitochondria in the
87 anisogamous brown alga *Mutimo cylindricus* (Shen et al. 2020). We concluded that maternal
88 inheritance of mitochondria could be universal in brown algae. In the case of *M. cylindricus*,
89 observations of ultrathin sections indicated that the size of the mitochondria in the male and
90 female gametes was almost identical. However, the average DNA content of one mitochondrion
91 in male gametes was one-seventh of that in female gametes. Mitophagic digestion of paternal
92 mitochondria occurs in zygotes. One of the mechanisms in uniparental inheritance of
93 mitochondria in this species is the reduction of mitochondrial DNA in male gametes. However,
94 the mechanism underlying the formation of robust mitochondria in female gametes is unclear.

95 All species of brown algae show multicellularity. Some of them alternate between the
96 generation of diploid sporophytes and haploid gametophytes. *M. cylindricus* is an anisogamous
97 species with a heteromorphic life cycle alternating between macroscopic gametophytes and
98 microscopic sporophytes (Kitayama et al. 1992; Kawai et al. 2012). In this study, we examined
99 mitochondrial morphology in gametophytes, gametangia, gametes, zygotes, and sporophytes
100 through 3D analysis using transmission electron microscopy (TEM) on serial section images. In
101 brown algae, 3D analysis has been performed using electron tomography (Terauchi et al. 2012;
102 Fu et al. 2013; Nagasato et al. 2014; Kinoshita et al. 2016). Those studies revealed the structure
103 of the plasmodesmata, transient membrane configuration during cytokinesis, and flagella. The

104 present study is the first to demonstrate the 3D reconstruction of a structure using serial sections,
105 focusing on the structure of brown algal mitochondria. These data enabled quantification of
106 mitochondria in each target stage.

107

108 **Material and Methods**

109 **Culture**

110 Mature male and female gametophytes of *M. cylindricus* (Okamura) H. Kawai and T.
111 Kitayama (Kawai et al. 2012) were collected at Odanohama, Toba, Mie, Japan (34°45' N
112 136°87' E), in March 2015 and April 2019. Male and female individuals were identified based
113 on the morphology of the plurilocular gametangia under a light microscope. Unialgal cultures of
114 *M. cylindricus* were established from gametes or trichothallic hairs of male and female
115 gametophytes (Shen et al. 2020) and incubated in sterilized seawater containing half-strength
116 Provasoil's enriched seawater medium (Provasoil 1968). Gametogenesis and maturation were
117 induced under the following conditions: 15 °C, using white-light-emitting diodes (LEDs, 20–40
118 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and long-day conditions (14 h light:10 h dark). The release of gametes
119 was stimulated by exchanging the fresh culture medium the day before and by light the next day.
120 Male and female gametes were collected separately in microtubes on ice. Fertilization was
121 induced by adding male gametes to settled female gametes on a gel support film (ATTO, Tokyo,
122 Japan) in Petri dishes.

123

124 **Cryofixation and freeze-substitution for TEM samples**

125 Rapid freezing and freeze substitution by the immersion method were applied to the
126 male and female gametes, 2-, 6-, and 24-h-old zygotes, and two-, four-, and seven-celled
127 sporophytes on the gel support film. The gametes were collected as pellets following
128 centrifugation at $500 \times g$ for 1 min using a KUBOTA model 4000 centrifuge (KUBOTA, Tokyo,
129 Japan). The pellets were then placed and spread on a formvar-coated gold ring immediately

130 before freezing. The gel support films containing the just fertilized and developing specimens
131 were cut into triangular shapes $< 1 \text{ cm}^2$ (Nagasato and Motomura 2002). The gametes on the
132 gold rings, or early zygotes on the gel support films, were rapidly immersed in a pre-cooled
133 liquid propane bath ($-186 \text{ }^\circ\text{C}$) and subsequently transferred into liquid nitrogen. Gametophytes
134 and 20-d-old crustose sporophytes were frozen using high-pressure freezing. Thalli fragments
135 were cut, placed into gold carriers, frozen using a high-pressure freezer (Leica Microsystems,
136 Vienna, Austria) at $-196 \text{ }^\circ\text{C}$ and 2,100 bar, and then stored in liquid nitrogen.

137 Freeze-substitution was performed by transferring the frozen samples from liquid
138 nitrogen to a freeze-substitution medium (2% osmium tetroxide [OsO_4] dissolved in anhydrous
139 acetone) pre-cooled in a liquid nitrogen cooling bath, and the samples were stored at $-85 \text{ }^\circ\text{C}$ for
140 2 d. The samples were gradually warmed to $-20 \text{ }^\circ\text{C}$ for 2 h, to $4 \text{ }^\circ\text{C}$ for 2 h, and then to room
141 temperature for approximately 30 min. Next, the samples were washed several times with
142 anhydrous acetone at room temperature and infiltrated with increasing concentrations (10%,
143 30%, 50%, 70%, 80%, 90%, and 100%) of low-viscosity Spurr's epoxy resin (Polysciences,
144 Warrington, PA, USA). Finally, the samples were embedded in Spurr's epoxy resin in
145 aluminum foil dishes.

146 Serial sections were cut using a NACC diamond knife (Micro Star Technologies,
147 Huntsville, TX, USA) with an ULTRACUT ultramicrotome (Reichert-Jung, Vienna, Austria),
148 mounted on formvar-coated slot grids, and stained using an EM stainer (Nisshin EM, Tokyo,
149 Japan). The sections were observed using a JEM-1011 electron microscope (JEOL, Tokyo,
150 Japan). Consecutive serial sections of each sample were examined. Images were acquired using
151 TEM films (Fujifilm, Tokyo, Japan). The developed films were scanned and converted into
152 digital data using an EPSON GT-X980 photo scanner (EPSON, Nagano, Japan) and images
153 were standardized (tiff format, resolution 300 dpi, 8-bit grayscale).

154

155 **Three-dimensional reconstruction**

156 Alignment of whole cell serial section images was performed using Fiji software
157 (<http://fiji.sc/Fiji>, Lowe 2004; Schindelin et al. 2012; Murtin et al. 2018). The alignment method
158 for the serial sections was modified from that described in previous studies. Stacks were made
159 using two adjacent (source and target) images, and feature extraction was automatically
160 performed using the scale-invariant feature transform (SIFT) algorithm. The images were
161 aligned using the “Linear Stack Alignment with SIFT” plugin. The stack was converted into
162 images, the transformed target image was treated as a new source image, and the next section
163 was aligned as the target image.

164 For the 3D reconstruction of whole-cell serial section images, the sequence of
165 transformed images was converted into stacks using the IMOD software package (version
166 4.9.12) (Kremer et al. 1996). The pixel sizes of several standardized section images were
167 obtained using the “set scale” Fiji plugin according to the scale bar in each image
168 (Supplementary Table S1). The thickness range of the sections was estimated based on the
169 interference color of the ultrathin sections (Peachey 1958). Therefore, it was impossible to set
170 the z-axis value, the ratio of section thickness (nm) to pixel size (nm), in the stacking of serial
171 section images as accurately as in electron tomography. For this reason, the thickness was
172 calibrated using spherical organelles, such as small vacuoles (Cui et al. 2019). Finally, the mean
173 thickness (approximately 100 nm) of each gold serial section was determined (Supplementary
174 Fig. S1a, b). The 3D models were generated from the outlines of each object in the serial
175 sections along the z-axis. The volumes and surface areas of individual intracellular structures
176 (nuclei, mitochondria, plasma membrane) were obtained from the IMOD software and
177 calculated using the program IMODINFO.

178 The mitochondrial complexity index (MCI) is a score between the mitochondrial
179 surface area (SA) and volume (V) and is associated with mitochondrial shape complexity
180 (Vincent et al. 2019). Based on 3D modeling of mitochondria, we calculated the index
181 according to a previously reported formula: $MCI = SA^3/16\pi^2V^2$ (Vincent et al. 2019). MCI is

182 0.71 for a spherically shaped mitochondrion and > 0.71 for an ellipsoid or tubular shape. A
183 higher value indicates greater elongation or branching of the mitochondrion.

184

185 **Statistical analysis**

186 All data shown are derived from three cells, with the exception of two- (24-h-old
187 zygotes) or four-celled structures (four daughter cells from two 24-h-old zygotes after
188 cytokinesis), from independent biological samples in each life-cycle stage (Supplementary
189 Table S1; the corresponding 3D images are shown in Supplementary Fig. S2). All results are
190 presented as mean \pm standard error of the mean (SEM). Significant differences among different
191 stages of the life cycle were determined using Tukey's multiple comparison test (Supplementary
192 Table S2). Statistical tests were performed using GraphPad Prism software (version 8.0.2,
193 GraphPad Software, Inc., San Diego, CA, USA).

194

195 **Results**

196 **Biological material used in this study**

197 *M. cylindricus* possesses a heteromorphic life cycle alternating between the
198 macroscopic haploid gametophytic generation and microscopic diploid sporophytic generation
199 (Kitayama et al. 1992; Kawai et al. 2012; Supplementary Fig. S3). Male and female plants were
200 independent, namely dioecious (Supplementary Fig. S3a, b). Gametophytes are parenchymatous,
201 and mature plants have sori with many plurilocular gametangia (Supplementary Fig. S3c, d).
202 Male gametophytes possess long, pale gametangia that produce male gametes, and female
203 gametophytes exhibit short, dark brown gametangia that produce female gametes
204 (Supplementary Fig. S3c–f). The cultured gametophytes established from gametes or
205 trichothallic hairs of the materials from the field sample showed uniseriate filaments, and
206 gametangia were directly formed on the filaments. Observation of the mitochondrial structure in
207 gametophyte cells was performed using the cultured materials. Supplementary Fig. S3g, h show

208 a 6-h-old zygote and a two-celled sporophyte, respectively. Two orange eyespots derived from
209 male and female gametes remained. One 20-d-old crustose sporophyte is shown in
210 Supplementary Figure S3i.

211

212 **Mitochondrial morphology in gametophytes**

213 We first observed the ultrastructure of uniseriate filamentous male and female
214 gametophytes (Fig. 1, Supplementary Movie S1, S2). The nucleus was generally located in the
215 middle part of the cell (Fig. 1a, b), and chloroplasts and mitochondria were positioned in the
216 perinuclear or pericytoplasmic regions by occupation of large vacuoles (Fig. 1a, b).

217 Reconstruction of 3D images was performed by tracing the outline of the nucleus, mitochondria,
218 and plasma membrane in the male and female gametophytes. Most mitochondria at this stage
219 were tubular, curved, V-, or Y-shaped (Fig. 1c–f). The mitochondria were not branched and did
220 not form interconnection networks.

221 We calculated the total number and total and mean volumes of mitochondria from three
222 cells in each of the male and female gametophytes (Supplementary Table S1). There were 50,
223 83, and 117 mitochondria in the male gametophytes and 42, 59, and 104 in the female
224 gametophytes. In the male gametophytes, the cells with the lowest number of mitochondria
225 exhibited the highest total mitochondrial volume in the cell (“Male_Gametophyte_3” in
226 Supplementary Table S1) because of variation in the mean volume of mitochondria in each
227 male gametophyte (Supplementary Table S1). The mean values of the mitochondrial volume in
228 the female gametophytes were not significantly different in each cell (Supplementary Table S1);
229 therefore, the number of mitochondria reflected the total volume of the mitochondria in the cell.
230 In this study, mitochondrial complexity was calculated to understand mitochondrial morphology
231 (Vicent et al. 2019). The analysis showed that the mitochondria in each cell of the
232 gametophytes exhibited considerable differences from spherical or elliptical geometries
233 (Supplementary Table S1).

234

235 **Mitochondrial morphology in mature gametangia**

236 Next, we examined mitochondrial morphology in mature male and female gametangia
237 (Fig. 2, Supplementary Movie S3, S4). Mitochondrial morphology and distribution were
238 determined through 3D analysis using three cells each of the male and female gametangia. In
239 the mature male gametangia, two flagella were present and an eyespot was observed within the
240 chloroplast adhered to the nucleus (Fig. 2a). Most mitochondria in the mature male gametangia
241 showed a discoid shape (Fig. 2b, c). Similarly, the flagella and eyespots were also seen in the
242 cells of mature female gametangia, and most mitochondria were tubular in shape with some
243 discoid (Fig. 2d–f). In the cells of male and female gametangia, some mitochondria were
244 located around the nucleus, while others were scattered in the cytoplasm. The number of
245 mitochondria examined in this study is summarized in Supplementary Table S1. Although the
246 number of mitochondria is different in each cell, the variability in the numbers was not as large
247 as in the gametophytes.

248 The average number of mitochondria in the cells of male and female gametangia was
249 approximately 6 and 38, respectively. The difference in the total volume of the mitochondria in
250 male or female gametangium was less compared to that observed in the gametophytic cells. In
251 addition, the average mitochondrial volume in the male or female gametangia was
252 approximately the same. The mitochondria in the cells of the male gametangia were slightly
253 larger ($0.162 \pm 0.013 \mu\text{m}^3$) than those in the cells of the female gametangia ($0.133 \pm 0.004 \mu\text{m}^3$);
254 however, there were no significant differences in the mitochondrial shape (Supplementary Table
255 S2), as indicated by the MCI, between the male (1.682 ± 0.091) and female (1.852 ± 0.074)
256 gametangia. The average MCI in the gametangia was lower than that in the gametophytes (male
257 gametophytes, 4.848 ± 0.282 ; female gametophytes, 6.190 ± 0.280 ; Supplementary Table S1),
258 indicating that the mitochondrial shape changed during gametogenesis.

259

260 **Mitochondrial morphology in male and female gametes**

261 3D reconstruction of the male and female gametes released from the gametangia was
262 performed next (Fig. 3, Supplementary Movie S5, S6). The shape of the mitochondria in the
263 male and female gametes were nearly spherical (Fig. 3a, b). Most mitochondria were distributed
264 in the anterior part of the gametes (Fig. 3c–f). The average mitochondrial number in three cells
265 each of the male and female gametes was 5 and 27, respectively (Supplementary Table S1). A
266 conspicuous decrease in the number of mitochondrial in female gametes prior to gamete release
267 was confirmed. However, the total mitochondrial volume did not change before and after the
268 release of gametes in both males and females. The average mitochondrial size was $0.173 \pm$
269 $0.022 \mu\text{m}^3$ in male gametes and $0.191 \pm 0.006 \mu\text{m}^3$ in female gametes, indicating that while
270 female mitochondria were slightly larger than male mitochondria, the difference was not
271 significant (Tukey's multiple comparison $P = 0.4593$; Supplementary Table S2). The MCI of
272 the male and female mitochondria was 0.758 ± 0.007 and 1.034 ± 0.042 , respectively.
273 According to these results, the shape of mitochondria in released gametes underwent a drastic
274 change either during or after gamete release. Mitochondria in male gametes were nearly
275 spherical (MCI ≈ 0.71) while the shape of mitochondria in female gametes was only slightly
276 spherical (Supplementary Table S1). However, based on Tukey's multiple comparison, the
277 shapes of mitochondria in male and female gametes were significantly different from each other
278 ($P < 0.0001$; Supplementary Table S2).

279

280 **Mitochondrial morphology in early sporophytes**

281 The disappearance of parental mitochondria in 2-h-old zygotes was observed (Shen et al.
282 2020). Mitochondrial morphology was examined in 2- and 6-h-old zygotes to compare the
283 configuration of the maternal mitochondria during or after the disappearance of the paternal
284 mitochondria (Fig. 4, Supplementary Movie S7, S8). Fertilization was confirmed by the number
285 of eyespots on the chloroplasts derived from male and female gametes (Shen et al. 2020). Two

286 eyespots were maintained until the formation of seven-celled sporophytes. The number of
287 mitochondria in the three 2-h-old zygotes was 25, 26, and 35 (Supplementary Table S1). The
288 zygote with 35 mitochondria (“2-h-old_Zygote_2” in Supplementary Table S1) appeared to
289 have male mitochondria that had not yet been digested. In three 6-h-old zygotes, the number of
290 mitochondria in each cell was 25, 29, and 30. The mean volume per mitochondrion in the 6-h-
291 old zygotes was $0.243 \pm 0.007 \mu\text{m}^3$, which indicated that these had mitochondria increased in
292 size compared to those in the 2-h-old zygotes ($0.190 \pm 0.006 \mu\text{m}^3$), and this increase was
293 statistically significant ($P < 0.0001$; Supplementary Tables S1 and S2). However, the MCI
294 values between the 2- and 6-h-old zygotes and the female gametes were not significantly
295 different ($P = 0.9997$; Supplementary Table S2).

296 The first cell division occurred symmetrically 24–48 h after fertilization. The
297 morphology of mitochondria in 24-h-old zygotes was compared before and after cytokinesis
298 (Fig. 5, Supplementary Movie S9, S10). The two zygotes examined before cytokinesis had
299 either 32 or 33 mitochondria while the two zygotes examined after cytokinesis had either 31 or
300 34 mitochondria. Almost half of the mitochondria in each daughter cell were distributed from
301 the mother cell (Supplementary Table S1). Dumbbell-shaped and elongated mitochondria were
302 observed in the cells before cytokinesis (Fig. 5a–c). The number of mitochondria did not change
303 toward the first cell division (Supplementary Table S1); however, the mitochondria became
304 larger and adopted more complex shapes than those from 2- (Fig. 4a–c) and 6-h-old (Fig. 4d–f)
305 zygotes. After cytokinesis, mitochondria in two-celled sporophytes were oval and tubular in
306 shape, and the dumbbell-shaped mitochondria were no longer observed (Fig. 5d–f).

307

308 **Mitochondrial morphology in sporophytes**

309 A cross-section of a 20-d-old crustose sporophyte is shown in Figure 6a. In the marginal
310 meristematic cell layer, longer cells with asymmetric cytoplasmic distribution and shorter cells
311 lacking large vacuoles were observed (Fig. 6b, c). We examined the mitochondria in a short cell

312 (Fig. 6c–e, “20-d-old_Sporophyte_1” in Supplementary Table S1; Supplementary Movie S11).
313 To compare the mitochondrial morphology among the cell layers, the cells positioned at the
314 subapical (Fig. 6f–h, “20-d-old_Sporophyte_2” in Supplementary Table S1; Supplementary
315 Movie S12) and central regions (Fig. 6i–k, “20-d-old_Sporophyte_3” in Supplementary Table
316 S1; Supplementary Movie S13) were examined. There were no significant differences in the
317 total number and volume of mitochondria among the three cells examined (Supplementary
318 Table S1). Tubular, curved, V-, and Y-shaped mitochondria were observed in all three cells (Fig.
319 6c–k). The MCI was highest in the subapical cell (4.180 ± 0.250), followed by the cell in the
320 central region (3.363 ± 0.203). The marginal apical cell had the lowest MCI (2.463 ± 0.147)
321 (Supplementary Table S1). There was little difference between the shapes of mitochondria in
322 the central and subapical regions ($P = 0.3047$; Supplementary Table S2); however, there was a
323 statistically significant difference in the shape of the mitochondrial in the apical region from
324 those in the subapical ($P < 0.0001$) and central regions ($P = 0.0264$; Supplementary Table S2).

325 The volume of each mitochondrion in non-gametophyte cells are summarized in Figure
326 7. This analysis showed that the volume of mitochondria increased before and after cytokinesis;
327 however, mitochondria in other stages were stable in volume.

328

329 **Discussion**

330 **Ultrastructural studies through the generations of the life cycle in *M. cylindricus***

331 To understand the dynamics of mitochondrial morphology across generations in brown
332 algae, we selected *M. cylindricus* because of the heteromorphic alternation of generations in its
333 life cycles and its anisogamous sexual reproduction patterns. The mitochondria in the uniseriate
334 filamentous gametophytes, mature gametangia, gametes, zygotes, and crustose sporophytes
335 were examined through consecutive serial sections using TEM. Moreover, quantitative analysis
336 based on the 3D images was performed to characterize the mitochondrial morphology at each
337 stage of the life cycle. Observation of the ultrastructure throughout the life cycle of brown algae

338 was previously performed in *Scytothamnus australis* and *Scytothamnus fasciculatus* (Clayton
339 1986) using cultured material. In that report, however, the mitochondrial structure was not
340 described. The ultrastructure of mitochondria in the gametangia, sporangia, and gametes of
341 brown algae have been reported (Brawley et al. 1976; La Claire and West 1978, 1979; Berkaloff
342 and Rousseau 1979; Henry and Cole 1982a, b; Clayton 1984; Katsaros and Galatis 1986; Maier
343 1997); however, our study was the first to report mitochondrial morphological observations
344 across generations and to quantify mitochondrial morphology in brown algae using 3D.

345

346 **Fusion of mitochondria at the final stage of female gametogenesis**

347 In this study, 3D image reconstruction allowed us to quantitatively analyze the volume
348 and complexity of the mitochondria. Mitochondria in gametes with two flagella and eyespots
349 within the locules of the male and female gametangia in *M. cylindricus* showed an elongated
350 tubular shape; however, they were almost spherical in the released male and female gametes.
351 The total number and volume of mitochondria contained in the released male gametes were
352 similar to those in the cells before release (Figs. 2a–c and 3a, c, e, Supplementary Table S1).
353 The mitochondrial morphology only changed immediately before release. In a similar analysis
354 of female gametes, the number of mitochondria diminished in the released gametes, and their
355 total volume did not change from the gametes before release (Figs. 2d–f and 3b, d, f). These
356 data suggest that mitochondrial fusion occurs in female gametes during the final stage of
357 gametogenesis.

358 Fission and fusion of mitochondria constitute the mitochondrial dynamics, which are
359 important processes for the maintenance of mitochondrial quality (Varuzhanyan and Chan
360 2020). In particular, fusion promotes the elimination of mitochondrial diversity, such as
361 mitochondrial DNA (mtDNA) and functional heterogeneity (Chan 2012). The fusion of
362 mitochondria is observed at a specific stage of spermatogenesis in *Drosophila melanogaster*
363 (Hales and Fuller 1997) in which the mitochondria aggregate and fuse to form the nebenkern, an

364 onion-like giant mitochondrion. Mitochondria elongate during pachytene in meiosis I and
365 fragment again after meiosis in rats (De Martino et al. 1979, Varuzhanyan et al. 2019).
366 Mitochondrial fusion is considered particularly important for the production of healthy sperms.
367 The mitochondrial fusion gene *fuzzy onions* (*Fzo*) was isolated from mutants showing male
368 sterility in *D. melanogaster* (Hales and Fuller 1997). Mammalian homologs of FZO are
369 mitofusins (Mfns) (Santel and Fuller 2001), and defects in the *MFN1* and *MFN2* genes induce
370 the failure of sperm production (Varuzhanyan et al. 2019). Mitochondrial fusion-related genes
371 were not found in the brown algae. However, based on a study on female gametogenesis in *M.*
372 *cylindricus*, mitochondrial fusion occurs immediately before gamete release. In *M. cylindricus*,
373 mtDNA is derived from female gametes, as reported previously (Shen et al. 2020). The mtDNA
374 copy number in each mitochondrion in a male gamete is less than one-seventh of that in a
375 female gamete. In addition, male mitochondria are digested by mitophagy after fertilization. It is
376 possible that the fusion of mitochondria before the release of the female gametes could be a
377 necessary final quality control step to unify the matrix and ensure the success of the maternal
378 inheritance of mitochondria in this species.

379

380 **Mitochondrial complexity and cell types**

381 Mitochondria in the vegetative gametophytes and sporophytes showed high MCI scores
382 (Supplementary Table S1). Tubular, curved, V-, and Y-shaped mitochondria observed in these
383 cells were not observed in other cells. The high MCI score for the mitochondria in these cells
384 may be related to the development of large vacuoles within the cells (Figs. 1, 6). In a 20-d-old
385 crustose sporophyte, mitochondrial morphology was analyzed using three cells collected from
386 different regions of the alga. The results showed that the total and individual mitochondrial
387 volumes were similar in all the cells. However, the MCI scores in the subapical and central
388 regions were obviously higher (“20-d-old Sporophyte_2” and “20-d-old Sporophyte_3” in
389 Supplementary Table S1) than in the marginal apical region (“20-d-old_Sporophyte_1” in

390 Supplementary Table S1). It was clear that the cytoplasm in the cell from the subapical region
391 was occupied by a large vacuole compared to cells in the other regions (Fig. 7a). The complex
392 morphology of mitochondria may thus be influenced to some extent by the development of
393 vacuoles.

394

395 **Mitochondrial structure in the vegetative and motile reproductive cells**

396 Generally, mitochondria in brown algae are tubular. Moreover, in the most motile
397 reproductive cells, the intercrystal structures—high electron-density structures—in the
398 mitochondrial cristae are observed, for example, in the male and female gametes of *Cutleria*
399 *hancockii* (La Claire and West 1978, 1979), *Scytosiphon lomentaria* (Clayton 1984), and *M.*
400 *cylindricus* (Shen et al. 2020); the male gametes of *Ectocarpus siliculosus* (Maier 1997);
401 zoospores of *Halopteris filicia* (Katsaros and Galatis 1986) and several species belonging to
402 *Laminariales* (Henry and Cole 1982a); and sperm of *Fucus serratus* (Berkaloff and Rousseau
403 1979). The function of the intercrystal structure has not yet been elucidated, and the presence of
404 this structure is limited in motile reproductive cells, not in eggs (Brawley et al. 1976). The
405 occurrence of this structure was observed in mature, but not immature, gametangia in *Pylaiella*
406 *littoralis* (Markey and Wilce 1975). The timing of the appearance of the tubular structure within
407 the mitochondrial cristae may indicate the preparation of a switch from the vegetative to the
408 reproductive type in the mitochondria. In this study, we did not examine the internal structure of
409 the mitochondria. The mitochondria in both male and female gametes changed from a dumbbell
410 or elongated shape to an almost spherical shape immediately before release. Morphological
411 changes at the final stage of gametogenesis may be linked to the completion of optimal
412 mitochondrial physiology in the motility cells. The timing of the disappearance of the
413 intercrystal structure after fertilization or settlement of zoospores is unknown. In *M. cylindricus*,
414 the mitochondrial size and MCI score increased 6 h after fertilization. It is thought that the
415 physiological condition or function of the mitochondria may change from reproductive to

416 vegetative type during this transition.

417

418 **Conclusion**

419 To understand the morphological dynamics in mitochondria across generations, we
420 performed a quantitative analysis on mitochondria from *M. cylindricus* based on 3D
421 reconstruction of serial sections imaged by TEM. It became clear that mitochondria in
422 gametophytes and sporophytes showed high MCI scores compared to those in the gametes and
423 during early development of the sporophytes, and this appeared to be related to the development
424 of vacuoles in the cells. In the final stages of gametogenesis, a conspicuous reduction in the
425 number of mitochondria in female gametes was observed. By comparing the total volume and
426 the number of mitochondria in pre- and post-release female gametes, there is a strong possibility
427 that mitochondria in the female gametes fuse immediately before release. This phenomenon
428 suggests that mitochondria fuse to reduce heterogeneity and enhance maternal inheritance of
429 mitochondria upon fertilization in this species. To verify this hypothesis, the continuous
430 sequence of dynamic morphological changes in mitochondria needs to be further investigated.

431

432

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437 gametophyte stage using high-pressure freezing.

438

439

440 **Author contributions**

441 Yuan Shen analyzed the data and maintained the *M. cylindricus* strains. Chikako Nagasato and
442 Taizo Motomura designed the experiments and critically reviewed the manuscript. All authors
443 have written and edited the manuscript.

444

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447

448

449 **Reference**

- 450 Aoyama H, Kuroiwa T, Nakamura S (2009) The dynamic behaviour of mitochondria in living
451 zygotes during maturation and meiosis in *Chlamydomonas reinhardtii*. Eur J
452 Phycol 44:497–507
- 453 Berkaloﬀ C, Rousseau B (1979) Ultrastructure of male gametogenesis in *Fucus serratus*
454 (Phaeophyceae). J Phycol 15:163–173
- 455 Brawley SH, Wetherbee R, Quatrano RS (1976) Fine-structural studies of the gametes and
456 embryo of *Fucus vesiculosus* L. (Phaeophyta). II. The cytoplasm of the egg and
457 young zygote. J Cell Sci 20:255–271
- 458 Chan DC (2012) Fusion and fission: interlinked processes critical for mitochondrial health.
459 Annu Rev Genet 46:265–287
- 460 Clayton MN (1984) An electron microscope study of gamete release and settling in the
461 complanate from *Scytosiphon* (Scytosiphonaceae, Phaeophyta). J Phycol 20:276–
462 285
- 463 Clayton MN (1986) Culture studies on the life history of *Scytothamnus australis* and
464 *Scytothamnus fasciculatus* (Phaeophyta) with electron microscope observations on
465 sporogenesis and gametogenesis. Br Phycol J 21:371–386
- 466 Cui Y, Cao W, He Y, Zhao Q, Wakazaki M, Zhuang X, Gao J, Zeng Y, Gao C, Ding Y, Wong
467 HY, Wong WS, Lam HK, Wang P, Ueda T, Rojas-Pierce M, Toyooka K, Kang BH,
468 Jiang L (2019) A whole-cell electron tomography model of vacuole biogenesis in
469 *Arabidopsis* root cells. Nat Plants 5:95–105
- 470 De Martino C, Floridi A, Marcante ML, Malorni W, Scorza Barcellona P, Bellocci M,
471 Silvestrini B (1979) Morphological, histochemical and biochemical studies on
472 germ cell mitochondria of normal rats. Cell Tissue Res 196:1–22
- 473 Ehara T, Osafune T, Hase E (1995) Behavior of mitochondria in synchronized cells of
474 *Chlamydomonas reinhardtii* (Chlorophyta). J Cell Sci 108:499–507

- 475 Fu G, Nagasato C, Ito T, Müller DG, Motomura T (2013) Ultrastructural analysis of flagellar
476 development in plurilocular sporangia of *Ectocarpus siliculosus*
477 (Phaeophyceae). *Protoplasma* 250:261–272
- 478 Hales KG, Fuller MT (1997) Developmentally regulated mitochondrial fusion mediated by a
479 conserved, novel, predicted GTPase. *Cell* 90:121–129
- 480 Henry EC, Cole KM (1982a) Ultrastructure of swimmers in the Laminariales (Phaeophyceae). I.
481 Zoospores. *J Phycol* 18:550–569
- 482 Henry EC, Cole KM (1982b) Ultrastructure of swimmers in the Laminariales (Phaeophyceae). II.
483 Sperm. *J Phycol* 18:570–579
- 484 Ho HC, Wey S (2007) Three dimensional rendering of the mitochondrial sheath morphogenesis
485 during mouse spermiogenesis. *Microsc Res Tech* 70:719–723
- 486 Katsaros C, Galatis B (1986) Ultrastructural studies on zoosporogenesis of *Halopteris filicina*
487 (Sphacelariales, Phaeophyta). *Phycologia* 25:358–370
- 488 Kawai H, Kogishi K, Hanyuda T, Kitayama T (2012) Taxonomic revision of the genus *Cutleria*
489 proposing a new genus *Mutimo* to accommodate *M. cylindricus* (Cutleriaceae,
490 Phaeophyceae). *Psychol Res* 60:241–248
- 491 Kinoshita N, Fu G, Ito T, Motomura T (2016). Three-dimensional organization of flagellar
492 basal apparatus in *Ectocarpus* gametes. *Phycol Res* 64:19–25
- 493 Kitayama T, Kawai H, Yoshida T (1992) Dominance of female gametophytes in field
494 populations of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in the Tsugaru
495 Strait, Japan. *Phycologia* 31:449–461
- 496 Kremer JR, Mastrorarde DN, McIntosh JR (1996) Computer visualization of three-dimensional
497 image data using IMOD. *J Struct Biol* 116:71–76
- 498 Kuroiwa T (2010) Mechanisms of organelle division and inheritance and their implications
499 regarding the origin of eukaryotic cells. *Proc Jpn Acad Ser B Phys Biol Sci*
500 86:455–471

501 La Claire JW, West JA (1978) Light-and electron-microscopic studies of growth and
502 reproduction in *Cutleria* (Phaeophyta). *Protoplasma* 97:93–110

503 La Claire JW, West JA (1979) Light–and electron–microscopic studies of growth and
504 reproduction in *Cutleria* (phaeophyta). *Protoplasma* 101: 247–267

505 Lowe DG (2004) Distinctive image features from scale-invariant keypoints. *Int J Comput*
506 *Vis* 60:91–110

507 Maier I (1997). The fine structure of the male gamete of *Ectocarpus siliculosus* (Ectocarpales,
508 Phaeophyceae). I. General structure of the cell. *Eur J Phycol* 32:241–253

509 Markey DR, Wilce RT (1975) The ultrastructure of reproduction in the brown alga *Pylaiella*
510 *littoralis*. I. Mitosis and cytokinesis in the plurilocular gametangia. *Protoplasma*
511 85:219–241

512 Murtin C, Frindel C, Rousseau D, Ito K (2018) Image processing for precise three-dimensional
513 registration and stitching of thick high-resolution laser-scanning microscopy image
514 stacks. *Comput Biol Med* 92:22–41

515 Nagasato C, Kajimura N, Terauchi M, Mineyuki Y, Motomura T (2014) Electron tomographic
516 analysis of cytokinesis in the brown alga *Silvetia babingtonii* (Fucales,
517 Phaeophyceae). *Protoplasma* 251:1347–1357

518 Nagasato C, Motomura T (2002) Ultrastructural study on mitosis and cytokinesis in *Scytosiphon*
519 *lomentaria* zygotes (Scytosiphonales, Phaeophyceae) by freeze-substitution.
520 *Protoplasma* 219(3–4):140–149

521 Nicander L, Ploen L (1969) Fine structure of spermatogonia and primary spermatocytes in
522 rabbits. *Z Zellforsch Mikrosk Anat* 99:221–234

523 Peachey LD (1958) Thin sections: I. A study of section thickness and physical distortion
524 produced during microtomy. *J Biophys Biochem Cytol* 4:233–242

525 Provasoli L (1968) Media and prospects for the cultivation of marine algae. In Watanabe A,
526 Hattori A (eds) Culture and collections of algae (Proc Jap Conf Hakone, 1966).
527 Japanese Society of Plant Physiology, Tokyo, pp 63–75

528 Rantanen A, Larsson NG (2000) Regulation of mitochondrial DNA copy number during
529 spermatogenesis. *Hum Reprod* 2:86–91

530 Santel A, Fuller MT (2001) Control of mitochondrial morphology by a human mitofusin. *J Cell*
531 *Sci* 114:867–874

532 Sato M, Sato K (2013) Maternal inheritance of mitochondrial DNA by diverse mechanisms to
533 eliminate paternal mitochondrial DNA. *Biochim Biophys Acta* 1833:1979–1984

534 Schindelin J, Arganda–Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S,
535 Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K,
536 Tomancak P, Cardona A (2012) Fiji: an open–source platform for biological-image
537 analysis. *Nat Methods* 28:676–682

538 Shen Y, Iwao T, Motomura T, Nagasato C (2020) Cytoplasmic inheritance of mitochondria and
539 chloroplasts in the anisogamous brown alga *Mutimo cylindricus*. *Protoplasma* 1–14

540 Terauchi M, Nagasato C, Kajimura N, Mineyuki Y, Okuda K, Katsaros C, Motomura T (2012)
541 Ultrastructural study of plasmodesmata in the brown alga *Dictyota dichotoma*
542 (Dictyotales, Phaeophyceae). *Planta* 236:1013–1126

543 Varuzhanyan G, Chan DC (2020) Mitochondrial dynamics during spermatogenesis. *J Cell Sci*
544 133:14

545 Varuzhanyan G, Rojansky R, Sweredoski MJ, Graham RL, Hess S, Ladinsky MS, Chan DC
546 (2019) Mitochondrial fusion is required for spermatogonial differentiation and
547 meiosis. *elife* 8:e51601

548 Vincent AE, White K, Davey T, Philips J, Ogden RT, Lawless C, Warren C, Hall MG, Ng YS,
549 Falkous G, Holden T, Deehan D, Taylor RW, Turnbull DM, Picard M (2019)
550 Quantitative 3D Mapping of the Human Skeletal Muscle Mitochondrial Network.

551 Cell Rep 26:996–1009
552 Yoshida Y, Kuroiwa H, Shimada T, Masaki Yoshida M, Mio Ohnuma M, Takayuki Fujiwara
553 T, Yuuta Imoto Y, Fumi Yagisawa F, Keiji Nishida K, Shunsuke Hirooka S, Osami
554 Misumi O, Yuko Mogi Y, Yoshihiko Akakabe Y, Kazunobu Matsushita K,
555 Tsuneyoshi Kuroiwa T (2017) Glycosyltransferase MDR1 assembles a dividing
556 ring for mitochondrial proliferation comprising polyglucan nanofilaments. Proc
557 Natl Acad Sci USA 114:13284–13289
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561 **Figure Legends**

562 **Fig. 1** Mitochondrial morphology in the somatic cells of gametophytes.

563 **a, c, e** male gametophyte, **b, d, f** female gametophyte.

564 **a** A thin-section image of male gametophyte.

565 **b** A thin-section image of female gametophyte.

566 **c** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
567 serial thin sections in the same cell as **a**.

568 **d** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
569 serial thin sections in the same cell as **b**.

570 **e** A complete 3D model of **c**.

571 **f** A complete 3D model of **d**.

572 **c**, chloroplast; **pm**, plasma membrane; **m**, mitochondrion; **n**, nucleus; **v**, vacuole. Scale bar: 1
573 μm .

574

575 **Fig. 2** Mitochondrial morphology in mature gametes from the plurilocular gametangia.

576 **a–c** male gamete in the gametangium, **d–f** female gamete in the gametangium.

577 **a** A thin-section image of male gamete.

578 **b** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
579 serial thin sections in the same cell as **a**.

580 **c** A complete 3D model of **b**.

581 **d** A thin-section image of female gamete.

582 **e** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
583 serial thin sections in the same cell as **d**.

584 **f** A complete 3D model of **e**.

585 **c**, chloroplast; **pm**, plasma membrane; **e**, eyespot; **f**, flagellum; **m**, mitochondrion; **n**, nucleus; **v**,
586 vacuole. Scale bar: 1 μm .

587

588 **Fig. 3** Mitochondrial morphology in gametes after release.

589 **a, c, e** male gamete, **b, d, f** female gamete.

590 **a** A thin-section image of male gamete.

591 **b** A thin-section image of female gamete.

592 **c** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
593 serial thin sections in the same cell as **a**.

594 **d** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
595 serial thin sections in the same cell as **b**.

596 **e** A complete 3D model of **c**.

597 **f** A complete 3D model of **d**.

598 Arrowheads indicate the anterior part of the cell.

599 c, chloroplast; pm, plasma membrane; e, eyespot; af, anterior flagellum; pf, posterior flagellum;
600 m, mitochondrion; n, nucleus; v, vacuole. Scale bar: 1 μ m.

601

602 **Fig. 4** Mitochondrial morphology in 2- and 6-h-old zygotes.

603 **a–c** a 2-h-old zygote, **d–f** a 6-h-old zygote.

604 **a** A thin-section image of a 2-h-old zygote.

605 **b** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
606 serial thin sections in the same cell as **a**.

607 **c** A complete 3D model of **b**.

608 **d** A thin-section image of a 6-h-old zygote.

609 **e** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
610 serial thin sections in the same cell as **d**.

611 **f** A complete 3D model of **e**.

612 c, chloroplast; pm, plasma membrane; e, eyespot; m, mitochondrion; n, nucleus; v, vacuole.

613 Scale bar: 1 μ m.

614

615 **Fig. 5** Mitochondrial morphology in 24-h-old zygotes.

616 **a–c** a 24-h-old zygote before cytokinesis, **d–f** a 24-h-old zygote after cytokinesis.

617 **a** A thin-section image of a 24-h-old zygote before cytokinesis.

618 **b** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
619 serial thin sections in the same cell as **a**.

620 **c** A complete 3D model of **b**.

621 **d** A thin-section image of the 24-h-old zygote after cytokinesis.

622 **e** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
623 serial thin sections in the same cell as **d**.

624 **f** A complete 3D model of **e**.

625 c, chloroplast; pm, plasma membrane; e, eyespot; m, mitochondrion; n, nucleus; v, vacuole.

626 Scale bar: 1 μ m.

627

628 **Fig. 6** Mitochondrial morphology in a crustose sporophyte.

629 **a** A thin-section image of a 20-d-old crustose sporophyte.

630 **b** A thin-section image of a longer apical cell located in the marginal meristematic region in **a**.

631 **c** A thin-section image of a shorter apical cell in the marginal meristematic region in **a**.

632 **d** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
633 serial thin sections in the same cell as **c**.

634 **e** A complete 3D model of **d**.

635 **f** A thin-section image of a cell in the subapical region in **a**.

636 **g** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
637 the serial thin sections in the same cell as **f**.

638 **h** A complete 3D model of **g**.

639 **i** A thin-section image of a cell in the central region in **a**.
640 **j** A partial 3D model of the nucleus, mitochondria, and plasma membrane reconstructed from
641 serial thin sections in the same cell as **i**.
642 **k** A complete 3D model of **j**.
643 c, chloroplast; pm, plasma membrane; h, hyaline hair; m, mitochondrion; n, nucleus; v, vacuole.
644 Scale bar: 10 μm in **a**; 1 μm in **b–k**.

645

646 **Fig. 7** Box plot presenting quantitative analysis of each mitochondrial volume examined in the
647 mature gametangia, released gametes, one-day-old zygotes, and two-day-old zygotes before and
648 after cytokinesis. All mitochondria used for quantitative analysis originated from
649 Supplementary **Table S1**: MPG, Male_Gametangium; FPG, Female_Gametangium; Mg,
650 Male_Gamete; Fg, Female_Gamete; 2hZ, 2-h-old_Zygote; 6hZ, 6-h-old_Zygote; 24hZ, 24-h-
651 old_Zygote; 24h2cS, 2-celled_Sporophyte; 20dS, 20-d-old_Sporophyte.
652 Within the box plot, the solid line represents the 50th percentile, the dashed line represents the
653 mean, the box delimits the 25th and 75th percentiles, and bars indicate the 10th and 90th
654 percentiles. Within the line plot, data are mean \pm SEM.

655

656 **Fig. S1** Serial ultrathin sections used for 3D reconstruction
657 a Ribbon of thin serial sections generated using a diamond knife. **b** Enlarged image of **a** with
658 interference color as gold.

659

660 **Fig. S2** Three-dimensional models used for quantitative analysis of mitochondrial morphology
661 across gametophyte-to-sporophyte life cycle of *M. cylindricus*. All mitochondria used for
662 quantitative analysis originated from **Table S1**. **a1–3** Male_Gametophyte_1–3. **b1–3**
663 Female_Gametophyte_1–3. **c1–3** Male_Gametangium_1–3. **d1–3** Female_Gametangium_1–3.
664 **e1–3** Male_Gamete_1–3. **f1–3** Female_Gamete_1–3. **g1–3** 2-h-old_Zygote_1–3. **h1–3** 6-h-

665 old_Zygote_1–3. **i1–2** 24-h-old_Zygote_1 to 2. **k1–4**: 2-celled_Sporophyte_1–2. **l1–3** 20-d-
666 old_Sporophyte_1 to 3. The plasma membrane is indicated in gray. Nuclei are indicated in blue.
667 Mitochondria are indicated in orange. Scale bar: 1 μ m.

668

669 **Fig. S3** Gametophyte-to-sporophyte life cycle of *M. cylindricus*

670 **a, b** Male and female gametophytes. Arrowheads indicate released swimming gametes. **c, d**

671 Male and female plurilocular gametangia. Black arrowheads indicate orange eyespots in

672 gametangia small loci. White arrowheads indicate somatic cells of gametophytes. **e, f** Released

673 swimming male and female gametes with one eyespot (arrowhead) in each cell. **g** Zygote with

674 two orange eyespots (arrowheads). **h** Two-celled sporophytes with two orange eyespots

675 (arrowheads). **i** Crustose sporophyte.

676 af, anterior flagellum; pf, posterior flagellum. Scale bar: 1 cm in **a, b**; 10 μ m in **c–h**.

677

678 **Movie S1** A 3D model of a male gametophyte

679

680 **Movie S2** A 3D model of a female gametophyte

681

682 **Movie S3** A 3D model of a male gametangium

683

684 **Movie S4** A 3D model of a female gametangium

685

686 **Movie S5** A 3D model of a male gamete

687

688 **Movie S6** A 3D model of a female gamete

689

690 **Movie S7** A 3D model of a 2-h-old zygote

691

692 **Movie S8** A 3D model of a 6-h-old zygote

693

694 **Movie S9** A 3D model of a 24-h-old zygote

695

696 **Movie S10** A 3D model of a two-celled sporophyte

697

698 **Movie S11** A 3D model of the marginal apical region in a 20-d-old sporophyte

699

700 **Movie S12** A 3D model of the subapical region in a 20-d-old sporophyte

701

702 **Movie S13** A 3D model of the central region in a 20-d-old sporophyte

Table S1 Quantitative analysis of mitochondrial morphology across gametophyte-to-sporophyte life cycle of *M. cylindricus*

Life-cycle stages and sample No.	Pixel size of each TEM image (nm)	Total number		Total volume (μm^3)		Average volume (μm^3)		Average MCI	
		Each sample	Average (mean \pm SEM)	Each sample	Average (mean \pm SEM)	Each sample (mean \pm SEM)	Average (mean \pm SEM)	Each sample (mean \pm SEM)	Average (mean \pm SEM)
Male_Gametophyte_1	3.318	117		11.114		0.095 \pm 0.004		3.684 \pm 0.172	
Male_Gametophyte_2*	3.318	83	83 \pm 19	9.123	10.925 \pm 0.991	0.110 \pm 0.004	0.131 \pm 0.006	2.796 \pm 0.132	4.848 \pm 0.282
Male_Gametophyte_3	3.318	50		12.539		0.251 \pm 0.016		10.980 \pm 0.911	
Female_Gametophyte_1*	4.921	104		13.522		0.130 \pm 0.005		5.708 \pm 0.475	
Female_Gametophyte_2	4.921	42	68 \pm 19	7.536	9.479 \pm 2.022	0.179 \pm 0.011	0.139 \pm 0.004	6.778 \pm 0.380	6.190 \pm 0.280
Female_Gametophyte_3	4.921	59		7.379		0.125 \pm 0.006		6.621 \pm 0.408	
Male_Gametangium_1*	3.318	7		0.840		0.120 \pm 0.007		1.869 \pm 1.682	
Male_Gametangium_2	3.318	5	6 \pm 1	1.024	0.918 \pm 0.055	0.205 \pm 0.024	0.162 \pm 0.013	1.337 \pm 0.133	1.682 \pm 0.091
Male_Gametangium_3	3.318	5		0.890		0.178 \pm 0.020		1.765 \pm 0.152	
Female_Gametangium_1*	4.921	40		5.049		0.126 \pm 0.007		2.563 \pm 1.852	
Female_Gametangium_2	4.921	38	38 \pm 1	5.109	5.042 \pm 0.041	0.134 \pm 0.005	0.133 \pm 0.004	1.448 \pm 0.057	1.852 \pm 0.074
Female_Gametangium_3	4.921	36		4.969		0.138 \pm 0.006		1.489 \pm 0.068	
Male_Gamete_1*	2.086	5		0.763		0.153 \pm 0.022		0.744 \pm 0.005	
Male_Gamete_2	2.086	5	5 \pm 0	0.834	0.867 \pm 0.072	0.167 \pm 0.046	0.173 \pm 0.022	0.745 \pm 0.006	0.758 \pm 0.007
Male_Gamete_3	2.086	5		1.004		0.201 \pm 0.045		0.785 \pm 0.014	
Female_Gamete_1	4.214	23		5.486		0.239 \pm 0.013		0.980 \pm 1.034	
Female_Gamete_2*	4.214	30	27 \pm 2	4.937	5.144 \pm 0.172	0.165 \pm 0.009	0.191 \pm 0.006	0.994 \pm 0.106	1.034 \pm 0.042
Female_Gamete_3	4.214	28		5.010		0.179 \pm 0.008		1.121 \pm 0.031	
2-h-old_Zygote_1	7.018	25		4.846		0.194 \pm 0.010		0.867 \pm 0.078	
2-h-old_Zygote_2	7.018	35	29 \pm 3	5.995	5.472 \pm 0.336	0.171 \pm 0.007	0.190 \pm 0.006	0.938 \pm 0.034	0.901 \pm 0.028
2-h-old_Zygote_3*	7.018	26		5.575		0.214 \pm 0.013		0.884 \pm 0.031	
6-h-old_Zygote_1*	4.243	29		7.859		0.271 \pm 0.012		0.887 \pm 0.069	
6-h-old_Zygote_2	4.243	30	28 \pm 2	6.590	6.802 \pm 0.559	0.220 \pm 0.010	0.243 \pm 0.007	0.857 \pm 0.027	0.943 \pm 0.031
6-h-old_Zygote_3	4.243	25		5.958		0.271 \pm 0.012		0.887 \pm 0.069	
24-h-old_Zygote_1*	5.255	33	33 \pm 1	13.634	13.734 \pm 0.100	0.413 \pm 0.030	0.424 \pm 0.018	1.308 \pm 0.108	1.387 \pm 0.065
24-h-old_Zygote_2	5.255	32		13.834		0.432 \pm 0.023		1.449 \pm 0.078	
2-celled_Sporophyte_1*	5.255	31		12.304		0.397 \pm 0.024		1.065 \pm 0.050	
2-celled_Sporophyte_2	5.255	(15 & 16)**	33 \pm 2	(5.914 & 6.390)**	11.406 \pm 0.899		0.370 \pm 0.019		1.114 \pm 0.047
		34	(16 \pm 1)**	10.507	(5.703 \pm 0.287)**	0.309 \pm 0.014		1.575 \pm 0.400	
		(18 & 16)**		(5.443 & 5.065)**					
20-d-old_Sporophyte_1*	7.918	36		6.349		0.176 \pm 0.008		2.463 \pm 0.147	
20-d-old_Sporophyte_2*	7.918	39	36 \pm 2	7.451	6.651 \pm 0.404	0.191 \pm 0.010	0.186 \pm 0.006	4.180 \pm 0.250	3.358 \pm 0.138
20-d-old_Sporophyte_3*	7.918	32		6.153		0.192 \pm 0.011		3.363 \pm 0.203	

*3D images are shown in **Figure 1, 2, 3, 4, 5, 6**

**The numerical values show the data from each daughter cell or from the zygote before fusion of male and female gamete nuclei (karyogamy)

Table S2 Partial pairwise multiple comparison using Tukey's multiple comparison test

Comparison	Difference of means	SE of difference	95.00% CIs (confidence intervals)	T-value	P-value	P < 0.050
Volume of each mitochondrion						
Male_Gametangium vs. Female_Gametangium	0.02931	0.0132	(0.001637,0.05698)	2.221	0.0391	Yes
Male_Gametangium vs. Male_Gamete	-0.01134	0.0252	(-0.09738, 0.07470)	0.4502	0.6568	No
Female_Gametangium vs. Female_Gamete	-0.05784	0.007323	(-0.07233, -0.04335)	7.898	<0.0001	Yes
Male_Gamete vs. Female_Gamete	-0.01719	0.2268	(-0.06514, 0.03077)	0.7577	0.4593	No
Female_Gamete vs. 2-h-old_Zygote	0.00192	0.008625	(-0.01511, 0.01895)	0.2226	0.8242	No
Female_Gamete vs. 6-h-old_Zygote	-0.05241	0.009791	(-0.07175, -0.03307)	5.353	<0.0001	Yes
2-h-old_Zygote vs. 6-h-old_Zygote	-0.05433	0.009371	(-0.07284, -0.03582)	5.797	<0.0001	Yes
6-h-old_Zygote vs. 24-h-old_Zygote	-0.1809	0.01962	(-0.2201, -0.1418)	9.223	<0.0001	Yes
24-h-old_Zygote vs. 2-celled_Sporophyte	0.05434	0.2598	(0.002749, 0.1059)	2.091	0.0392	Yes
2-celled_Sporophyte vs. 20-d-old_sporophyte	0.1831	0.0194	(0.1442, 0.2219)	9.436	<0.0001	Yes
Morphological complexity index of each mitochondrion						
Male_Gametangium vs. Female_Gametangium	-0.1703	0.1172	(-0.5691, 0.2285)	1.453	0.9268	No
Male_Gametangium vs. Male_Gamete	0.9237	0.09103	(0.5859, 1.261)	10.15	<0.0001	Yes
Female_Gametangium vs. Female_Gamete	0.818	0.08504	(0.5406, 1.095)	9.619	<0.0001	Yes
Male_Gamete vs. Female_Gamete	-0.2759	0.04216	(-0.4154, -0.1364)	6.545	<0.0001	Yes
Female_Gamete vs. 2-h-old_Zygote	0.1201	0.04942	(-0.04170, 0.2819)	2.43	0.3558	No
Female_Gamete vs. 6-h-old_Zygote	0.09075	0.05178	(-0.07855, 0.2601)	1.752	0.8054	No
2-h-old_Zygote vs. 6-h-old_Zygote	-0.02934	0.0409	(-0.1629, 0.1042)	0.7173	0.9997	No
6-h-old_Zygote vs. 24-h-old_Zygote	-0.4432	0.07155	(-0.6811, -0.2053)	6.194	<0.0001	Yes
24-h-old_Zygote vs. 2-celled_Sporophyte	0.2731	0.08004	(0.008581, 0.5375)	3.412	0.037	Yes
2-celled_Sporophyte vs. 20-d-old_Sporophyte_1	-2.25	0.2088	(-2.969, -1.531)	10.77	<0.0001	Yes
2-celled_Sporophyte vs. 20-d-old_Sporophyte_2	-3.067	0.2548	(-3.935, -2.199)	12.04	<0.0001	Yes
2-celled_Sporophyte vs. 20-d-old_Sporophyte_3	-1.35	0.1546	(-1.875, -0.8241)	8.731	<0.0001	Yes
20-d-old_Sporophyte_1 vs. 20-d-old_Sporophyte_2	-1.717	0.2904	(-2.688, -0.7459)	5.913	<0.0001	Yes
20-d-old_Sporophyte_1 vs. 20-d-old_Sporophyte_3	-0.9	0.2510	(-1.741, -0.05895)	3.585	0.0264	Yes
20-d-old_Sporophyte_2 vs. 20-d-old_Sporophyte_3	0.8169	0.3225	(-0.2571, 1.891)	2.533	0.3047	No

Data source is corresponding with **Table S1**;

Overall significance level = 95.00%;

When a $P < 0.050$, there is a statistically significant difference