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Atypical development of *Chaetomorpha antennina* in culture (Cladophorales, Chlorophyta)

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SUMMARY

The green seaweed genus *Chaetomorpha* is characterized by unbranched filaments. Molecular phylogenetic data indicate that *Chaetomorpha* forms a clade that is nested in a paraphyletic assemblage of branched species (*Cladophora*). It follows that the unbranched condition is evolutionarily conserved and likely evolved early in the evolution of this clade. In this study we show that under laboratory culture conditions, the filaments of *C. antennina* frequently produce lateral branches, similar to *Cladophora*. Our results thus indicate that the unbranched thallus architecture is not entirely genetically constrained, but at least in part subject to morphological plasticity. Additionally, culture observations of *C. antennina* allowed a detailed study of rhizoidal development, which seems unique among Cladophorales.

Key words: culture, green algae, morphological development, phenotypic plasticity.

INTRODUCTION

Chaetomorpha is a common and widespread green seaweed genus, characterized by unbranched filaments. Molecular phylogenetic data indicate that *Chaetomorpha* forms a clade, which is nested in a paraphyletic assemblage of branched *Cladophora* species (Leliaert *et al*. 2009). This suggests that the unbranched filamentous architecture evolved from branched forms early in the evolution of the *Chaetomorpha* clade and that this morphological character likely has a strong genetic basis. It should be noted, however, that apart from *Chaetomorpha*, unbranched forms have evolved several times independently in the Cladophorales, i.e. in a clade containing *Rhizoclonium riparium* (Roth) Harvey and in *R. africanum* Kützing (Leliaert *et al*. 2009).

About 50 *Chaetomorpha* species are currently recognized based on a few morphological features, such as growth form, cell dimensions and shape of the basal attachment cell. The scarcity of diagnostic characters, combined with ecologically induced variations accounts for vague boundaries in several species (Leliaert & Boedeker 2007). However, some species are clearly circumscribed and exhibit a fairly constant morphology throughout their ecological and geographical range, for example *C. antennina* (Bory de Saint-Vincent) Kützing, *C. moniligera* Kjellman, *C. robusta* (Areschoug) Papenfuss, *C. spiralis* Okamura and *C. vieillardii* (Kützing) Wynne.

Chaetomorpha antennina has a pantropical distribution and is easily identifiable by its characteristic erect, brush-like tufts, composed of straight, rigid filaments borne on a long clavate basal cell with annular constrictions (Børgesen 1913; Abbott & Hollenberg 1976; Abbott & Huisman 2004; Coppejans *et al*. 2005; Kraft 2007; Coppejans *et al*. 2009). The species typically grows on intertidal to shallow subtidal rocks that are surf-exposed or subject to strong surge. The plants firmly attach by rhizoids developing from the proximal pole of the basal cells, resulting in a profusely branched, stoloniferous rhizoidal system.

In this paper we describe atypical development of *C. antennina* under laboratory culture conditions, including formation of branches in the upright filaments. Culture observations also allowed a detailed study of the development of the stoloniferous rhizoidal system in this species.

MATERIALS AND METHODS

Chaetomorpha antennina plants were collected along the Pacific coast of Mexico (Table 1). Portions of each specimen were preserved in 5% formalin in seawater, dried in silica gel, and kept alive in seawater. After thalli were transferred to fresh seawater in the lab, sporangia developed within 1–2 weeks, and unialgal cultures were established by isolation of spores. Algal cultures were grown in sterile 1× modified Provasoli enriched seawater (West 2005) under 'high' and 'low' light intensity (25–30 and 5–10 μE m^{-2} s^{-1}) and 'high'

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Table 1. Specimens from which partial large subunit (LSU) nrDNA sequences were newly determined

Species	Collection information	EMBL accession numbers
Chaetomorpha antennina	GUAM117, Guam: Talofofo Bay (Schils T., 23 Jul. 2007)	FN687234
C. antennina	GUAM118, Guam: Talofofo Bay (Schils T., 23 Jul. 2007)	FN687235
C. antennina	HEC15206, Madagascar : Tolanaro: Plage de Libanona, wave-exposed intertidal rocky flat (Coppejans E., 30 Aug. 2002)	FN687236
C. antennina	KZN2047, South Africa: Kwazulu-Natal: Palm Beach, intertidal (Leliaert F., 7 Feb. 2001)	FN687237
C. antennina	KZN2322, South Africa (Indian Ocean): Kwazulu-Natal: Durban: Isipingo (Leliaert F., 16 Jun. 2003)	FN687238
C. antennina	MX0172, Mexico (Pacific Ocean): Guerrero: Acapulco: Canal de Boca Chica: Isla Roqueta, exposed intertidal rock on vertical wall facing ocean (Verbruggen H. & Tyberghein L., 19 Feb. 2009)	FN687239
C. antennina	MX0220, Mexico (Pacific Ocean): Guerrero: Ixtapa: Playa del Palmar, on seaward side of intertidal rock boulder on the beach (Verbruggen H. & Tyberghein L., 21 Feb.2009)	FN687240
C. antennina	MX0275, Mexico (Pacific Ocean): Colima: Manzanillo: Playa la Audiencia, intertidal wave channel, exposed to heavy wave action (Tyberghein L. & Verbruggen H., 23 Feb. 2009)	FN687241
C. gracilis	HEC12944, Tanzania: Mtwara: Mana Hawanja, intertidal rock flat; shallow pool (Coppejans E., 29 Jul. 2000)	FN687242
Chaetomorpha sp.	Bunker5, United Kingdom: Wales: Pembrokeshire: Milford Haven, on maerl, 2–4 m deep (Bunker F., 6 Nov. 2007)	FN687243
Chaetomorpha sp.	HV709, Philippines: Bohol: Panglao, sandy intertidal flat, loosely attached to a seagrass (Verbruggen H., 1 Feb. 2004)	FN687244
Rhizoclonium africanum	FL703, Tanzania: Zanzibar: Nungwi, supralitoral, epilithic (Leliaert F., 26 Jul. 1997)	FN687245

and 'low' temperature (23°C and 17°C). Cultures were not shaken or aerated. Photographs were taken with a ColorView (Olympus) digital camera mounted on an Olympus BX51 bright field microscope or Olympus SZX10 stereo microscope (Olympus Co., Tokyo, Japan).

Morphology-based species identification was verified by sequencing the variable C1D2 region of the large subunit (LSU) nrDNA of the Mexican plants (field-collected specimens and cultures) along with additional specimens of *C. antennina* from South Africa, Madagascar and Guam (Table 1). DNA extraction, polymerase chain reaction (PCR) amplification and sequencing were performed as described in Leliaert *et al*. (2007). The newly generated sequences were combined with available sequences from 10 *Chaetomorpha* species and other representatives of the *Cladophora* clade (as circumscribed by Leliaert *et al*. 2009). Sequences were aligned using MUSCLE (Edgar 2004), and visually inspected. The dataset was analyzed with Bayesian inference (BI), using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003), under a GTR+G model as determined by the Akaike Information Criterion in JModeltest v0.1.1 (Posada 2008). The analysis consisted of 4 million generations with sampling every 1000 generations. A burn in sample of 2000 trees was removed before constructing the majority rule consensus tree.

RESULTS

The BI tree (Fig. 1) placed all *Chaetomorpha antennina* specimens in a clade of closely related sequences, preceded by a well supported branch, confirming the morphology-based species identifications. LSU nrDNA sequences of the field-collected plants and cultures were identical.

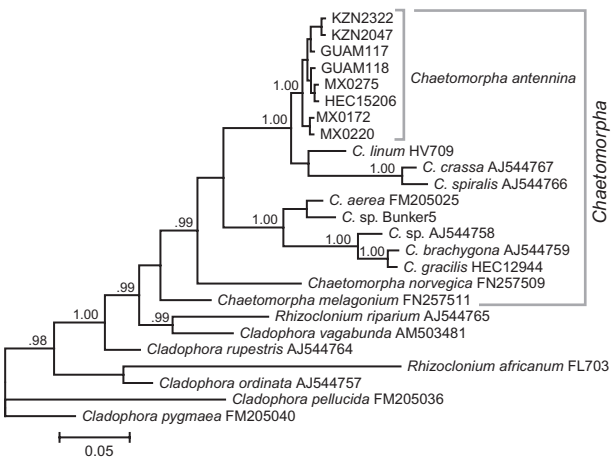
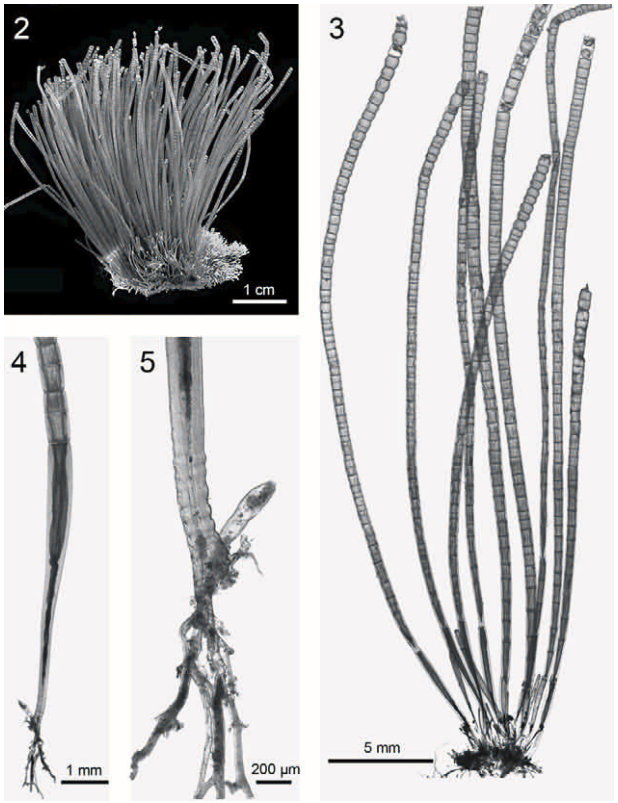


Fig. 1. Bayesian phylogenetic tree inferred from partial large subunit (LSU) nrDNA sequences, showing possible monophyly of *Chaetomorpha*, and all *C. antennina* specimens clustering in a well supported clade of closely related sequences.

Field collected plants from Mexico have a typical *C. antennina* morphology (Figs 2–5), forming erect brush-like tufts, 1–5(–8) cm high, composed of straight, rigid filaments. Plants are firmly attached to the substrate by rhizoids sprouting from the proximal pole of the basal cells, forming a compact basal mat. The elongated,



Figures 2–5. Field collected *Chaetomorpha antennina* from Mexico (MX0172). 2–3. Erect, brush-like tuft composed of straight filaments developing from a compact mat of rhizoids. 4. Elongated, club-shaped basal holdfast cell of a filament. 5. Detail of the proximal pole of a holdfast cell showing a thick striated cell wall with annular constrictions.

thick-walled holdfast cells are slightly club-shaped, with a few annular constrictions near the base, 400–700 µm in diameter at the distal end and up to 7.5 mm long. Other cells are subcylindrical, 400–750 µm in diameter, 700–1000 µm long, gradually becoming broader and barrel-shaped upward.

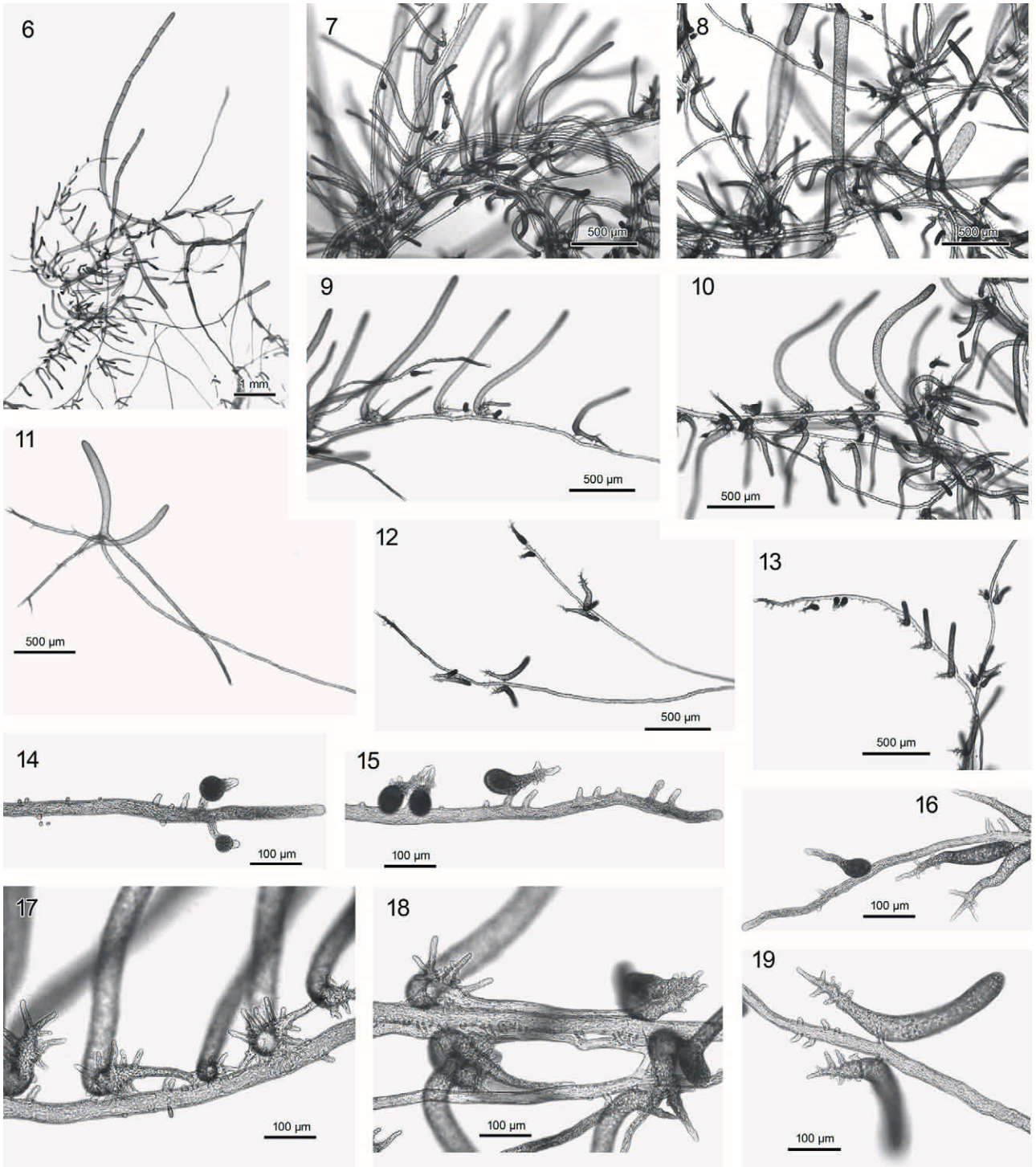
Similar to natural populations, plants in culture form profusely branched, stoloniferous rhizoids from which upright filaments develop (Figs 6–8). The creeping rhizoids are aseptate and extend by apical growth. Below the extending tips, short lateral branches are formed that enlarge into bulbous structures with a dense chloroplast layer, and are later cut off from the rhizoid by a cross-wall (Figs 14–16). At one end, these spherical bulges produce a hapteroid structure that attaches to the culture dish (Figs 14,15 and 17), or a long rhizoid that remains unattached (Fig. 11). The hapteroid structures later extend into new stoloniferous rhizoids (Fig. 18), which often grow adjacent to older rhizoids (Figs 7,9 and 18). The other end of the bulges extends into elongated upright cells (Figs 6–10). Haptera and upright cells are either formed unilaterally (Figs 9,13) or in pairs along the stoloniferous rhizoids (Figs 10,12 and 19). The upright cells divide repeatedly by intercalary cell division (centripetal invagination of the cell wall), resulting in unbranched, septate filaments (Figs 20,34). Cells of the upright filaments are cylindrical, 150–250 µm in diameter and 300–900 µm long. The basal cells of the filaments are up to 3.5 mm long and lack annular constrictions. In numerous filaments, cells produce lateral branches at their apical pole (Figs 21–28). Cross-wall formation at the base of the branches is markedly delayed (Figs 22,27 and 28). Branches are sometimes produced by cells that appear to become transformed into zoosporangia (noticeable by the domed pores and reticulation of the chloroplast layer) (Figs 26–28). Older filaments produce one or two bulges at the basal pole of cells (Figs 23 and 29–31), which are cut off by a cross-wall (Fig. 32, arrow). These structures are similar to the bulbous structures produced by rhizoids, and also form a basal rhizoid (which reattaches to the substrate and develops into a new prostrate rhizoidal system) and an upright filament that re-divides by intercalary cell divisions (Figs 24,25 and 33). Culture isolates have only been observed reproducing asexually by zoospores. Biflagellate zoospores (Fig. 39) are formed by transformation of apical or intercalary cells into zoosporangia; which emerge through one to several domed pores (Figs 20,26 and 35–38). Zoospores settle on the culture dish, shed off their flagella and attach by a hapteroid structure (Figs 40–42). These attached sporelings elongate and produce new upright filaments, while the haptera grow into new rhizoidal systems (Fig. 11).

Under low light and/or low temperature conditions morphological development was similar (but growth rate remarkably lower) as under high light and temperature (above description and Figs 6–42), indicating that these factors do not influence branch formation.

DISCUSSION

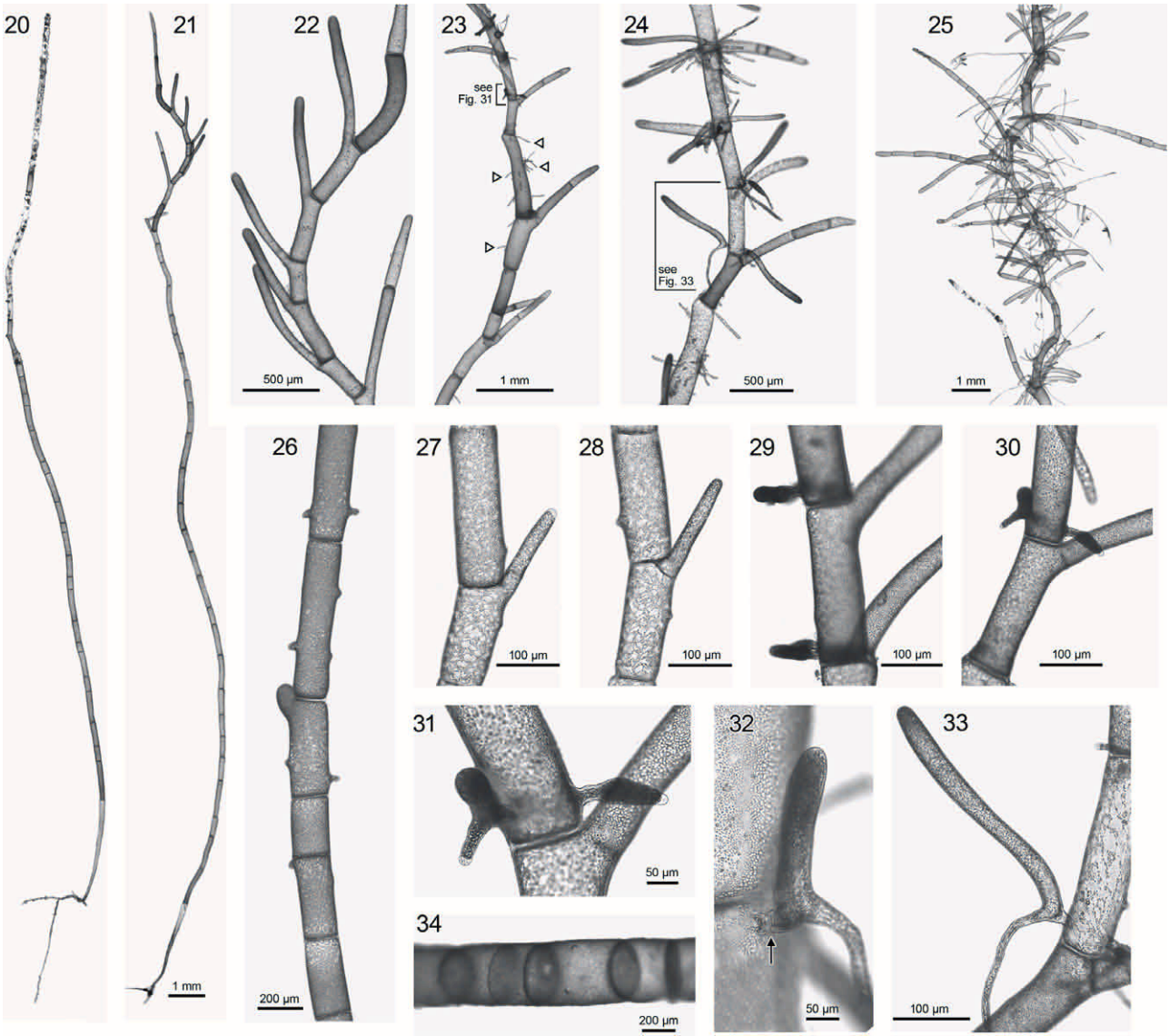
The morphology of *Chaetomorpha antennina* plants in culture differs markedly from that of natural populations. The most surprising observation is the formation of lateral branches by cells of the upright filaments, similar to *Cladophora* or *Cladophoropsis*. Other differences include longer and narrower cells, production of rhizoids in the upright filaments, and absence of annular constriction in the basal holdfast cell. Details of rhizoid formation are not well documented in *Chaetomorpha* or related genera but the observed rhizoidal development in *C. antennina* cultures seems unique among Cladophorales.

The atypical development of *C. antennina* in culture contrasts with the apparent constant morphology of



Figures 6–19. Culture of *Chaetomorpha antennina* (MX0172), development of rhizoidal system (see text for explanation).

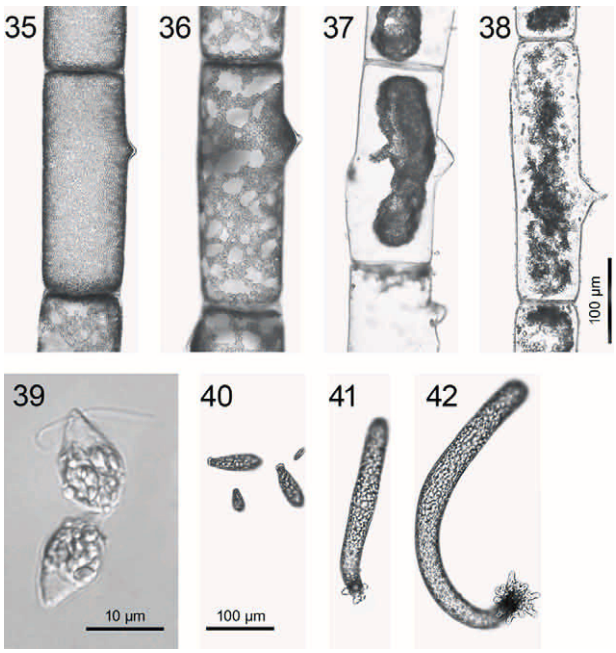
the species in nature. *Chaetomorpha antennina* typically grows on surf-exposed intertidal rocks, and the streamlined, unbranched filaments might be an adaptation or a plastic response to such dynamic environments, although it should be noted that several *Cladophora* species grow in similar environments. Several seaweeds and benthic, colonial marine animals (e.g. arborescent hydroids and bryozoans, gorgonian sea whips and sea fans) have been shown to display morphological plasticity in response to ambient water currents and grow into more streamlined shapes as flow velocity increases. Such reconfiguration by flowing water reduces the size of the wake downstream of a body, thereby reducing form drag (Carrington



Figures 20–34. Culture of *Chaetomorpha antennina* (MX0172), atypical development of filaments, including formation of branches and production of rhizoids by the filament cells (see text for explanation). Arrowheads in Fig. 23 indicate sporelings attached to the filaments, rather than rhizoids.

1990; Koehl *et al*. 2001). Similarly, our culture observations indicate that the unbranched thallus architecture of *C. antennina* might not be entirely genetically constrained but at least partly a plastic response to strong wave action and surge in natural habitats, suggesting that the genetic potential for branching is still present in *Chaetomorpha*. This assumption, however, is difficult to reconcile with the fact that all known *Chaetomorpha* species invariably form unbranched filaments in nature (including taxa that typically grow in calm water conditions).

Morphological plasticity in response to various environmental conditions has been demonstrated in laboratory culture experiments for several green macroalgae. For example in *Caulerpa*, extensive variability has been shown in response to different light intensities and temperatures (Calvert 1976; Ohba *et al*. 1992). In several *Cladophora* species (e.g. *C. albida*, *C. glomerata* and *C. sericea*) plants grown in laboratory cultures were found to be morphologically similar to natural forms from quiet, sheltered habitats (van den Hoek 1963). These experiments have indicated that, although morphological variability under culture conditions is often substantial, the variation generally falls within the species' natural morphological range. In this study we show that the morphology of *C. antennina* in culture is highly atypical for the species and even crosses the generic boundary. Unfortunately, we were not able to show which factors induce the formation of branches. More elaborate experiments will be needed as several



Figures 35–42. Culture of *Chaetomorpha antennina* (MX0172), development of zoosporangia, settlement of zoospores, and initial growth of sporelings (see text for explanation).

studies have shown that branching and rhizoidal development in green algae is controlled by complex processes (Inoue *et al*. 2002; Yoshida *et al*. 2003).

Branch formation in most Cladophorales (e.g. *Cladophora*, *Cladophoropsis*, *Chamaedoris*, *Boodlea* and *Phyllodictyon*) is initiated by lateral cell wall expansion in the apical cell pole, and is independent of cell division (Okuda *et al*. 1993). This type of cell morphogenesis is regulated by cytoskeletal and endomembrane rearrangements, which are triggered by various internal regulatory pathways involving signal transduction and hormonal control, as well as various external physical stimuli (Mine *et al*. 2008). Laboratory culture experiments have indeed shown that physical factors, such as light and temperature are important in controlling cell and thallus morphogenesis in green seaweeds (see above paragraph). However, information on environmental cues inducing branch formation in Cladophorales is scarce. Further studies, using culture experiments of unbranched and branched taxa, will be required to address this question. In addition future comparative genomic studies will facilitate the identification of the genetic bases controlling cell morphogenesis in siphonocladous green algae.

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REFERENCES

Abbott, I. A. and Hollenberg, G. J. 1976. *Marine Algae of California*. Stanford University Press, Stanford.

Abbott, I. A. and Huisman, J. M. 2004. *Marine Green and Brown Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu.

Børgesen, F. 1913. The marine algae of the Danish West Indies. Part 1. Chlorophyceae. *Dansk Bot. Ark.* **1**: 1–158.

Calvert, H. E. 1976. Culture studies on some Florida species of *Caulerpa*: morphological responses to reduced illumination. *Br. Phycol. J.* **11**: 203–14.

Carrington, E. 1990. Drag and dislodgment of an intertidal macroalga – consequences of morphological variation in *Mastocarpus papillatus* Kützing. *J. Exp. Mar. Biol. Ecol.* **139**: 185–200.

Coppejans, E., Leliaert, F. and Verbruggen, H. 2005. Green algae. Chlorophyceae. Guide to the seaweeds of KwaZulu-Natal. *Scr. Bot. Belg.* **33**: 38–93.

Coppejans, E., Leliaert, F., Dargent, O., Gunasekara, R. and De Clerck, O. 2009. *Sri Lankan Seaweeds. Methodologies and Field Guide to the Dominant Species*. ABC Taxa, Brussels.

Edgar, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**: 1–19.

Inoue, N., Yamada, S., Nagata, Y. and Shimmen, T. 2002. Rhizoid differentiation in *Spirogyra*: Position sensing by terminal cells. *Plant Cell Physiol.* **43**: 479–83.

Koehl, M. A. R., Helmuth, B. and Carpenter, R. 2001. Growing and Flowing. In Kaandorp, J. A. and Kubler, J. E. (Eds) *The Algorithmic Beauty of Seaweeds, Sponges and Corals*. Springer-Verlag, Heidelberg, pp. 17–29.

Kraft, G. T. 2007. *Algae of Australia: Marine Benthic Algae of Lord Howe Island and the Southern Great Barrier Reef. 1. Green Algae*. CSIRO Publishing, Melbourne.

Leliaert, F. and Boedeker, C. 2007. Cladophorales. In Brodie, J., Maggs, C. A. and John, D. (Eds) *Green Seaweeds of Britain and Ireland*. The British Phycological Society, London, pp. 131–83.

Leliaert, F., De Clerck, O., Verbruggen, H., Boedeker, C. and Coppejans, E. 2007. Molecular phylogeny of the Siphonocladales (Chlorophyta: Cladophorophyceae). *Mol. Phylogenet. Evol.* **44**: 1237–56.

Leliaert, F., Rueness, J., Boedeker, C. *et al*. 2009. Systematics of the marine microfilamentous green algae *Uronema curvatum* and *Urospora microscopica* (Chlorophyta). *Eur. J. Phycol.* **44**: 487–96.

Mine, I., Menzel, D. and Okuda, K. 2008. Morphogenesis in giant-celled algae. *Int. Rev. Cell Mol. Biol.* **266**: 37–83.

Ohba, H., Nashima, H. and Enomoto, S. 1992. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) III. Reproduction, development and morphological variation of

laboratory-cultured *C. racemosa* var. *peltata*. *Bot. Mag. Tokyo* **105**: 589–600.

Okuda, K., Matsuo, K. and Mizuta, S. 1993. The meridional arrangement of cortical microtubules defines the site of tip growth in the coenocytic green alga, *Chamaedoris orientalis*. *Bot. Mar.* **36**: 53–62.

Posada, D. 2008. jModelTest: Phylogenetic Model Averaging. *Mol. Biol. Evol.* **25**: 1253–6.

Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–4.

van den Hoek, C. 1963. *Revision of the European Species of Cladophora*. E. J. Brill, Leiden.

West, J. A. 2005. Long term macroalgal culture maintenance. In Andersen, R. A. (Ed.) *Algal Culturing Techniques*. Academic Press, New York, pp. 157–63.

Yoshida, K., Inoue, N., Sonobe, S. and Shimmen, T. 2003. Involvement of microtubules in rhizoid differentiation of *Spirogyra* species. *Protoplasma* **221**: 227–35.