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Gymnochlora dimorpha sp. nov., a chlorarachniophyte with unique daughter cell behaviour

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A new chlorarachniophyte species, *Gymnochlora dimorpha* sp. nov., was described. This new species was isolated from an enrichment preculture of *Padina* sp. collected from a subtidal coral reef zone in Republic of Palau. The new strain, P314, was characterized by light and electron microscopy in the present study. Under the culture conditions used here, the amoeboid stage was dominant. Two types of amoeboid cells were found in the cultures: motile and flattened nonmotile (sessile) cells. The motile cells typically multiplied via binary cell division. The sessile cells were always present in the cultures, but they never became dominant under the culture conditions. Time-lapse video microscopic observations revealed that after cell division of a sessile cell, one daughter cell became motile, while the other remained sessile. According to ultrastructural characteristics of the pyrenoids and nucleomorphs, the new chlorarachniophyte strain belongs to the genus *Gymnochlora*. However, P314 differed from *G. stellata*, the only hitherto known species of that genus, by forming flattened sessile cells in culture and having smaller cell dimensions (7–14 μm). Therefore, P314 is described here as a new species of *Gymnochlora*. This conclusion is supported by previously reported 18S rDNA phylogenies of chlorarachniophytes. The genus *Gymnochlora* as defined by morphology accorded with the molecular phylogenies.

Key Words: Chlorarachniophytes, Daughter cell behaviour, *Gymnochlora dimorpha*, Life cycle, Taxonomy

INTRODUCTION

Chlorarachniophytes are unicellular algae, all of which live in marine environments, e.g. coastal regions and open ocean waters (e.g. Ishida *et al.* 2007). Up to now they have comprised 12 species in eight genera. In higher-level eukaryote systematics, this algal group is phylogenetically positioned within the cercozoan clade that consists of filoseans and endomyxans as major lineages; the chlorarachniophytes are filoseans (e.g. Cavalier-Smith 2002, 2003; Cavalier-Smith & Chao 2003; Bass *et al.* 2005). The cells of chlorarachniophytes possess one or more chloroplasts, which are surrounded by four membranes and contain chlorophyll *a* and *b* as major pigments (Hibberd & Norris 1984). The chloroplast is assumed to be derived from a green algal secondary endosymbiosis (e.g. Ishida *et al.* 1999; Takahashi *et al.* 2007), and a remnant nucleus of the endosymbiont (nucleomorph) is still present in the periplastidial compartment, the space between the inner and outer pairs of the chloroplast membranes (Hibberd & Norris 1984). Nucleomorph-containing algae like chlorarachniophytes and cryptophytes are currently used to study genome and chromosome evolution (e.g. Archibald & Lane 2009; Silver *et al.* 2010; Tanifuji *et al.* 2010, 2011).

Although eight genera have been described as belonging to the Chlorarachniophyta, six of the genera are monotypic (Geitler 1930; Hibberd & Norris 1984; Calderon-Saenz & Schnetter 1987; Ishida *et al.* 1996, 2011; Ota *et al.* 2007b, 2009a). The generic delimitation of the chlorarachniophytes, except for *Cryptochlora* Calderon-Saenz & Schnetter for which ultrastructural data are unavailable, are defined by a set of ultrastructural and life cycle characteristics as follows: (1) ultrastructure of the pyrenoids; (2) nucleomorph position in the periplastidial compartment; and (3) the dominant cell type in the vegetative stage (Ishida *et al.* 1996; Ota *et al.* 2007b, 2009a; Ishida *et al.* 2011). The life cycle patterns are mainly used as diagnostic features for species delimitation (Dietz *et al.* 2003; Ota *et al.* 2005, 2007a, 2009b).

Unique behaviour of daughter cells has been found in several chlorarachniophytes based on time-lapse video microscopic observations. Amongst *Lotharella polymorpha* Dietz, Ehlers, Wilhelm, Gil-Rodríguez & Schnetter and *L. vacuolata* S. Ota & Ishida, daughter cells have shown unique post cell-division behaviours; specifically, one of the daughter cells becomes an amoeboid cell and hatches from the parental coccoid cell, while the other stays within the parental cell (Dietz *et al.* 2003; Ota *et al.* 2005). In *Bigelowiella longifila* S. Ota & Ishida, one of the two

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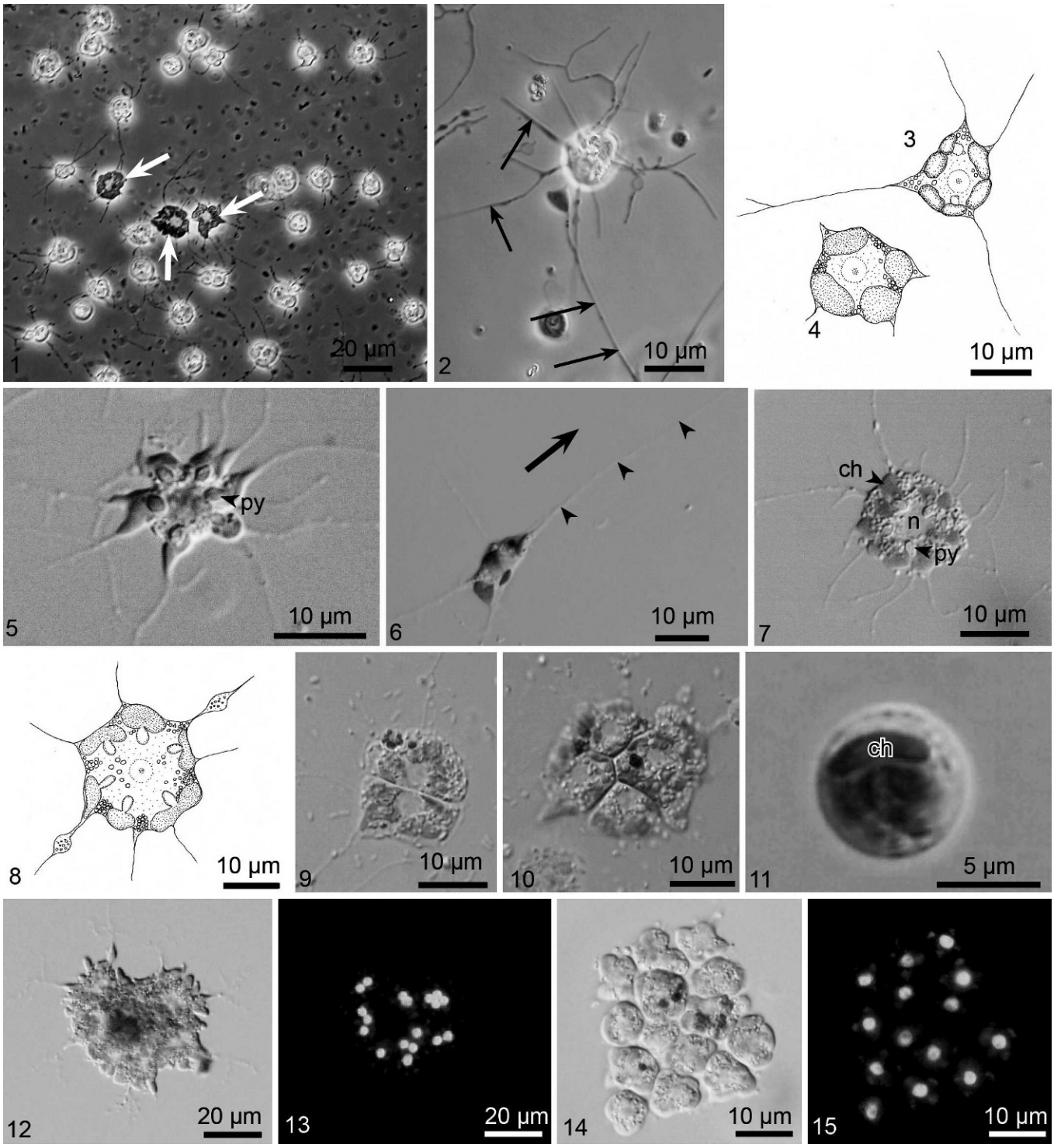

Figs 1–15. Light micrographs and line drawings of *Gymnochlora dimorpha* sp. nov.

Fig. 1. Low magnification view of the culture (phase contrast). Arrows indicate sessile amoeboid cells, all the other cells are motile amoeboid cells.

Fig. 2. Motile amoeboid cells (phase contrast). Arrows indicate fine filopodia.

Figs 3, 4. Line drawings of motile amoeboid cells.

Fig. 5. Stellate vegetative cell with filopodia (DIC). py = pyrenoid.

Fig. 6. Migrating vegetative cell extending a filopodium (arrowheads) toward the direction of movement (arrow) (DIC).

Fig. 7. Flattened sessile amoeboid cell (DIC). ch = chloroplast, n = nucleus, py = pyrenoid.

Fig. 8. Line drawing of a sessile amoeboid cell.

Fig. 9. Binary division of a sessile amoeboid cell (DIC).

Fig. 10. Quaternary division of a sessile amoeboid cell (DIC).

Fig. 11. Small naked spherical cell (DIC). ch = chloroplast.

daughter cells inherits a long filopodium after cytokinesis, while the other daughter cell does not. Subsequently, the daughter cell with a long filopodium transports its cell contents (i.e. organelles) through the filopodium at the distal end of the filopodium (Ota *et al.* 2007a). In the present study based on time-lapse microscopic observations we report a third type of unique daughter cell behaviour amongst chlorarachniophytes. We also examine the taxonomic position of a new chlorarachniophyte strain, P314, by using light and transmission electron microscopy and describe it as a new species of *Gymnochlora*.

MATERIAL AND METHODS

Sampling and culture

Strain P314 was established using a micropipette isolating method from an enrichment culture containing *Padina* sp. which was collected from a subtidal coral reef zone, near Ongael Island, Palau (7°25′N, 134°37′E), on 28 May 2003. The culture was maintained in ESM (Kasai *et al.* 2009) or K medium (Keller *et al.* 1987) at 20°C under a 14:10 light:dark cycle with 20–40 μmol photons m^{-2} s^{-1} from cool-white bulbs.

Light and transmission electron microscopy

Cells were observed with Nomarski differential interference contrast and phase contrast optics using a Leica DMR microscope (Leica, Wetzlar, Germany). Light micrographs were taken using a DP70 CCD camera (Olympus, Tokyo, Japan) or a VB6010 CCD camera (Keyence, Osaka, Japan). For transmission electron microscopy, cells were fixed and prepared as described by Ota *et al.* (2009b). Observations for TEM were carried out using a JEM-1010 electron microscope (JEOL, Tokyo, Japan).

Time-lapse microscopy

Cells were grown for several days on a small, sterile coverslip (18 × 18 mm) which was placed in a culture dish. The coverslip was removed and placed on a microscope slide, and a larger coverslip (24 × 24 mm) was then placed over the smaller coverslip. To reduce evaporation, the edges of the large coverslip were sealed with 'VALAP', a 1:1:1 mixture of paraffin wax, lanolin and vaseline. The cells were examined with Nomarski differential interference contrast optics using a Nikon Optiphot microscope (Nikon, Tokyo, Japan). Sequential images were taken with a QICAM CCD camera (model: QIC-CLR-12, QImaging, British Columbia, Canada) and edited and assembled using ImageJ v.1.43u (National Institutes of Health, Bethesda, MD, USA).

RESULTS AND OBSERVATIONS

Gymnochlora dimorpha S. Ota *sp. nov.*

Figs 1–15, 17–63

Cellulae solitariae, amoeboideae, stellatae ad circulares, mobiles ('cellulae mobiles'); vel circulares ad ellipticae, complanatae, sessiles ('cellulae complanatae'). Cellulae mobiles, 7–14 μm in diam., filopodiis ramosis ver inramosis, dominans in cultura. Nucleus unicus. Cellulae plurichloroplastis ad peripheriam. Chloroplasti bilobati, virides, pyrenoide projecta. Cellulae complanatae, 12–18 μm diam., ~12 filopodiis; Chloroplasti complanati, virides, pyrenoide projecta. Cellulae complanatae post divisionem, filia una sessilis, filia diversa mobilis. Pyrenoidis projectus; membrana intima chloroplasti invasa in matricem pyrenoidem. Nucleomorphus in base pyrenoidis in spatio periplasti. Cellulae giganteae multinucleo in culturis vetustioribus.

Cells solitary, amoeboid, stellate to circular with motility ('motile cells'); or circular to elliptic and flattened, sessile ('flattened cells'). Motile cells, 7–14 μm in diameter, with branched or unbranched filopodia, dominant in culture. Nucleus one. Cell with several chloroplasts at cell periphery; chloroplasts bilobed, green, with a projecting pyrenoid. Flattened sessile cells, 12–18 μm in diameter, filopodia ~12 in number. After division of a flattened sessile cell, one daughter cell remains sessile while the other becomes motile. Pyrenoid with many tubular intrusions of the innermost membrane of the chloroplast envelop. A nucleomorph located in the pyrenoid base in the periplastidial compartment. Multinuclear giant cells emerging in older cultures.

HOLOTYPE: One microscope slide (PC0146173), deposited in the Muséum National d'Histoire Naturelle, Paris (PC). Figs 3 and 4 are designated as iconotype images.

ISOTYPE: One microscope slide (TNS-AL-56963) is deposited at the National Museum of Nature and Science, Tsukuba, Japan (TNS). One TEM block (TNS-AL-56964) is also deposited at TNS.

AUTHENTIC CULTURE: RCC1935. The culture used here (original strain name P314) is deposited at Roscoff Culture Collection (RCC), France.

DNA SEQUENCE INFORMATION: The accession number EF622547 is a nuclear SSU rDNA sequence of *G. dimorpha* sp. nov. (Silver *et al.* 2007).

TYPE LOCALITY: Off Ongael Island, Republic of Palau, Pacific Ocean (7°25′N, 134°37′E), 28 May 2003, collected by S. Ota, Y. Hirakawa and K. Ishida.

HABITAT: Littoral, marine.

ETYMOLOGY: The species epithet refers to the two types of amoeboid cells.

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Fig. 12. Large multinucleate amoeboid cell (DIC).
Fig. 13. DAPI stained cell of Fig. 12, showing many nuclei in the amoeboid cell.
Fig. 14. Large multinucleate cell after cytokinesis (DIC).
Fig. 15. DAPI stained cell of Fig. 14, showing single nucleus in a daughter cell.

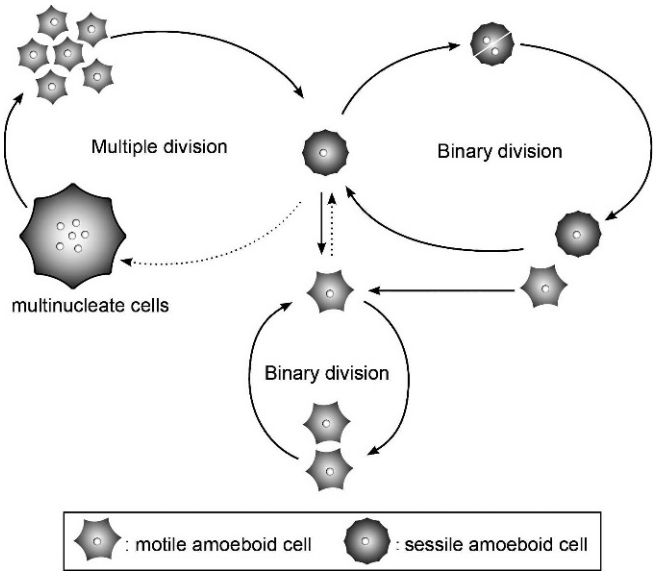
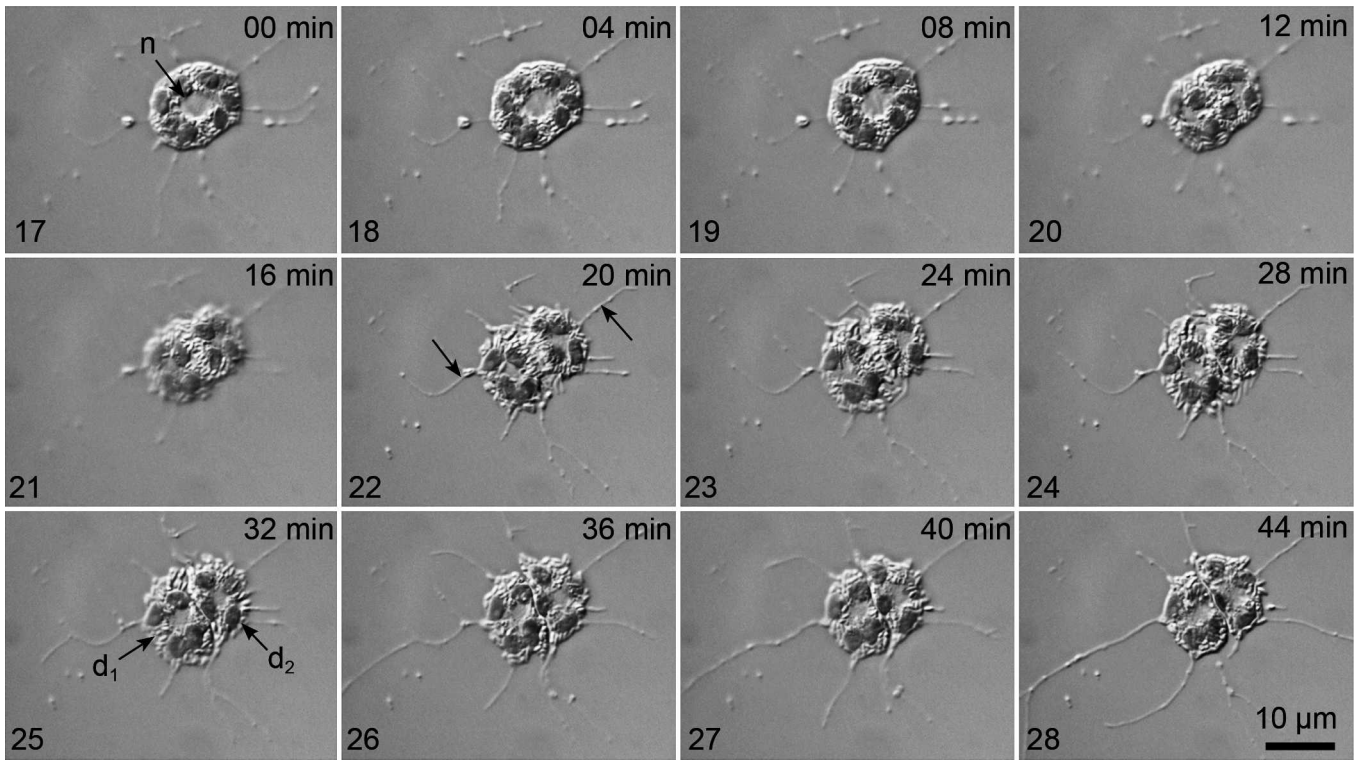


Fig. 16. Interpretative diagram of the life cycle of *Gymnochlora dimorpha* sp. nov. Solid line arrows indicate the processes confirmed in the present study, and dotted line arrows indicate predicted processes.

OTHER STRAINS EXAMINED: **Ongael, Palau:** Strain 'P310', from a sample of *Padina* sp. on coral reef, 28 May 2003, leg. Ota, S., Hirakawa, Y. & Ishida, K.; **Ngerechong Inside, Palau:** Strain 'P328', from sand grains on *Halimeda* sp. in coral reef, 25 May 2003, leg. Ota, S., Hirakawa, Y. & Ishida, K.

Remarks

LIGHT MICROSCOPY: Under the culture conditions used in the present study, amoeboid cells were the dominant form (Fig. 1). We observed two types of amoeboid cells, motile (nonlabelled cells in Fig. 1) and flattened nonmotile (sessile; arrow-labelled cells in Fig. 1). The motile amoeboid cells were dominant in the culture and were able to migrate on substrata. The main bodies of the motile amoeboid cells were almost spherical to star-shaped with several (~12 in number) filopodia (Figs 1–5). The cell size ranged (without filopodia) from 7 to 14 μm (mean = 10 μm, n = 50) in diameter. When the cells were moving, they were nearly fusiform and extended a filopodium in the direction of the movement (Fig. 6). The amoeboid cells possessed branched (or sometimes unbranched) filopodia extending radially from the cell periphery (Fig. 2). Each cell usually possessed two to six chloroplasts that were green and bilobed (Figs 2–5). The chloroplast possessed a single spherical to ovoid



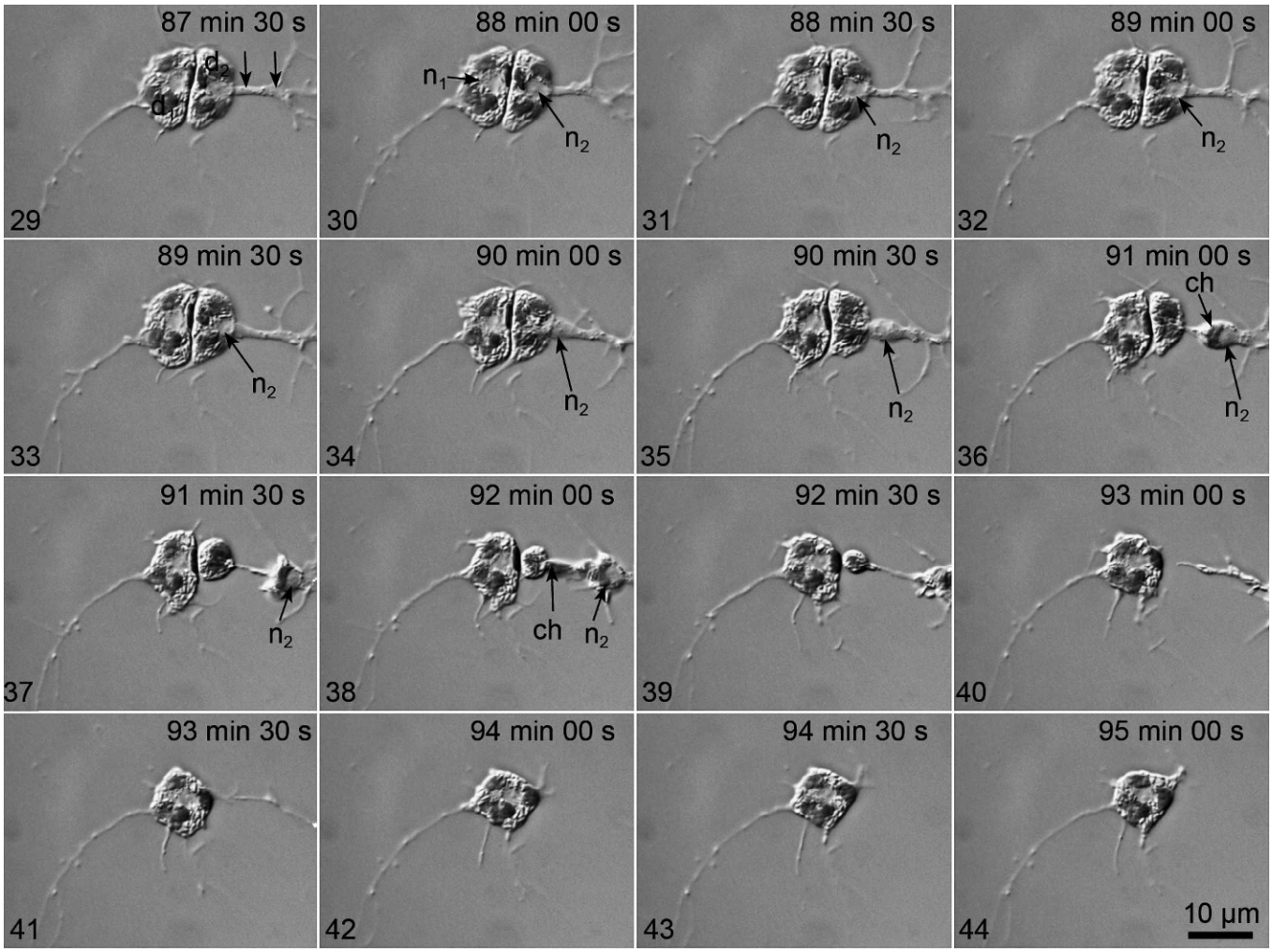
Figs 17–28. Time-lapse video sequence, showing cell division of a sessile amoeboid cell of *Gymnochlora dimorpha* sp. nov. The time-lapse sequence is shown at 4 min intervals. The times are shown on each frame (T = 0 at Fig. 17).

Fig. 17. Prophase cell. n = nucleus.

Figs 19–20. Metaphase cell.

Figs 21–24. Anaphase cell. After nuclear division, cytokinesis occurs. Note that the filopodia emerge again at the beginning of cytokinesis (arrows in Fig. 22).

Figs 25–28. When cytokinesis is completed, two identical daughter cells are formed (d_1 and d_2), both of which show no amoeboid locomotion for about 1 h.



Figs 29–44. Time-lapse video sequence, showing cell division of a sessile amoeboid cell of *Gymnochlora dimorpha* sp. nov. The sequence is shown 43.5 min after the frame of Fig. 28. The time-lapse sequence is shown at 0.5 min intervals. The times are shown on each frame.

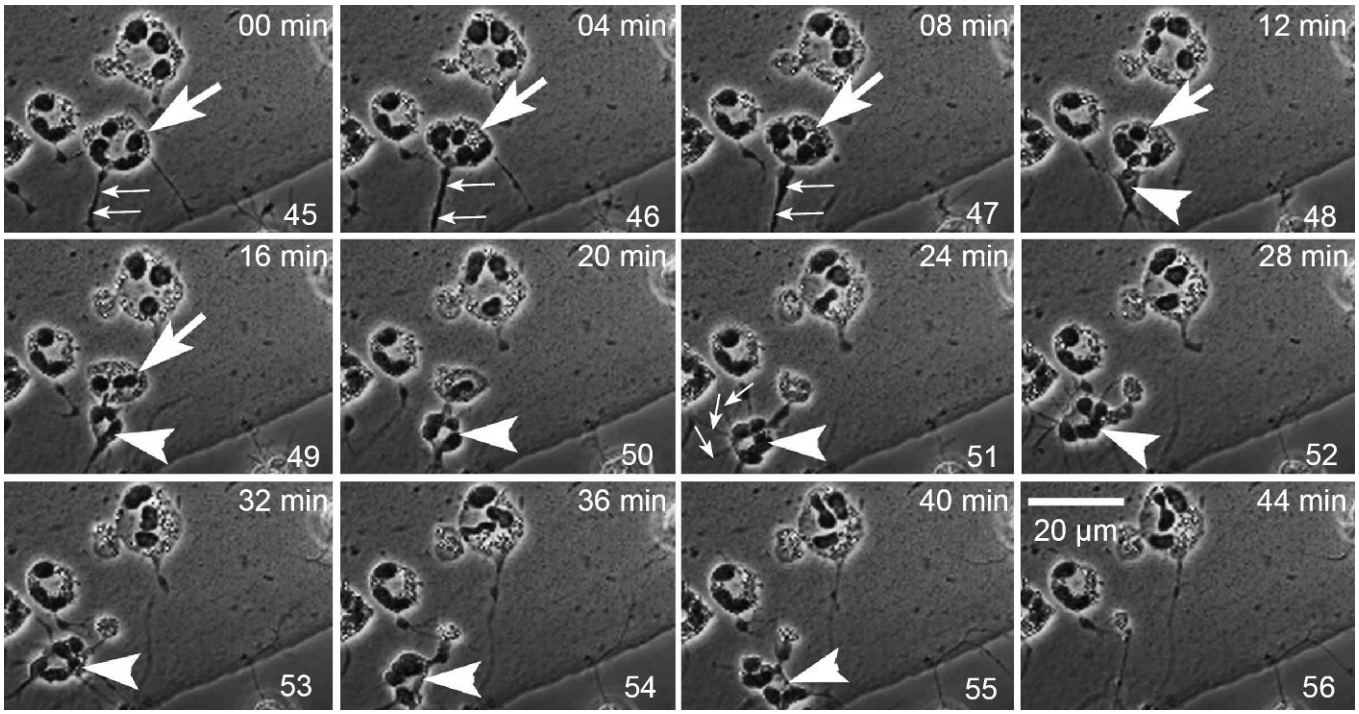
Fig. 29. One of the daughter cells (d_2) thickens its filopodium (arrows).

Figs 30–38. When transporting the organelles, the nucleus n_2 is at first moved distally. Following the nucleus movement, chloroplasts (ch) and other organelles (e.g. mitochondria) are transported distally through the filopodium. n_1, n_2 = nuclei of daughter cells.

Figs 39–44. Once all the organelles are transported, one of the daughter cell becomes a motile amoeboid cell, while the other daughter cell remains sessile.

pyrenoid, which was often observed between the chloroplast lobes (Fig. 5). A spherical nucleus was located in the middle region of the cell (Figs 3–5). The sessile amoeboid cells were flattened and attached to substrata such as glass tubes or culture flasks (Figs 7, 8). The main bodies of the sessile amoeboid cells were almost circular to slightly polygonal, 12–18 μm (mean = 15 μm, n = 14) in diameter (Figs 7, 8). This cell type was never dominant in the culture but was always observed in cultures at any stage (i.e. fresh, middle-aged or old). The filopodia, 0–12 per cell, were of varying lengths and very fine and emerged radially from the cell body (Figs 7, 8). Each sessile amoeboid cell possessed several parietal green chloroplasts, some of which possessed a single spherical to ovoid pyrenoid between the chloroplast lobes (Figs 7, 8). In the cytokinesis of the sessile amoeboid cells, binary cell divisions were often observed, but quaternary cell divisions were sometimes also observed (Figs 9, 10). Both types of amoeboid cells (i.e. motile and sessile amoeboid cells) did not form a meroplasmodium (reticulopodial network). In all stages of cultures, small (< 10 μm in diameter) naked spherical cells were sometimes observed (Fig. 11), but the cells never became dominant in the culture. No walled cells were observed under the culture conditions. In old cultures (1 month or older), somewhat large amoeboid cells appeared (Figs 12, 13). They were multinucleate, spherical to irregularly shaped, and ranged from 15 to 40 μm in diameter. The cells possessed numerous chloroplasts and many filopodia of varying lengths (Figs 12, 13). DAPI staining of the multinucleate cells showed ~17 nuclei (Figs 12, 13) or more (data not shown). Cytokinesis was observed in the multinucleate cells when the culture was replenished. After cytokinesis of a multinucleate cell, the daughter cells finally became motile amoeboid cells, each of which possessed a single nucleus (Figs 14, 15).

LIFE CYCLE: The life cycle of *G. dimorpha* is summarized in Fig. 16. Motile amoeboid cells, which were able to divide into two identical daughter cells, were dominant in the cultures.



Figs 45–56. Time-lapse video sequence of a sessile amoeboid cell of *Gymnochlora dimorpha* sp. nov., showing the direct transformation from a sessile amoeboid cell (large arrow) to a motile amoeboid cell (arrowhead). The time-lapse sequence is shown at 4 min intervals. The times are shown on each frame ($T = 0$ at Fig. 45).

Figs 45–47. Sessile amoeboid cell thickens its filopodium (small arrows).

Figs 48–51. Unidirectional cytoplasmic streaming through the filopodium. Note that several fine filopodia are visible around the cell (small arrows in Fig. 51).

Figs 52–56. When the cytoplasmic streaming is completed, the flattened amoeboid cell becomes a motile amoeboid cell and commences movement.

Therefore, the amoeboid cells represented the vegetative stage. In the cell division of sessile amoeboid cells, binary cell divisions were mainly observed. After the binary divisions of the sessile amoeboid cell, one daughter cell remained sessile and amoeboid; whereas, the other became motile and amoeboid. The sessile amoeboid cell sometimes became motile even without cell division. In contrast, transformation of a motile to a sessile amoeboid cell was not observed. However, sessile amoeboid cells were always observed in cultures originating from an isolated motile amoeboid cell, thus a motile amoeboid cell may become a sessile amoeboid cell. With increasing age of the culture, large multinucleate cells were sometimes observed. They were able to divide into several daughter cells possessing a single nucleus. Zoospores or flagellate cells were not observed at any point in the life cycle. No sexual reproduction was observed under the culture conditions applied.

BEHAVIOUR OF DAUGHTER CELLS AFTER CYTOKINESIS: Time-lapse video sequences of cytokinesis and postcytokinesis behaviour are shown in Figs 17–28 and Figs 29–44. During prophase of a sessile amoeboid cell, the nucleus was located in the centre of the cell, and several chloroplasts were located around it (Figs 17, 18). In metaphase, the first karyokinesis occurred (Figs 19, 20) followed by cytokinesis (Figs 21–24). After completion of cytokinesis, two morphologically identical daughter cells were formed, and neither showed amoeboid movement for about 1 h after

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Figs 57–63. Transmission electron micrographs of *Gymnochlora dimorpha* sp. nov.

Fig. 57. General ultrastructure, showing a nucleus (n) with an electron opaque nucleolus (arrow), mitochondria (m), chloroplasts (ch), and transverse section of pyrenoid (py). The boxed area is enlarged in Fig. 59.

Fig. 58. Mitochondria with tubular cristae.

Fig. 59. Detail view of the four membranes surrounding the chloroplast, showing outer two membranes (arrowheads) and inner two membranes (arrows), a nucleomorph (nm) in the periplastidial compartment is visible. ch: chloroplast.

Fig. 60. Longitudinal section of chloroplast and pyrenoid. The boxed area is enlarged in Fig. 61. Nm = nucleomorph.

Fig. 61. Detail view of the pyrenoid matrix, showing many tubular structures (arrows) originating from the innermost membrane of the chloroplast envelope.

Fig. 62. Transverse section of pyrenoid, showing many tubular structures (arrows) originating from the innermost chloroplast membrane.

Fig. 63. Longitudinal section of chloroplast and pyrenoid, showing that a nucleomorph is located near the pyrenoid base in the periplastidial compartment. Some electron-opaque globules (arrows) are visible in peripheral region of the nucleomorph.

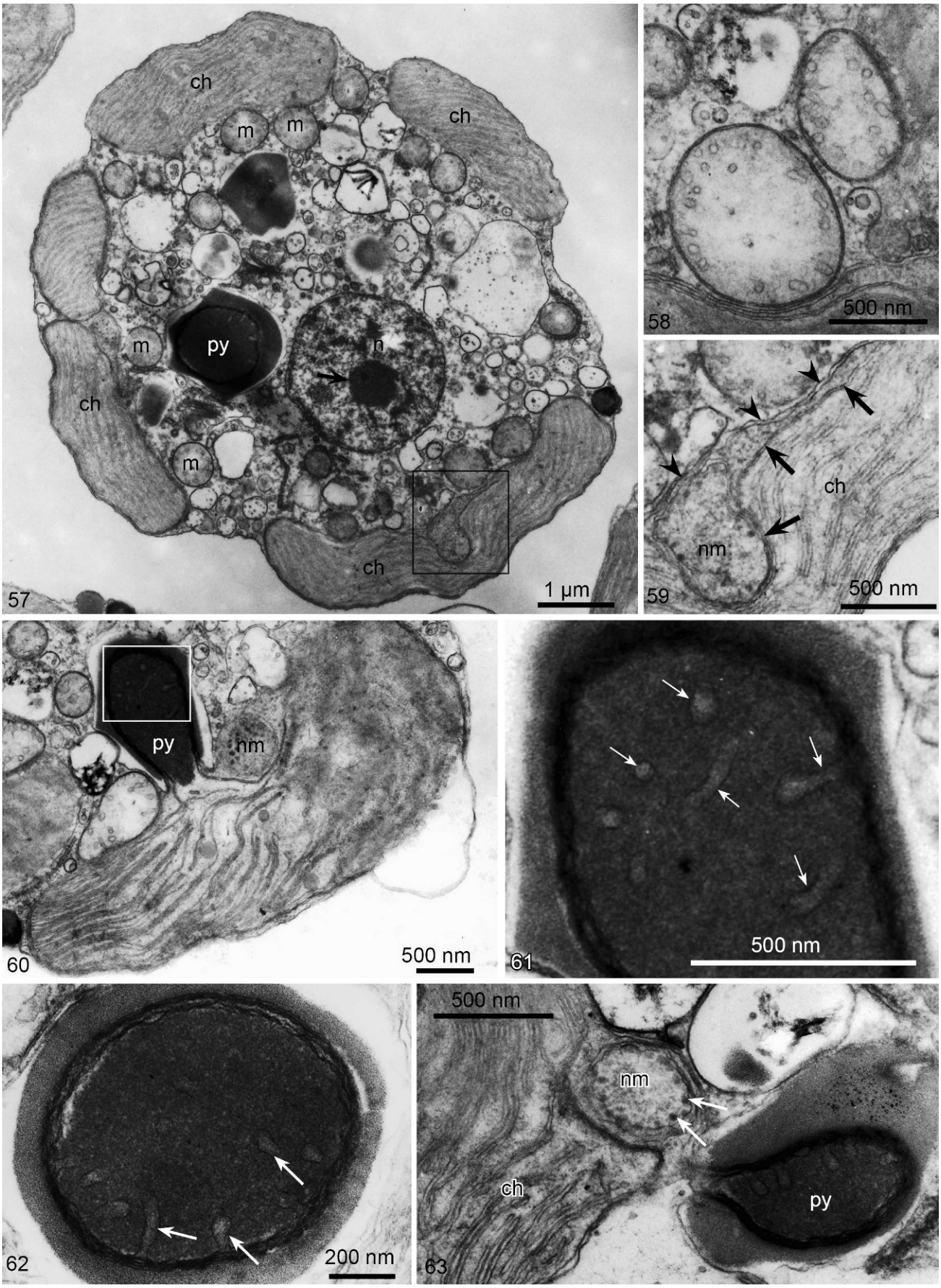
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Table 1. Comparison of life cycles, morphology and ultrastructure among *Gymnochlora* species.

	Cell diameter (vegetative cells)	Flat sessile amoeboid cells	Pyrenoid type	Main vegetative stage
G. stellata[1]	10–20 μm	absent	tubular invagination	amoeboid
G. dimorpha sp. nov.[2]	7–14 μm	present	tubular invagination	amoeboid

[1] Ishida *et al.* (1996).
[2] This study.

cytokinesis (Figs 25–28, 29). Subsequently the filopodium of one daughter cell thickened (Figs 29–32) and unidirectional cytoplasmic streaming occurred inside the filopodium. The nucleus was first to move and then the other organelles (e.g. chloroplast and mitochondria) were transported distally (Figs 33–38). Once all the organelles were transported, the daughter cell became a motile amoeboid cell which was morphologically identical to a normal vegetative cell, while the other daughter cell showed almost no movement and remained sessile (Figs 39–44). The cytoplasmic streaming after cytokinesis took about 4 min to complete in the present time-lapse video observation.

DIRECT TRANSFORMATION FROM A SESSILE AMOEBOID CELL TO A MOTILE AMOEBOID CELL: Direct transformation from a sessile amoeboid cell to a motile amoeboid cell was sometimes observed; that is, a sessile amoeboid cell sometimes became a motile amoeboid cell without cell division. Time-lapse video sequence of this scenario is shown in Figs 45–56. Once the sessile amoeboid cell thickened its filopodium (Figs 45–47), unidirectional cytoplasmic streaming occurred. First, the nucleus was transported distally through the thickened filopodium, then the chloroplasts and other organelles were transported distally (Figs 48–52). When the cytoplasmic streaming was complete, the flattened amoeboid cell became a motile amoeboid cell which was morphologically identical to a normal vegetative cell (Figs 53–56). The transformation took about 40 min to complete in the present time-lapse video observation.

ULTRASTRUCTURE: The general ultrastructure of an amoeboid cell is shown in Fig. 57. The cell was naked and possessed a nucleus, several chloroplasts and mitochondria (Fig. 57). The nucleus was spherical and located in the centre of the cell. An electron-opaque nucleolus was observed in the centre of the nucleus (Fig. 57). Several mitochondria with tubular cristae were observed in the cytoplasm (Figs 57, 58). The chloroplasts were located in the parietal region of the cell (Fig. 57). They were surrounded by four chloroplast membranes (Fig. 59). The chloroplast matrix was traversed by stacks of two or three thylakoids (Fig. 59). The periplastidial compartment, i.e. the space between the second and the third membrane, was partially observed (Fig. 59). A bulbous pyrenoid projecting toward the centre of the cell was observed in the longitudinal section of the chloroplast (Fig. 60). The pyrenoid matrix was filled with electron-opaque material and was invaded by many tubular structures originating from the innermost membrane of the chloroplast envelope (Figs 61, 62). A nucleomorph was located in the periplastidial compartment near the base of the pyrenoid (Figs 60, 63). The nucleomorph was surrounded by double membranes and possessed several small electron-opaque globules in a parietal position (Fig. 63).

DISCUSSION

Taxonomy

Gymnochlora dimorpha (strain P314) possesses green chloroplasts surrounded by four membranes, a nucleomorph in the periplastidial compartment and mitochondria with tubular cristae. These are significant characters that we used to assign the strain to the phylum Chlorarachniophyta. Ishida *et al.* (1996, 2011) and Ota *et al.* (2007b, 2009a) recognized the following set of features for assignment at the generic level (except for *Cryptochlora* for which ultrastructural information is yet unavailable): (1) pyrenoid ultrastructure if the pyrenoid is present; (2) nucleomorph position; and (3) the dominant cell type in the vegetative stage. According to these criteria, the present chlorarachniophyte has to be classified within *Gymnochlora* because it has the diagnostic features which define the genus. There is (1) a pyrenoid with tubular invaginations; (2) a nucleomorph located near the pyrenoid base in the periplastidial compartment; and (3) the vegetative cells are amoeboid. A recently published molecular phylogeny of chlorarachniophytes showed the strain P314 (*G. dimorpha*) and its close relatives (strains P310 and P328) in a well-supported clade that includes *G. stellata* Ishida & Y. Hara, the type species of *Gymnochlora* (Silver *et al.* 2007; Ota *et al.* 2009a). This supports our present taxonomic conclusion as based on morphological observations.

At the species level, however, *G. dimorpha* is clearly different from *G. stellata* in morphological and life cycle characteristics (Table 1). In the life cycle of *G. dimorpha*, two cell types of the amoeboid stage (a motile and sessile amoeboid cell) are observed, while *G. stellata* has only one cell type (a motile amoeboid cell). In addition, although there is some overlap in cell diameter between *G. stellata* and *G. dimorpha*, cell diameters of *G. dimorpha* are smaller than those of *G. stellata* (Table 1). A recently published molecular phylogenetic tree showed the *Gymnochlora* clade divided into three subclades: CCMP2014, *Gymnochlora stellata* and P314 (i.e. *G. dimorpha*; Ota *et al.* 2009a). This molecular study suggested that *G. dimorpha* was phylogenetically distant from the type species. Based on the morphological, ultrastructural and molecular studies (Silver *et al.* 2007; Ota *et al.* 2009a), the chlorarachniophyte examined in the present study must be placed in the genus *Gymnochlora* and described as a new species, *Gymnochlora dimorpha* sp. nov.

LIFE CYCLE AND DAUGHTER CELL BEHAVIOUR: Under the culture conditions applied in the present study, *G. dimorpha* completely lacks a flagellate stage in its life cycle. *Gymnochlora stellata*, the type species of *Gymnochlora*, also

lacks the zoospore stage (Ishida *et al.* 1996). In contrast, other chlorarachniophytes possess zoospore or flagellate stage in their life cycle (Calderon-Saenz & Schnetter 1987; Ishida & Hara 1994; Ishida *et al.* 2000; Moestrup & Sengco 2001; Dietz *et al.* 2003; Ota *et al.* 2005, 2007a, b, 2009a, b). Thus, the lack of zoospores may represent a synapomorphy for *Gymnochlora* species.

We observed multinucleate amoeboid cells in older cultures of *G. dimorpha*. The multinucleate cells were relatively large (~40 μm) and possessed many nuclei per cell (up to 17 were observed here), and thus it is easy to distinguish the multinucleate from the vegetative stage. *Gymnochlora stellata* also forms multinucleate amoeboid cells in old cultures (M. Kaneda, unpublished observations). Like the *Gymnochlora* species, the chlorarachniophyte *Norrisiella sphaerica* S. Ota & K. Ishida also possesses a multinucleate stage (Ota *et al.* 2007b). Bass *et al.* (2009) reported that some endomyxan species formed multinucleate amoeboid cells in old cultures. The endomyxans are the sister group of filoseans, both of which are major components of the cercozoans. Because the multinucleate stage is observed in various lineages of the cercozoans, the life cycle feature of the multinucleate stage may accordingly be a primitive state in the cercozoans. However, the biological implications of multinucleate amoeboid cells are not known. Further studies are needed to understand the life cycle evolution and the biological implications of multinucleate cells in the cercozoans.

The present time-lapse video observation shows that two different types of daughter cells are formed after cytokinesis; that is, one becomes a motile amoeboid cell which is identical to a vegetative cell and the other remains sessile. The three chlorarachniophytes, *Lotharella polymorpha*, *L. vacuolata* and *Bigelowiella longifila*, also show similar post-cytokinesis behaviour (Dietz *et al.* 2003; Ota *et al.* 2005, 2007a). However, the behaviour differs somewhat depending on the species. For example, *L. vacuolata* possesses coccoid cells as its vegetative stage, and the behaviour is always observed in the coccoid cells; whereas, in *G. dimorpha*, the behaviour is observed only in amoeboid cells. In spite of the differences, 'unilateral' cytoplasmic streaming always occurs in all species possessing the behaviour (Ota *et al.* 2005, 2007a; this study). These observations suggest that the behaviour may come from a common origin rather than having arisen independently amongst the chlorarachniophytes. However, it is still unclear whether heterotrophic cercozoans possess the unique daughter cell behaviour. In addition, the biological implications of this ability are less well-understood. In order to understand the evolution of the life cycle, the behaviour and its biological implications, wider taxon sampling and further life cycle surveys using video microscopy are needed not only in chlorarachniophytes but also in heterotrophic cercozoans.

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