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Phylogenetic appraisal of the genus *Platoma* (Nemastomatales, Rhodophyta), including life history and morphological observations on *P. cyclocolpum* from the Azores

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The red algal genus *Platoma* Schmitz (Schizymeniaceae, Nemastomatales) with type *Platoma cyclocolpum* (Montagne) Schmitz was originally described from the Canary Islands. Life history studies were conducted on *P. cyclocolpum* from the Azores under 10°C/8:16 light (L):dark (D), 15°C/16:8 L:D, and 23°C/natural daylight regimes in culture. Three nonreproductive modes of thallus development resulted from germinating carpospores: (1) a permanent crust, (2) a filiform, nonfoliose erect thallus occasionally attached by a holdfast composed of cell aggregations, and (3) a filiform, nonfoliose erect thallus has been reported previously for populations from the Canary Islands, but the latter two have not been described before. *Platoma cyclocolpum* is widely reported in the literature, but specimens under that name from Madagascar are identified here as *Platoma chrysymenioides* Gavio *et al.*, a deepwater species first described from the northern Gulf of Mexico and now recorded for the entire gulf. Genuine *P. cyclocolpum* specimens from the Azores, Madeira, and the Canary Islands were sequenced for chloroplast-encoded *rbc*L, and phylogenetic relationships of four *Platoma* species are discussed. A table listing diagnostic morphological and anatomical features of *Platoma* species is provided.

Key Words: Atlantic Ocean, Azores, Life history, Nemastomatales, *Platoma*, *rbc*L, Rhodophyta, Schizymeniaceae

INTRODUCTION

The red algal genus *Platoma* (Schizymeniaceae, Nemastomatales) was established by Schmitz in 1894, on the basis of a type specimen from the Canary Islands [*Platoma cyclocolpum* (Montagne) Schmitz]. *Platoma* species have frequently been confused with genera of similarly gelatinous or soft consistency, such as *Nemastoma* and *Predaea* (e.g. Huisman 1999), especially when sterile or in the absence of clear generic boundaries (e.g. Kraft & John 1976). The genus has historically been allied to *Nemastoma* in the family Nemastomataceae, order Gigartinales (Kylin 1956), but is now included in the Schizymeniaceae (Schmitz & Hauptfleisch) Masuda & Guiry (1995), a family that, together with Nemastomataceae Schmitz (1892), comprises the order Nemastomatales Kylin (1925). The Nemastomatales was merged with the Gigartinales by Kylin (1932) but was recently resurrected by Saunders & Kraft (2002) on the basis of molecular studies of small-subunit ribosomal DNA sequences to accommodate the Nemastomataceae and Schizymeniaceae, a taxonomic decision supported by Gavio (2002) and Gavio *et al.* (2005) on the basis of comparative *rbc*L sequence analysis. Gavio *et al.* (2005) described a new species of *Platoma* from the Gulf of Mexico and stated that the Nemastomatales belongs to a large supraordinal complex.

The tribe Schizymeniae Schmitz & Hauptfleisch (1897) was elevated to familial rank by Masuda & Guiry (1995) to encompass a group of genera distinct from those of the Nemastomataceae, the members of which possess darkly staining nutritive auxiliary cells adjacent to the carpogonial branch and have carpogonia that divide after fertilization, one or both products fusing with a contiguous subsidiary cell that generates connecting filaments to effect diploidization of auxiliary cells. The Schizymeniaceae currently comprises *Platoma*, *Schizymenia* (including its *Haematocelis* tetrasporophyte phase), *Titanophora* and *Wetherbeella* (Masuda & Guiry 1995, Saunders & Kraft 2002, Gavio *et al.* 2005).

The genus *Platoma* currently consists of seven species from warm-temperate to tropical waters of both hemispheres (Table 1; Guiry & Guiry 2008). They are mostly differentiated by vegetative features such as type of branching pattern, stipe presence or absence, extent of degree of fusion of branches, presence or absence of surface proliferation, or habit colour. These characters may not bear much taxonomic weight (Kajimura 1997). Species previously placed in *Platoma* that have since been found not to belong in this genus include *Platoma australicum* Womersley & Kraft and *Platoma foliosa* Womersley & Kraft (1994), which are currently placed in the genus

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Wetherbeella (Saunders & Kraft 2002), and *Platoma fanii* Dawson is now considered a synonym of *Sarcodiotheca linearis* Setchell & Gardner (Guiry & Guiry 2008).

The revised Nemastomatales contains taxa with variable form and texture, all bearing discrete pseudo-dichotomous cortical branch systems, a lax intertwining medulla of narrow filaments and rhizoids that are not connected with one another by means of secondary pit connections, and carpogonial branches and auxiliary cells located in separate cortical filaments, an arrangement termed 'nonprocarpic' (Saunders & Kraft 2002, Gavio *et al.* 2005). Of all Nemastomatales genera, only *Schizymenia* is known to produce secondary pit connections between cortical cells. A distinctive feature of the Schizymeniaceae is the presence of nutritive auxiliary cells, two cells borne immediately distally on the supporting cell of the carpogonial branch that receive zygote-derived diploid nuclei from the fertilized carpogonium before issuing the connecting filaments that convey zygote nuclei to often distant generative auxiliary cells. In the sister family Nemastomataceae, connecting filaments are directed to auxiliary cells from carpogonia without the intervention of what Kylin termed 'nutritive' auxiliary cells (Ardré 1980; Masuda & Guiry 1995). Life history studies conducted in both families indicate so far that erect cylindrical to foliose thalli are gametophytes; the alternate tetrasporophytic phases are crustose in the Schizymeniaceae (Ardré 1977, 1980; Masuda & Guiry 1994) and filiform in the Nemastomataceae (Lemus & Ganesan 1977; Athanasiadis 1988; Millar & Guiry 1989; Vergés *et al.* 2004). *Platoma* can be separated from superficially similar genera by a combination of vegetative and reproductive characters, such as cell location and shape. Tetrasporophytes have not been recorded from nature (Masuda & Guiry 1994).

The aims of this paper are as follows: (1) circumscribe *P. cyclocolpum* on the basis of morphological and molecular evidence, and assess its biogeographic distribution worldwide; (2) clarify the life history of *P. cyclocolpum* on the basis of the development of presumed tetrasporophytes in culture; (3) infer the phylogenetic position and relationships of the genus *Platoma* within the Nemastomatales on the basis of comparative chloroplast-encoded *rbc*L sequence analysis; and (4) assess the diagnostic characteristics of the known species of *Platoma*.

MATERIAL AND METHODS

Material for the present study was collected from 1990 to 2006. Most collections were made at several sites in the Archipelago of the Azores from April to September because of their occurrence at this time of the year in both the intertidal and subtidal (to depths of 18 m) habitats, the latter by scuba diving. Samples of *Platoma chrysymenioides* were collected on unconsolidated rubble throughout the Gulf of Mexico with box dredges or 'scrapes' (Joyce & Williams 1969) at depths ranging from 40 to 90 m during cruises of the R/V *Pelican* sponsored primarily by the National Science Foundation, USA.

Collections were numbered, pressed as voucher herbarium sheets, and stored as subsamples in 5% formalin–seawater or silica gel. Vouchers are deposited in the Herbarium of the Department of Biology, University of the Azores, and the Herbarium at the Natural History Museum of London. The accession numbers of the most representative specimens are given in the text.

Identification of species was based on the original descriptions, a critical review of the literature and the type method (Silva 1952). Microscope slides of squash mounts were stained with 1% aniline blue with a drop of 1% HCl mordant and mounted in 50% Karo® corn syrup–water solution (containing a few drops of phenol).

Photomicrographs were taken using several digital cameras connected to light microscopes. Measurements of cells and other structures were made using a micrometer eyepiece (presented in the text as length per width). Around 300 specimens were studied, the most representative of which are listed below (P = pressed, F = formalin-fixed, SMG = São Miguel Island, GRW = Graciosa Island).

Life history investigations were based on unialgal cultures. Female gametophytes bearing carposporophytes were wiped gently with cotton wool and thallus fragments measuring about 0.5 cm^2 each were then placed into Petri dishes containing sterilized coverslips and culture medium (von Stosch 1963). Fertile portions were subjected to one of two treatments: (1) material was squashed between two slides to release the carpospores, then immersed in culture medium, and (2) material was kept in the dark and cold (8°C) for 20 hours in Petri dishes filled with culture medium, followed by 24 hours of light under 22°C, to induce sporulation by stress (Vergés *et al.* 2003); triplicates of each treatment were incubated in three sets of environmental regimes: (1) 10°C 8 light (L):16 dark (D); (2) 15°C 16L:8D; and (3) 23°C natural daylight in a Petri dish near the window. Digital micrographs of the development stages were taken. During the first 3 weeks, spore development was followed daily and then every 2 weeks when the cultures were cleaned. Slides and coverslips were placed at the bottom of the Petri dishes to act as substratum for spore attachment and to facilitate the microscopic observations.

Cultures of vegetative fragments and of fragments containing carpospores were started from each sample collected at São Miguel Island from the West Coast (Mosteiros: 05 June 2004, 07 September 2005), North Coast (São Vicente: 02 June 2004), and South Coast (Caloura: 07 July 2004, and Ponta Garça: 20 August 2004). Twenty-three plants were used to establish cultures, with a minimum of three replicates distributed in the three culture regimes.

DNA of the specimens was extracted using the DNAeasy Plant Minikit (Qiagen, Valencia, CA). Silica gel-dried specimens and DNA extracts are deposited at the University of Louisiana at Lafayette (LAF) and stored at −20°C. Plastid-encoded *rbc*L was selected to infer the phylogeny of *Platoma* species as this gene has proved successful in assessing systematic relationships in the group (Gavio *et al.* 2005). Protocols for DNA extraction, gene amplification and sequencing are described in Gavio & Fredericq (2002). Polymerase chain reaction primers

Table 1. Comparison of vegetative and reproductive features of *Platoma* (adapted from Kajimura 1997).

Feature	*P. abbottianum* Norris & Bucher 1977	*P. ardreanum* Kraft & Abbott 1997	*P. chrysymenioides* Gavio, Hickerson & Fredericq 2005	*P. cyclocolpum* (Montagne) Schmitz 1894	*P. fanii* E.Y. Dawson 1961	*P. heteromorphum* Schils & Coppejans 2002	*P. incrassatum* Schousboe & De Toni 1905	*P. izunosimense* Segawa 1938	*P. tenue* Howe & Taylor 1931
Branching pattern	dichotomous, often with forked branch apices	broadly lobed, with deep incisions	subcylindrical, elongated, irregularly lobed	irregularly dichotomous or pinnate, with rounded bifurcations at apices, some with deep incisions	irregularly and sparingly lobed	flattened, subcylindrical, irregularly lobed	irregularly lobed, thickened margins, nonundulate	irregularly pinnate, often with forked branch apices, also palmate or irregular and surfaces undulate	suborbicular or subreniform, subentire or sparingly lobed, undulate–crenulate
Thallus shape	foliose	foliose with apparent calluses, blunt lobes, and dentate to narrowly proliferous margins or ruffles	foliose	foliose to subcylindrical, with marginal proliferations	foliose	foliose, occasionally with proliferations	foliose	foliose, with or without proliferations	foliose
Stipe	present	absent	present	absent	absent	present	absent	present or absent	present
Anastomosing of branches	absent	present	absent	present	absent	absent	absent	absent	absent
Thallus color and texture	rose red, gelatinous but firm	deep reddish-brown, soft	bright pink, mucilaginous	light pink to reddish-brown, fleshy but firm	rose red, exceedingly gelatinous	deep red, gelatinous	yellowish brown to purple	reddish-brown to pinkish-red, fleshy and gelatinous	purplish-vinaceous, extremely soft and gelatinous
Gland cells	absent	present, intercalary	present, intercalary	present, intercalary	absent	present, intercalary	absent	present, intercalary, singly or in adjacent pairs	Absent
X-shaped medullary cells	present	?	present	?	?	?	?	present	?
Carpogonial branch	three-celled	three (four)-celled	three-celled	three-celled	three-celled	three-celled	?	three-celled	?
Sterile cells on carpogonial branch	absent	present, one or two	absent	probably absent	absent	occasionally present	?	absent	?
Shape of trichogyne	straight	straight	straight	straight	coiled	straight	?	straight	?
Division of fertilized carpogonium	?	yes	yes	no, direct fusion to one subsidiary auxiliary cell and secondary pit connection to the other	?	yes	?	no, direct fusion to one subsidiary auxiliary cell and secondary pit connection to the other	?

Table 1. Continued

Feature	*P. abbottianum* Norris & Bucher 1977	*P. ardreanum* Kraft & Abbott 1997	*P. chrysymenioides* Gavio, Hickerson & Fredericq 2005	*P. cyclocolpum* (Montagne) Schmitz 1894	*P. fanii* E.Y. Dawson 1961	*P. heteromorphum* Schils & Coppejans 2002	*P. incrassatum* Schousboe & De Toni 1905	*P. izunosimense* Segawa 1938	*P. tenue* Howe & Taylor 1931
Origin of connecting filament	?	from one of the two contacted subsidiary auxiliary cells	from both subsidiary auxiliary cells	from one or both subsidiary auxiliary cells and the cortical cell distal to it	?	from one contacted subsidiary auxiliary cell and the cortical cell distal to it	?	from one or both subsidiary auxiliary cells	?
Mature carposporophyte diameter	130–195 µm	80–120 µm	90–200 µm	140–200 µm	*c.* 60 µm	90–210 µm	?	60–140 µm	*c.* 50 µm
Number of spermatangia/spermatangial parent cell	?	two to three	two	one to two	?	?	?	one to two (four)	?
Distribution	French Polynesia, Gulf of California	Fiji, Hawaiian Islands	Gulf of Mexico, Madagascar	Mediterranean, N Atlantic Islands, Caribbean Islands, W & South Africa, W.Australia, Fiji	Gulf of California	Arabian Sea (Oman)	S Spain, Egypt, W Coast of Morocco	S Japan, New South Wales (Australia)	Caribbean Islands, Brazil

(F7-R753, F57-R557, F645-R1150, F993-R*rbc*Sstart) and sequencing primers (F7, F57, F645, F993, R376, R557, R753, R1150, R*rbc*Sstart) are listed in Lin *et al.* (2001) and Gavio & Fredericq (2002).

Phylogenies were based on 55 *rbc*L sequences (Table 2) of which 39 are new, 16 were downloaded from Genbank, and one was obtained from the *rbc*L database of the Seaweed Lab at LAF. The newly generated DNA sequences were deposited in GenBank. A data set was assembled for 54 Schizymeniaceae, with representatives of the Nemastomataceae serving as outgroups. Outgroup species were selected on the basis of phylogenetic findings of earlier analyses of the Nemastomatales (Gavio *et al.* 2005).

The generated *rbc*L sequences were compiled, edited and aligned using Sequencher software (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis in PAUP* v.4.0 beta 10 (Swofford 2003) and MacClade v.4 (Maddison & Maddison 2000). Because many *rbc*L sequences used in this study were incomplete at the 5′ coding region of the gene, the data set was restricted to the last 1367 base pairs (bp) of the 1467 *rbc*L coding region. As some sequences were incomplete around the 600th base, an additional 100 bp were removed (from the 565th to the 655th).

Phylogenetic analyses were conducted with the maximum parsimony (MP) and maximum likelihood (ML) algorithms as implemented in PAUP, with Bayesian inferences implemented in MrBayes 3.0 (Hall 2001; Huelsenbeck & Ronquist 2001). Parsimony trees obtained under the Fitch (1971) criterion of equal weights for all substitutions were inferred from a heuristic search, excluding uninformative characters consisting of 1000 random sequence additions holding 10 trees at each step, MULPARS and tree bisection–reconnection (TBR) algorithms with the MULTREES (saving multiple trees) and STEEPEST DESCENT option. Consistency and retention indices were calculated (Kluge & Farris 1989). Support for nodes in the MP and ML analyses were assessed by calculating bootstrap proportion values (Felsenstein 1985) on the basis of 1000 and 100 resamplings, respectively.

The optimal model of sequence evolution to fit the sequence alignment estimated by hierarchical likelihood ratio tests performed by Modeltest v.3.6 (Posada & Crandall 1998) was the TIM + I + G (transition model with variable base frequencies, variable transitions, equal transversion substitution matrix) for the data set. The Akaike information criterion parameters for the Schizymeniaceae data set were as follows: assumed nucleotide frequencies A = 0.3057; C = 0.1487; G = 0.1979; T = 0.3477; substitution rate matrix A–C substitutions = 1.0, A–G = 5.0837, A–T = 2.0484, C–G = 2.0484, C–T = 13.6014, G–T = 1.0; proportion of sites assumed to be invariable = 0.5081, and rates for variable sites assumed to follow a gamma distribution with shape parameter = 1.2595. These likelihood parameters were also applied in the Bayesian analysis [Lset base = (0.3057, 0.1487, 0.1979, 0.3477), nst = 6, rmat = (1.0, 5.0837, 2.0484, 2.0484, 13.6014, 1.0), rates = gamma, shape = 1.2595, pinvar = 0.5081].

The ML phylogram was inferred using the general time-reversible model from trees generated by a heuristic search

Table 2. List of species used in the *rbc*L sequence analysis with collection information and GenBank accession number.

Species	Collection identification	Collection locality	Collection data	GenBank accession number
Nemastoma canariense (Kützing) Montagne		Canary Islands	R. Haroun, s.d.	AY294370
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-5-27-00-11-1	Offshore Louisiana, USA (60-m depth), 28°03.492′N, 92°27.665′W	S. Fredericq, B. Gavio, C.F. Gurgel & J. Lopez-Bautista, 27 May 2000	AY294362
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-5-27-00-12-11	Offshore Louisiana, USA (66-m depth)	S. Fredericq, B. Gavio, C.F. Gurgel & J. Lopez-Bautista, 27 May 2000	AY294359
Platoma chrysymenioides Gavio, Hickerson & Fredericq	HEC14805	Chaland, Rodrigues (15-m depth), 19°40.243′S, 63°25.019′E	E. Coppejans, s.d.	FJ878843
Platoma chrysymenioides Gavio, Hickerson & Fredericq	HEC15094b	Balise Nord of Tuléar, Madagascar (10–15-m depth)	E. Coppejans, D. Douterlungne & I. Razanakoto, s.d.	FJ878844
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-5-31-04-2-6 (NSF-I-5-6)	Dry Tortugas, FL, USA (85–86-m depth), 24°49.53′N, 83°51.71′W	S. Fredericq, 31 May 2004	FJ878845
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-6-4-05-3-7 (NSF-II-3-7)	Offshore Louisiana, USA (62–112-m depth), 28°06.12′N, 91°02.45′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 4 Jun 2005	FJ878846
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-6-17-05-7-4 (NSF-II-100-4)	Campeche Banks, Mexico (49–53-m depth), 22°11.33′N, 91°08.71′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 17 Jun 2005	FJ878847
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-6-18-05-8-19 (NSF-II-109-19)	Campeche Banks, Mexico (54–56-m depth), 22°16.08′N, 90°42.89′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 18 Jun 2005	FJ878848
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-6-22-05-2-7 (NSF-II-123-7)	Campeche Banks, Mexico (58–71-m depth), 27°55.07′N, 92°23.08′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 22 Jun 2005	FJ878849
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-6-23-05-5-16 (NSF-II-128-5)	Offshore Louisiana, USA (64–67-m depth), 28°03.21′N, 92°27.10′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 23 Jun 2005	FJ878850
Platoma chrysymenioides Gavio, Hickerson & Fredericq	LAF-7-9-06-5-1 (NSF-III-7.09.06.5.1)	Florida Middle Ground, FL, USA (68–72-m depth), 27°86.46′N, 92°00.31′W	S. Fredericq, D. Krayesky, N. Arakaki, W. Schmidt, 9 Jul 2006	FJ878851
Platoma cyclocolpum (Montagne) Schmitz	LAF-sn	Canary Islands, s.l.	Y. de Jong, s.d.	AY294385
Platoma cyclocolpum (Montagne) Schmitz	PIX-05-02	Porto da Calheta, Pico, Azores (11-m depth)	P. Madeira, Jun 2005	FJ878852
Platoma cyclocolpum (Montagne) Schmitz	SMG-04-80	Porto de São Vicente, São Miguel, Azores	D. Gabriel & M.I. Parente, 2 Jun 2004	FJ878853
Platoma cyclocolpum (Montagne) Schmitz	SMG-04-82	Porto de São Vicente, São Miguel, Azores	D. Gabriel & M.I. Parente, 2 Jun 2004	FJ878857
Platoma cyclocolpum (Montagne) Schmitz	SMG-04-116	Mosteiros (Poças), São Miguel, Azores	D. Gabriel, 5 Jun 2004	FJ878854
Platoma cyclocolpum (Montagne) Schmitz	SMG-04-117	Mosteiros (Poças), São Miguel, Azores	D. Gabriel, 5 Jun 2004	FJ878855
Platoma cyclocolpum (Montagne) Schmitz	SMG-04-202	Ponta Garça, Vila Franca, São Miguel, Azores (8-m depth)	D. Gabriel & P. Madeira, 20 Aug 2004	FJ868809
Platoma cyclocolpum (Montagne) Schmitz	SMG-04-205	Ponta Garça, Vila Franca, São Miguel, Azores (8- m depth)	D. Gabriel & P. Madeira, 20 Aug 2004	FJ878858
Platoma cyclocolpum (Montagne) Schmitz	SMG-06-80	São Miguel, Azores		FJ878856
Platoma ardreanum Kraft & Abbott	LAF-8-25-93-1-3	Wan Li Dung, Kenting National Park, Taiwan (7-m depth)	S. Fredericq & S. M. Lin	AY294368
Platoma sp	HEC10222	Barrier reef in front of Port Moresby (Motupore Island), Papua New Guinea (15–30-m depth)	E. Coppejans & O. De Clerck, s.d.	FJ878859
Predaea feldmannii Børgesen	LAF-10-17-99-1-1	Long Bay Point, Isla Colon, Caribbean Panama	B. Wysor, 17 Oct 1999	AY294366
Schizymenia apoda (J. Agardh) J. Agardh	LAF-7-6-93-1-1	Swakopmund, Namibia	M.H. Hommersand, 6 Jul1993	AY294401

Table 2. Continued

Species	Collection identification	Collection locality	Collection data	GenBank accession number
Schizymenia apoda (J. Agardh) J. Agardh	LAF-6-94-1-1	Taiping Cape, Shandong prov., China	M.H.Hommersand, Jun1994	AY294392
Schizymenia apoda (J. Agardh) J. Agardh	GRW-04-87	Barro Vermelho, Graciosa, Azores	D. Gabriel, 10 Jun 2004	FJ878860
Schizymenia apoda (J. Agardh) J. Agardh	GRW-04-88	Barro Vermelho, Graciosa, Azores	D. Gabriel, 10 Jun 2004	FJ878861
Schizymenia apoda (J. Agardh) J. Agardh	GRW-06-786	Baía da Fonte, Graciosa, Azores	9 Jul 2006	FJ878862
Schizymenia apoda (J. Agardh) J. Agardh	SMG-04-164	Praia do Pópulo, São Miguel, Azores	M.I. Parente, 14 Jun 2004	FJ878863
Schizymenia apoda (J. Agardh) J. Agardh	SMG-04-165	Praia do Pópulo, São Miguel, Azores	M.I. Parente, 14 Jun 2004	FJ878864
Schizymenia apoda (J. Agardh) J. Agardh	SMG-05-145	São Roque, São Miguel, Azores	D. Gabriel, 9 Aug 2005	FJ878865
Schizymenia apoda (J. Agardh) J. Agardh	SMG-05-226	Mosteiros, São Miguel, Azores	D. Gabriel & M.I. Parente, 07 Sep 2005	FJ878866
Schizymenia apoda (J. Agardh) J. Agardh	SMG-05-259	São Vicente, São Miguel, Azores	D. Gabriel, 21 Sep 2005	FJ878867
Schizymenia apoda (J. Agardh) J. Agardh	SMG-06-84	São Miguel, Azores		FJ878868
Schizymenia dubyi (Chauvin ex Duby) J. Agardh	LAF-6-22-93-1-1	Pighet, Brittany, France	J. Cabioch, 22 June 1993	AY294389
Schizymenia dubyi (Chauvin ex Duby) J. Agardh	LAF-7-10-94-1-1	Jodogahama, Miyako, Iwate-ken, Japan	M.H. Hommersand & M. Yoshizaki, 10 Jul 1994	AY294388
Schizymenia dubyi (Chauvin ex Duby) J. Agardh	LAF-7-10-94-1-2	Jodogahama, Miyako, Iwate-ken, Japan	M.H. Hommersand & M. Yoshizaki, 10 Jul 1994	FJ878869
Schizymenia pacifica (Kylin) Kylin	LAF-7-26-95-1-1	Kanaha Bay, W. Juan Island, WA, USA	M.J. Wynne, 26 Jul 1995	AY294393
Schizymenia pacifica (Kylin) Kylin	LAF-4-15-94-1-1	Vancouver, BC, Canada (drift)	S.C. Lindstrom, 15 Apr 1994	AY294394
Schizymenia pacifica (Kylin) Kylin	LAF-7-24-98-1-1 (TC101)	North Boardman St. Park, OR, USA	T.O. Cho & G.I. Gayle, 24 Jul 1998	FJ878870
Schizymenia pacifica (Kylin) Kylin	LAF-7-24-98-1-2 (TC130)	North Boardman St. Park, OR, USA	T.O. Cho & G.I. Gayle, 24 Jul 1998	FJ878871
Schizymenia sp.	LAF-9-5-93-1-1	Tokawa, Choshi, Chiba Pref.	S. Fredericq & M. Yoshizaki, 2 Sept 1993	AY294391
Titanophora incrustans (J. Ag.) Børgesen	LAF-7-30-01-1	Offshore Louisiana, USA, 58-m depth, 28°06.470′N, 90°55.359′W	S. Fredericq, J. Lopez-Bautista & F. Rita, 30 Jul 2001	AY294365
Titanophora incrustans (J. Ag.) Børgesen	LAF-6-13-05-7-2 (NSF-II-67-2)	Campeche Banks, Mexico (38–50-m depth), 21°49.30′N, 92°05.59′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 13 Jun 2005	FJ878872
Titanophora incrustans (J. Ag.) Børgesen	LAF-6-14-05-3-1 (NSF-II-79-1)	Campeche Banks, Mexico (49–47-m depth), 22°10.39′N 91°09.69′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 14 Jun 2005	FJ878873
Titanophora incrustans (J. Ag.) Børgesen	LAF-6-17-05-7-1 (NSF-II-100-1)	Campeche Banks, Mexico (49–53-m depth), 22°11.33′N, 91°08.71′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 17 Jun 2005	FJ878874
Titanophora sp.1	LAF-6-13-05-5-1 (NSF-II-69-1)	Campeche Banks, Mexico (36–54-m depth), 21°49.42′N, 92°05.54′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 13 Jun 2005	FJ878875
Titanophora sp.2	LAF-6-17-05-1-2 (NSF-II-94-2)	Campeche Banks, Mexico (48–51-m depth), 22°07.98′N, 91°23.75′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 17 Jun 2005	FJ878876
Titanophora pikeana (Dickie) J. Feldmann	LAF-2-11-01-1-1	Deep Sponge Reef, Sodwana Bay, KwaZulu-Natal, South Africa (27-m depth)	S. Fredericq & O. DeClerck, 11 Feb 2001	AY294364
Titanophora weberae Børgesen	LAF-8-7-94-1-1	Wang Hai Xiang, N. Taiwan (12-m depth)	S. Fredericq & S.M. Lin, 7 Aug 1994	AY294387

Table 2. Continued

Species	Collection identification	Collection locality	Collection data	GenBank accession number
Titanophora weberae Børgesen	LAF-5-29-04-3-1 (NSF-I-3-1)	Offshore Louisiana, USA (68–58-m depth), 28°05.22′N, 91°01.41′W	S. Fredericq, 29 May 2004	FJ878877
Titanophora weberae Børgesen	LAF-6-17-05-7-11 (NSF-II-100-11)	Campeche Banks, Mexico (49–53-m depth), 22°11.33′N, 91°08.71′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 17 Jun 2005	FJ878878
Titanophora weberae Børgesen	LAF-6-22-05-2-8 (NSF-II-123-8)	Offshore Louisiana, USA (58–71-m depth), 27°55.07′N, 92°23.08′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 22 Jun 2005	FJ878879
Titanophora weberae Børgesen	LAF-6-23-05-3-17 (NSF-II-129-17)	Offshore Louisiana, USA (61–66-m depth), 28°03.64′N, 92°27.72′W	S. Fredericq, D. Gabriel, F. Gurgel & T.O. Cho, 23 Jun 2005	FJ878880
Titanophora weberae Børgesen	LAF-7-8-06-3-3 (NSF-III-7.08.06.3.3)	Offshore Louisiana, USA (55–57-m depth), 28°05.54′N, 91°01.38′W	S. Fredericq, D. Krayesky, N. Arakaki, W. Schmidt, 8 Jul 2006	FJ878881

using an 'as is' sequence addition option. Bootstrapping was inferred from a fast heuristic search, consisting of 100 replicates, retaining groups with a frequency greater than 50%, MULPARS and TBR algorithms.

For the Bayesian analysis, four chains of the Markov chain Monte Carlo were run, sampling one tree every 100 generations for 2,000,000 generations starting with a random tree for the *rbc*L data sets. The first generations were discarded as the 'burn-in' period to reach equilibrium. A 50% consensus tree (majority rule as implemented by PAUP*) was computed from the trees saved after the burn-in point, 44,400 generations for the Schizymeniaceae. Reliability of the Bayesian consensus tree is given by the frequency at which each node appears among all saved trees after the burn-in generation. This frequency corresponds to the posterior probability of the clades (Hall 2001).

RESULTS

Platoma cyclocolpum (Montagne) Schmitz 1894

Figs 11–17

SPECIMENS EXAMINED: SMG-93-29 (F), SMG-94-141 (F), SMG-94-141 a, b (P), SMG- 94-239 (F), SMG-95-49 (F), SMG-97-178 (F), SMG-97-273 a (P), SMG-97-273 b (F), SMG-98- 833 d (F), SMG-00-201 (F), SMG-00-202 (F), SMG-00-211 (F), SMG-00-231 (F), PIX-05-01(F) , PIX-05-06(F), GRW-06- NHM 000044528 (P), NHM 000044509 (P), NHM 000519586 (P) (See Table 1).

REPORTED DISTRIBUTION IN THE CURRENT LITERATURE: *Platoma cyclocolpum* has been reported from the following areas: ***Europe***: Adriatic Sea (Giaccone 1978), Balearic Islands (Seoane-Camba 1969; Ribera Siguán 1983; Ribera-Siguán & Gómez-Garreta 1984), Greece (Gerloff & Geissler 1974; Athanasiadis 1988), Italy (Giaccone 1969; Cinelli 1971; Feoli & Bressan 1972; Cinelli *et al*. 1976; Furnari *et al*. 1999, 2003; Rindi *et al*. 2002), Spain (Ballesteros 1981; Ballesteros & Romero 1982; Gallardo *et al*. 1985). ***Atlantic Islands***: Azores (Neto 1994), Bermuda (Taylor 1960), Canary Islands (Gil-Rodríguez & Afonso-Carrillo 1980; Lawson *et al*. 1995; Haroun *et al*. 2002; John *et al*. 2004), Madeira (Levring 1974; Neto *et al*. 2001; John *et al*. 2004), Salvage Islands (John *et al*. 2004). ***Caribbean Islands:*** Lesser Antilles (Taylor 1960; Littler & Littler 2000), Netherlands Antilles (Taylor 1960). ***Africa***: Morocco (Benhissoune *et al.* 2002; John *et al.* 2004), Rodrigues (De Clerck *et al.* 2004), Sierra Leone (John *et al.* 2004), South Africa (De Clerck *et al.* 2005). ***Australia and New Zealand***: Western Australia (Huisman 2000; Huisman & Borowitzka 2003). ***Pacific Islands***: Fiji (South & Skelton 2003), Wallis and Futuna Islands (N'Yeurt & Payri 2004) (see Guiry & Guiry 2008).

Habitat and phenology

Plants are rare at all places visited in the Azores except for one general diversity-rich algal site at Mosteiros. Occurring in the lower intertidal zone to depths of 18 m, thalli were always epilithic and generally attached to the sides or at the

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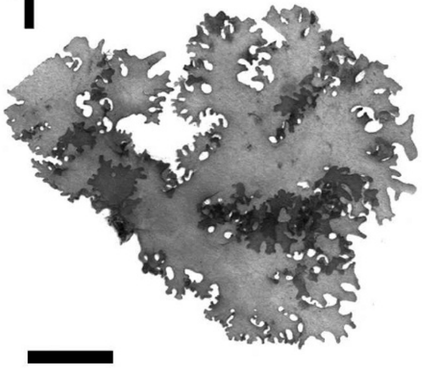

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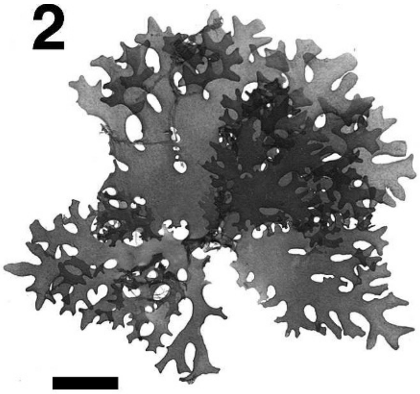

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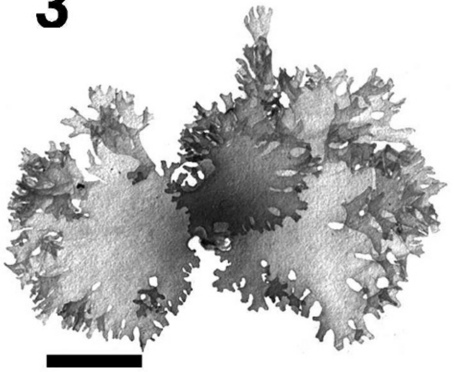

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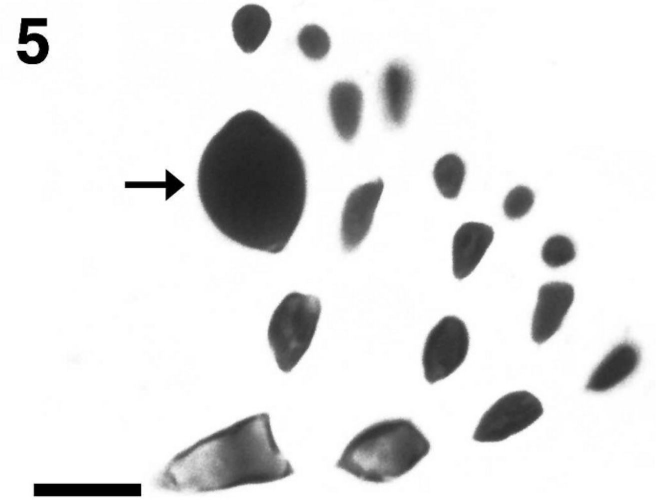

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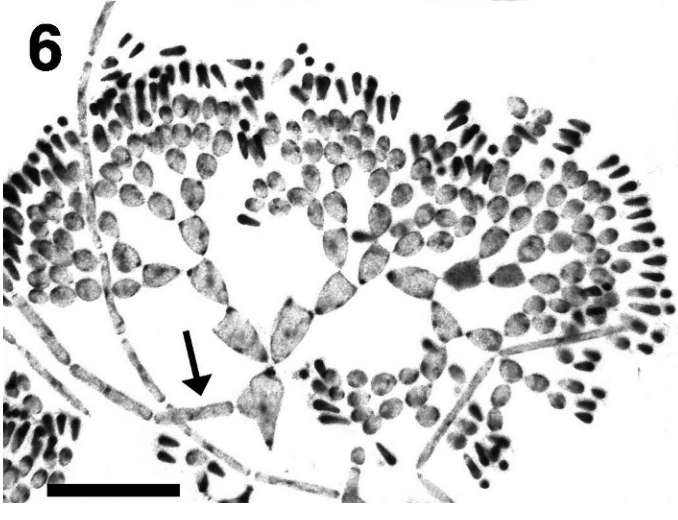

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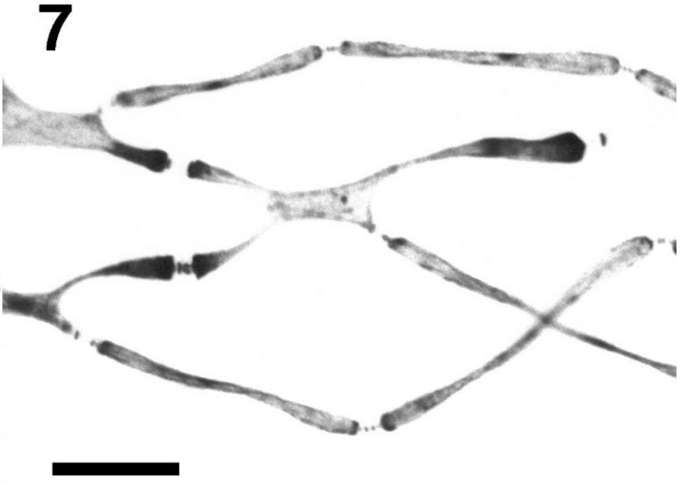
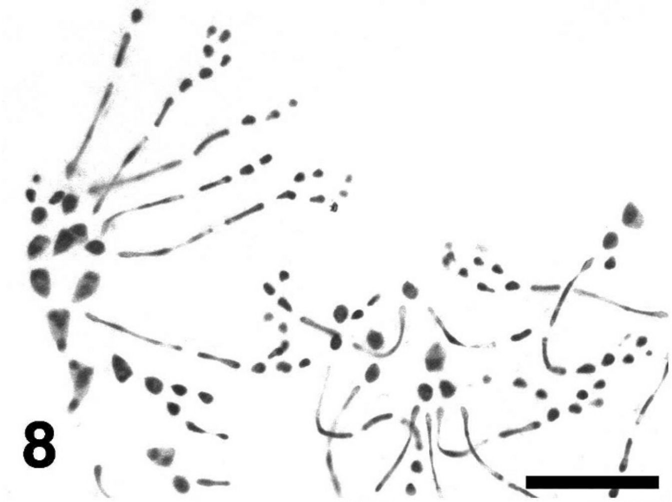
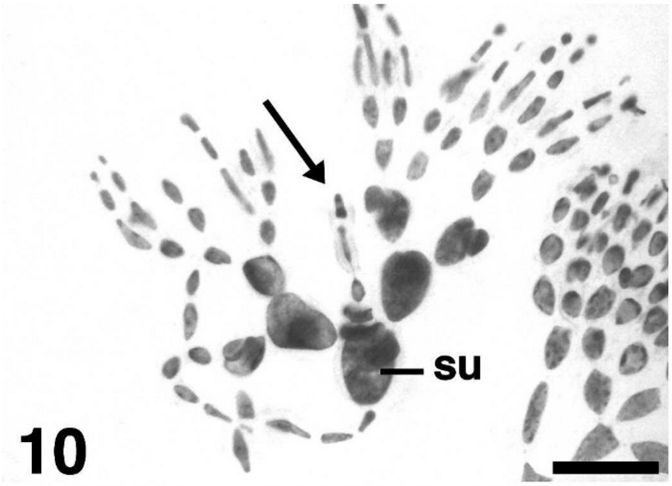
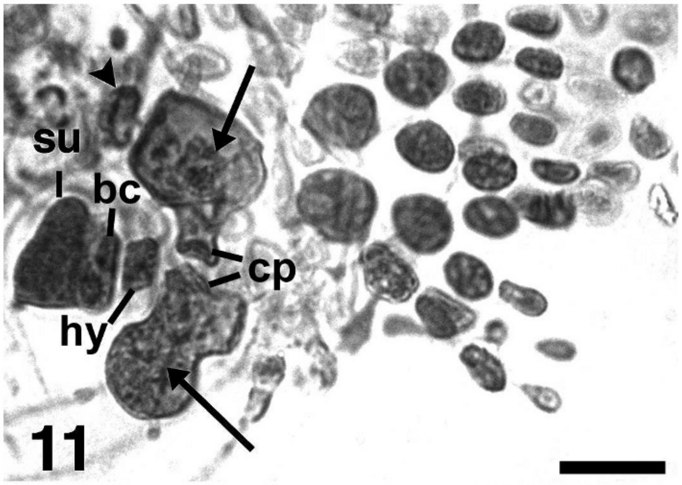


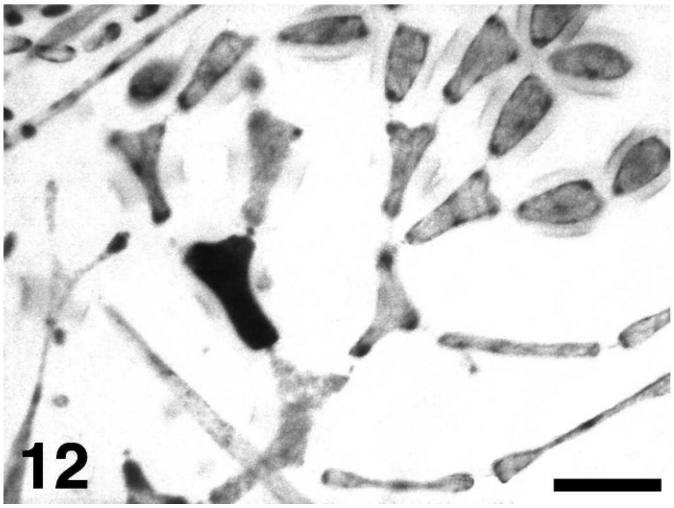
Figs 1–3. Habit of *Platoma cyclocolpum* from the Azores.
  Fig. 1. Cystocarpic specimen from São Miguel, SMG-93-126. Scale bar = 1 cm.
  Fig. 2. Cystocarpic specimen from São Miguel, SMG-99-840. Scale bar = 1 cm.
  Fig. 3. Cystocarpic specimen from Formigas, FOR-90-172. Scale bar = 1 cm.
Figs 4–7. *Platoma cyclocolpum* from São Miguel. Vegetative morphology.
  Fig. 4. Regular pseudichomotous branching of cortical fascicles and a septate medullary filament (arrow). SMG-97-178. Scale bar = 50 μm.
  Fig. 5. Intercalary cortical ‘gland’ cell (arrow) SMG-00-281. Scale bar = 10 μm.
  Fig. 6. A cortical fascicle with adventitious rhizoids (arrow) borne on cuneate inner cortical cells. SMG-00-204. Scale bar = 50 μm.
  Fig. 7. X-shaped medullary cells and septate medullary filaments. Scale bar = 25 μm. SMG-00-211.

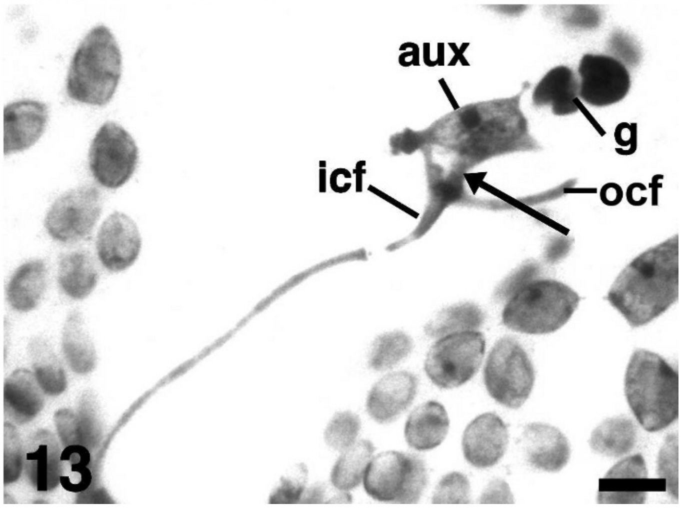
base of rocks in relatively low light conditions and in places of moderate wave action. Thalli were collected from mid-spring to late summer throughout the entire archipelago but were conspicuously absent at other times of the year. The plants grew on bare rock and were frequently associated with encrusting Corallinaceae, sometimes on top of patches of *Codium adhaerens* C. Agardh and less frequently among *Corallina* turfs. Female reproductive structures were found











Figs 8–13. Squash preparation of *Platoma cyclocolpum* from São Miguel.

Fig. 8. Adventitious regrowth of filaments from subsurface cortical cells regenerating from possible wounding or mechanical damage. SMG-00-205. Scale bar = 50 μm.

Fig. 9. Opposite and unilateral branching of adventitious cortical filaments. SMG-02-197. Scale bar = 50 μm.

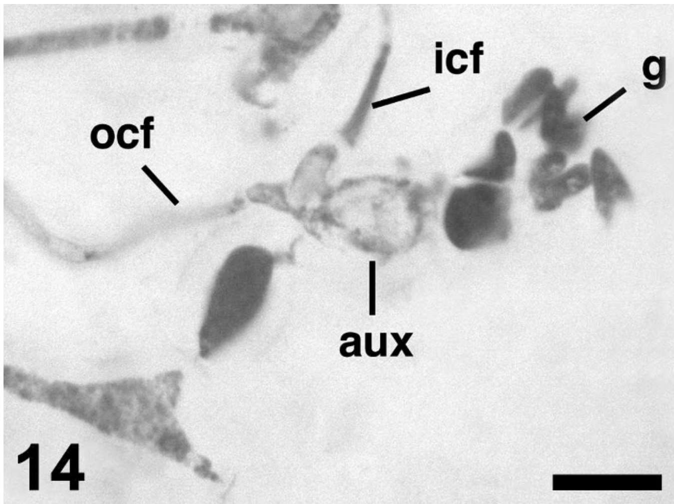
Figs 10–13. Pre- and early postfertilization structures.

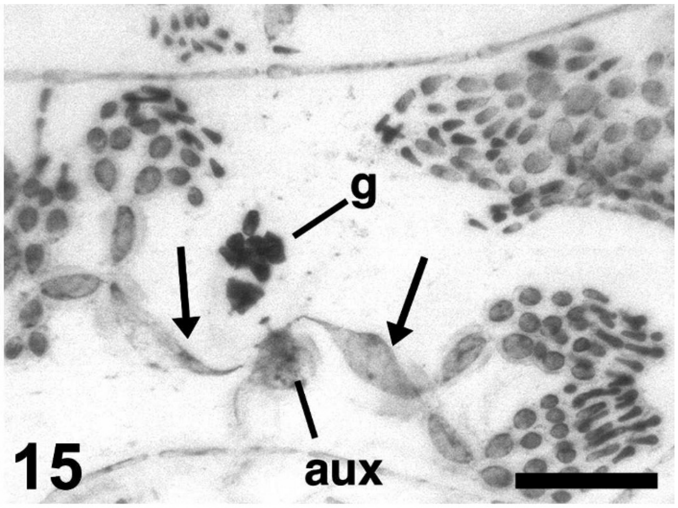
Fig. 10. Abortive carpogonial branch system, with two layers of darkly staining cells of cortical branches borne on the supporting cell (su), and the trichogyne reverting to a septate vegetative filament (arrow). SMG-00-201. Scale bar = 25 μm.

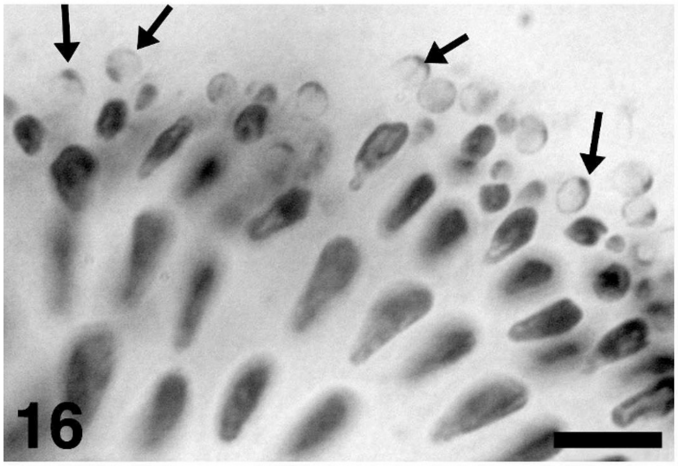
Fig. 11. Supporting cell (su) bearing a functional three-celled carpogonial branch consisting of a basal cell (bc), hypogynous cell (hc) and a fertilized carpogonium (cp), showing the division of the carpogonium in two halves and their fusion with the two contiguous nutritive auxiliary cells (arrows). A single connecting filament initial (arrowhead) has been cut from one of the nutritive auxiliary cells. SMG-95-49. Scale bar = 25 μm.

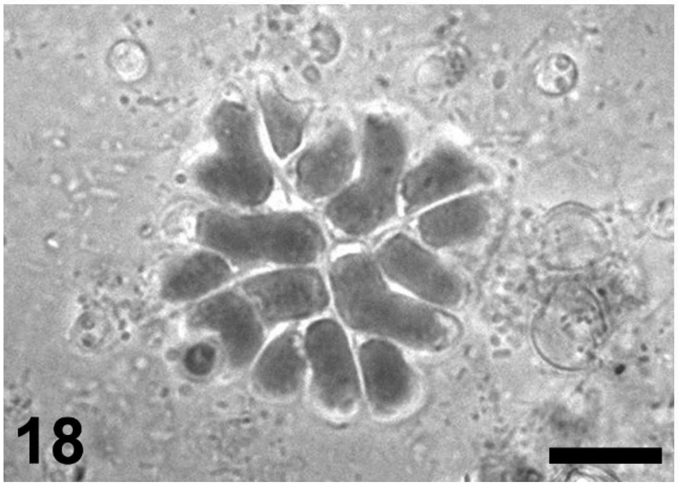
Fig. 12. Darkly staining intercalary generative auxiliary cell. SMG-00-211. Scale bar = 25 μm.

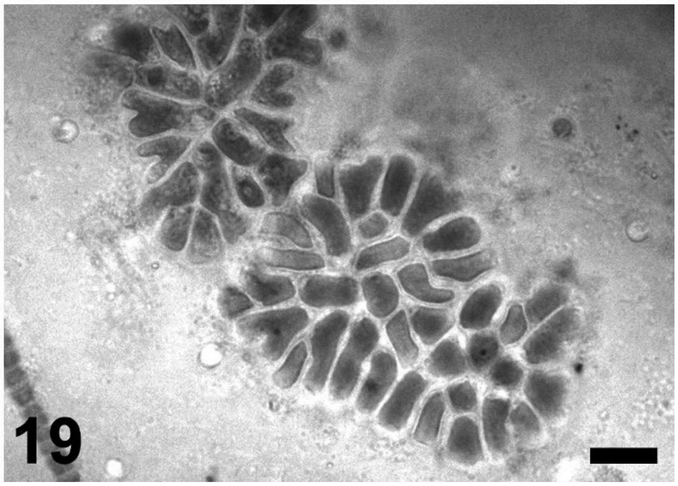
Fig. 13. A diploidized generative auxiliary cell (aux) on which a gonimoblast initial (g) and first gonimolobe initial have arisen after contact and lateral fusion (arrow) with an incoming connecting filament (icf) that has continued on to effect further diploidizations to the outgoing connecting filament (ocf). SMG-00-204. Scale bar = 10 μm.











Figs 14, 15. Squash preparations of *Platoma cyclocolpum* from São Miguel.

Fig. 14. Early gonimoblast (g) growth, the carposporophyte oriented toward the thallus surface and attached to an auxiliary cell (aux) largely emptied of contents and basally attached to incoming (icf) and outgoing (ocf) connecting filaments SMG-98-833. Scale bar = 25 μm.

Fig. 15. Gonimolobe (g) of a maturing carposporophyte, and cortical cells linked to the auxiliary cell (aux) having elongated (arrows) to form a bracket around the mature carposporangia. SMG-00-204. Scale bar = 50 μm.

Fig. 16. Spermatangia (arrows) borne singly and in pairs at the ends of cortical filaments. GRW-04-117. Scale bar = 10 μm.

Figs 17–19. *Platoma cyclocolpum* from São Miguel. Carpospore development in culture. SMG-04-118.

Fig. 17. First divisions of a carpospore settled on a glass coverslip. Scale bar = 10 μm.

Figs 18, 19. Centrifugal growth of early monostromatic crusts. Scale bar = 10 μm.

in all plants taller than 1.5 cm. Male structures were rarely observed but, when present, they were found on monoecious gametophytes.

Habit

Plants are foliose, the flattened to undulate blades lubricous and soft-textured, estipitate, to 9.5 cm in length. There is a primary holdfast and occasionally up to five secondary ones near the base where the fronds touch the substratum. Fronds are irregularly lobed and occasionally deeply cleft, the incisions lined with dentate or blunt proliferations of varying length and width (Figs 1–3). When blades of the same frond overlap, they may coalesce. Thalli are reddish-brown, becoming yellowish-pink when exposed to direct light, irrespective of the depth in which the plants grow.

Vegetative structure

Thalli are composed of loose aggregations of pseudodichotomously branched cortical filaments composed of regular-sized cylindrical cortical cells (~ 29–216 µm × 1.7–12 µm) and filiform, septate medullary filaments (Figs 4, 6). The cortical fascicles are pseudodichotomous and branch 6 to 12 times (Figs 4, 6, 8), with nearly every intercalary cell bearing a fork. Inner cortical cells tend to become cuneate where they subtend pseudodichotomies (Fig. 6). Surface cells measure about 3–15 µm long and 1.7–6 µm wide. Spherical to oblong darkly staining ‘gland’ cells 9–62 µm × 7–47 µm (Fig. 5) differentiate in small numbers from intercalary cortical cells two to four cell layers in the surface. X-shaped cells (Fig. 7) form when an intercalary cell that bears a pseudodichotomy initiates an inwardly directed adventitious rhizoid (Fig. 6) that grows into and augments the medulla. Short cortical filaments may also be formed secondarily on uniseriate filaments, probably as a response to mechanical injury or herbivory; these adventitious structures typically elongate, narrow, and ultimately are capable of ‘‘normal’’ cortical branching (Figs 8, 9).

Pre- and early postfertilization structures

Most carpogonial branch systems are nonfunctional and abortive, as indicated by the fact that mature unfertilized carpogonia apparently reverted to septate cortical filaments (Fig. 10). Regardless of whether they are functional (Fig. 11) or aborted (Fig. 10), the complex of cells consisting of the carpogonial branch, its supporting cell, and the two distal vegetative cells (the ‘nutritive’ auxiliary cells) borne immediately on the supporting cell all stain darkly with aniline blue. Carpogonial branches are three-celled and borne distally on a supporting cell between two basal cells of the pseudodichotomy (Figs 10, 11). Supporting cells are 12–25 × 8–28 µm. Carpogonia are small and conical, measuring 6–14 × 2.5–8.5 µm, and extend into a straight trichogyne directed toward the thallus surface. The hypogynous cell is flattened (3.5–13 × 5.5–15 µm); whereas, the basal cell is hemispherical and 4–15 × 7–20 µm (Figs 10, 11).

Spermatangia are borne terminally singly or in pairs (rarely in threes) on parent cells, measuring 3.5–6.5 × 2.5–4 µm (Fig. 16), occurring in patches on the thallus surface of plants that also bear carpogonial branch systems. Spermatia (3–4.5 × 2–4 µm) differentiate within each spermatangium (Fig. 16) and contain a darkly stained, distally situated nucleus. Tetrasporophytes are unknown in the field and were not obtained in culture.

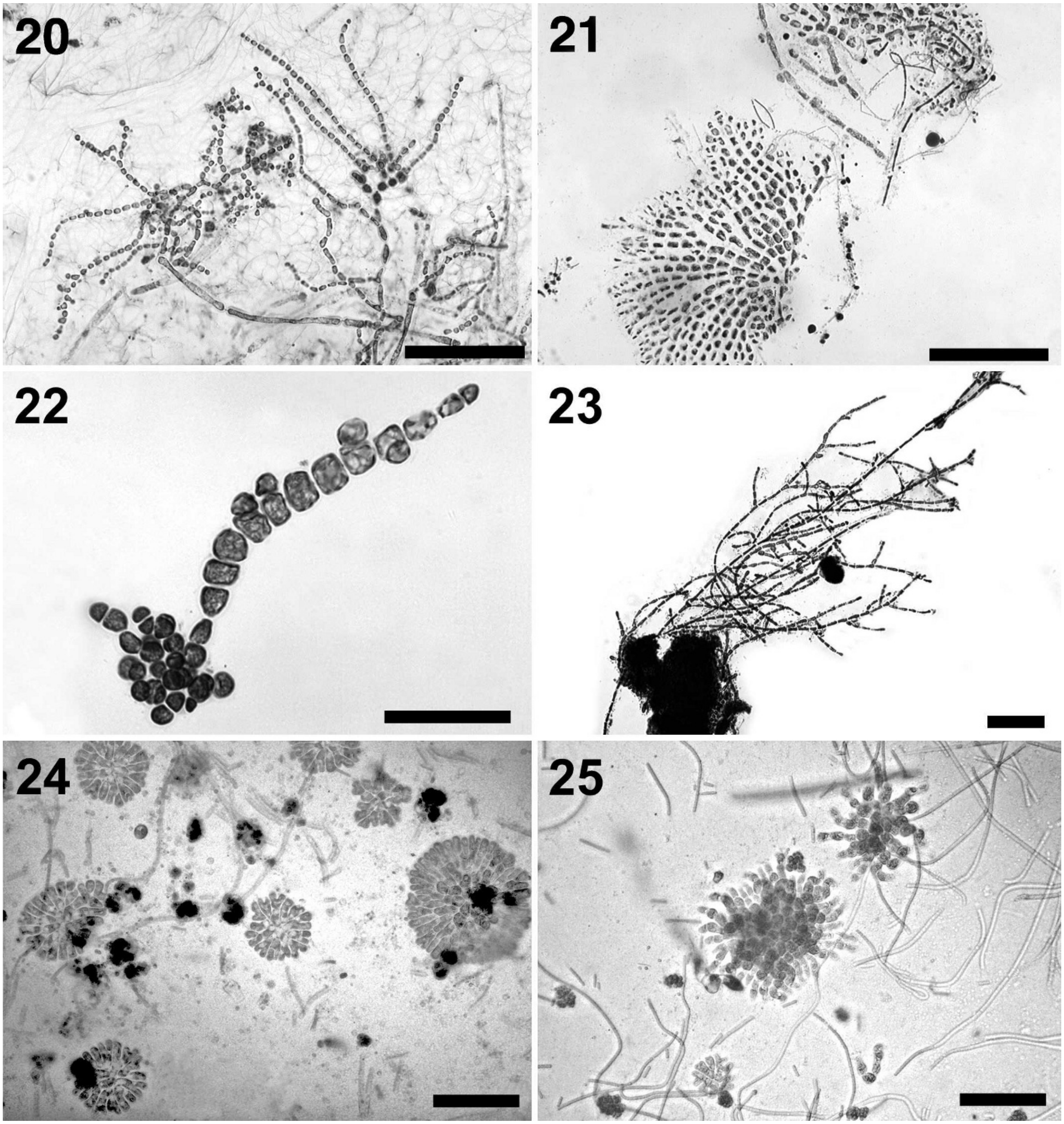
Postfertilization structures

The trichogyne may persist for a while after fertilization, and more than one attached spermatium has been observed. Upon presumed fertilization, the cytoplasm of the carpogonium is cut off from that of the trichogyne by a constriction that apparently prevents further passage of male nuclei; the carpogonium then divides into two cells, each of which fuses with one or both adjacent nutritive auxiliary cells (Fig. 11). These nutritive auxiliary cells expand in size, becoming irregular in outline, and each produces a primordium that extends into a branched septate-connecting filament (Fig. 11). As they traverse the thallus, often for considerable distances, the connecting filaments attach to generative auxiliary cells on their proximal surfaces (Figs 12–15), partially fuse and transfer a diploid zygote nucleus. Upon fusion, connecting filaments continue to grow to effect further diploidizations.

Diploidized auxiliary cells are 18–40 µm long and 11–20 µm wide and intercalary in cortical filaments that initially are darkly staining (Figs 12, 13) but subsequently become more lightly staining (Figs 14, 15) as gonimoblast growth progresses. During carposporophyte development, the auxiliary cell becomes cytoplasmically depleted (Fig. 14). One or two cells borne distally on the generative auxiliary cell lengthen apace with the growing carposporophyte and wrap around the gonimoblast as a lax and rudimentary involucre (Fig. 15). The single gonimoblast initial is 12–15 × 6–10 µm and arises on the distal end of the generative auxiliary cell. A single gonimolobe develops from the gonimoblast initial and consists of initially small cells (Figs 13–15) that divide and expand into uninucleate subspherical carposporangia measuring 10–30 × 9–22 µm (Fig. 15). Carposporophytes reach 125–160 × 95–140 µm and consist entirely of carposporangia that release carpospores through lacunae between cortical fascicles rather than by differentiated ostioles.

Life history studies: development of carpospores

Released carpospores were spherical, light red in colour and contain several discoid plastids. Certain carpospores attached firmly to the substratum and divided rapidly; whereas, others remained undivided for months and their development started much later. Most spores developed within a few days into one of three types of sporeling: (1) two equal- or unequal-sized cells derived from the carpospore and that subsequently divided radially to form small discs or pseudodiscs, the latter irregularly shaped discs lacking an outer synchronised growth margin, and with limited lateral cohesion of adjacent filaments (Figs 17–19); (2) a single germ tube that grew from the germinating spore and divided transversely into a uniseriate filament of two to



Figs 20–25. *Platoma cyclocolpum* from São Miguel. Carpospore development in culture. SMG-04-118.

Fig. 20. Uniseriate filaments derived from a single germ tube growing on glass coverslip. Scale bar = 100 µm.

Fig. 21. Consolidation of uniseriate filaments into spreading monolayered disks. Scale bar = 100 µm.

Figs 22, 23. Stages in carpospore development leading to erect filament formation.

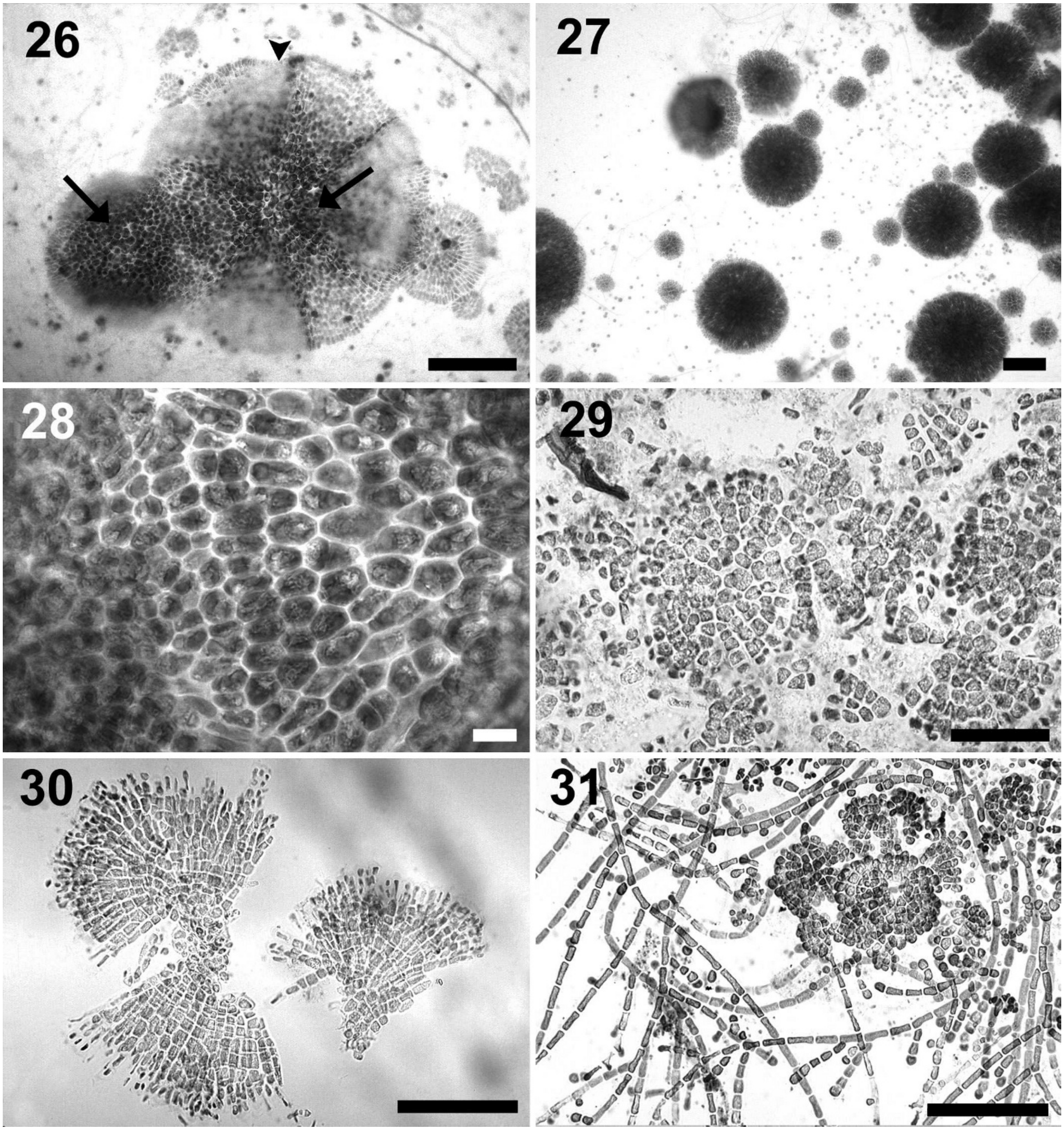
Fig. 22. Erect branched filament growing from an irregular aggregation of cells. Scale bar = 40 µm.

Fig. 23. Erect branched filaments growing from irregular aggregation of cells. Scale bar = 100 µm.

Fig. 24. Regular discs germinated from carpospores. SMG-04-118. Scale bar = 50 µm.

Fig. 25. Pseudodiscs germinated from carpospores. SMG-04-78. Scale bar = 50 µm.

several cells long (Fig. 20) developing distally into a disc or pseudodisc (Figs 21); and (3) an irregular aggregation of cells measuring 4.5–13 × 5–11 µm formed by transverse and longitudinal divisions developing in either aggregates spread out into horizontal filaments of comparable length and cell shapes/sizes (Fig. 22) or erect branched filaments to 205 µm in length composed of cells of variable shape and size (6–10 × 5–10 µm; Fig. 23).



Figs 26–31. *Platoma cyclocolpum* from São Miguel. Carpospore development in culture.

Fig. 26. Regular discs that have become confluent, with multistratose layers in center (arrows) and monostromatic layer at margin (arrowhead). SMG-04-118. Scale bar = 50 µm.

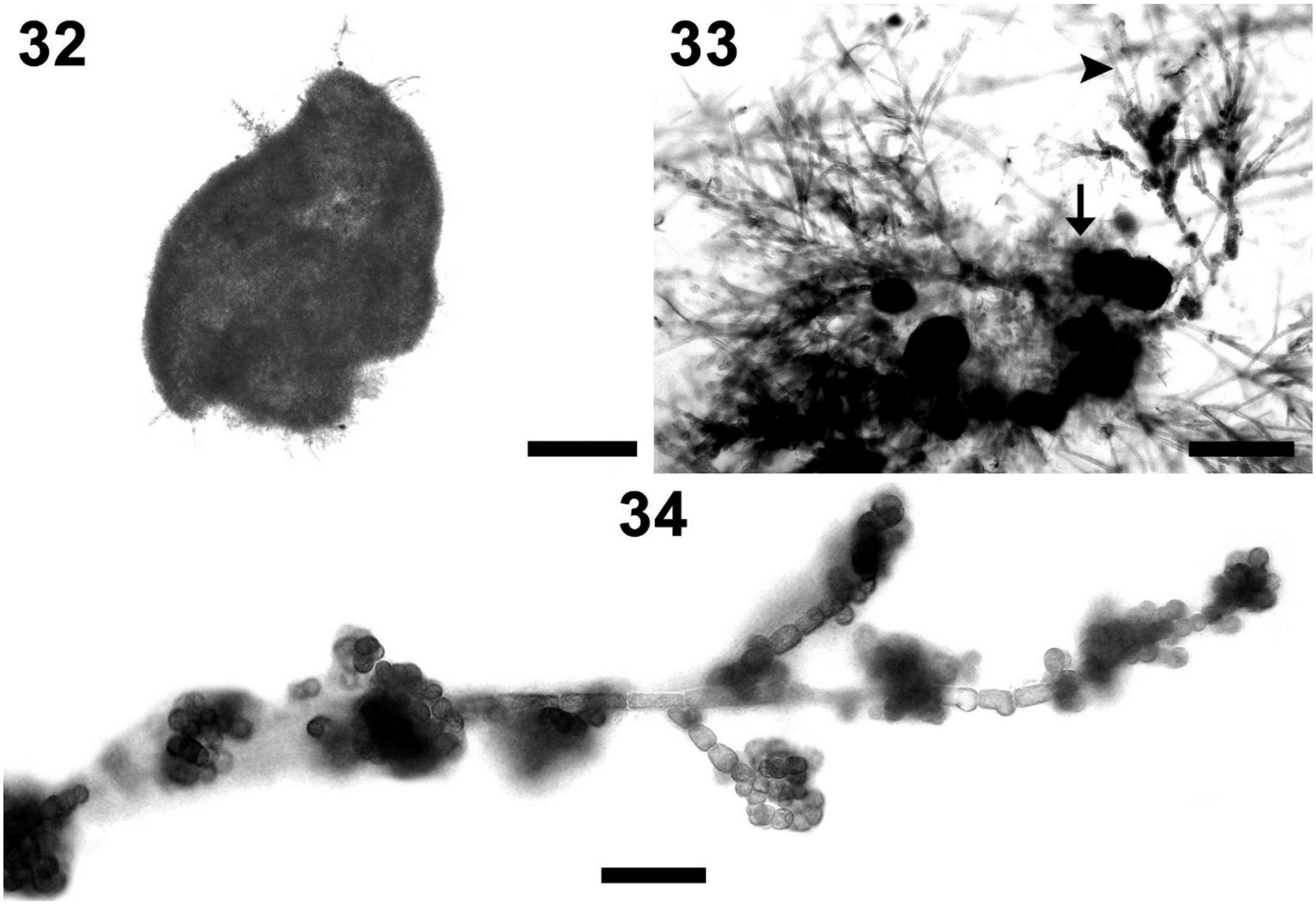
Fig. 27. Regular discs of different sizes growing on the same glass coverslip. SMG-04-81. Scale bar = 100 µm.

Fig. 28. Close-up of confluence in Fig. 26. SMG-04-118. Scale bar = 10 µm.

Fig. 29. Squash preparation of a multilayered crust consisting of central base and radially expanding erect filaments, each up to six cells long. Scale bar = 40 µm.

Fig. 30. Squash preparation of knot ball showing pseudoparenchymatous multilayered crust consisting of central base and radially expanding erect filaments, each up to 12 cells long. SMG-04-118. Scale bar = 100 µm.

Fig. 31. Squash preparation of erect filaments growing from crusts. Scale bar = 100 µm.



Figs 32–34. *Platoma cyclocolpum* from São Miguel. Development of thalli in culture.
Fig. 32. Erect thalli resulted from the original blade used in culture. SMG-04-118. Scale bar = 0.5 mm.
Fig. 33. Squash preparation of the thallus part depicted in Fig. 32. Erect filaments (arrowheads) resulting secondarily from recovery from squashed portions (arrows) of the original frond. SMG-04-205. Scale bar = 200 μm.
Fig. 34. Close-up of erect filament in Fig. 33. SMG-04-81. Scale bar = 50 μm.

Development of crusts and knot balls

The regular discs or pseudodiscs adhered strongly to the substratum and were comprised of loosely aggregated filaments of elongated, colourless distal cells and of shorter, darker, more compact proximal cells (Figs 24–27). Some discs remained monostromatic (Fig. 24) throughout the culture study; whereas, others became multilayered crusts (Fig. 26) at a later stage (ranging from 4 to 6 months). Occasionally, crusts with irregular outlines (Fig. 25) were observed in all culture regimes. Abutting discs and crusts occasionally became confluent (Figs 26–28). The basal cells of multilayered crusts repeatedly divided parallel to the surface to produce a mound of cells (Fig. 29) up to 12 cell layers in thickness. Quadrate to rectilinear basal cells 7–10.5 × 7–11 μm gave rise to erect and closely packed pseudodichotomous filaments up to 165 μm long. Cell sizes progressively decreased toward the surface, apical cells measuring 5–15 × 5–10 μm.

A few crusts detached from the substratum, rounded off and became free-forming knot balls formed by a series of periclinal cell divisions of filament axial cells. Cells of knot balls continued dividing, forming new rows of cells outwardly. When squashed, these pseudoparenchymatous mounds revealed a central base with radially expanding erect filaments up to 12 cells long (Fig. 30); some of the crusts remained in this form until they died off, lasting up to 1 year.

Development of erect thalli

Three patterns of erect thallus development were observed: (1) carpospores developed directly into erect filaments (Fig. 23); (2) erect filaments were produced from the thicker regions of discs and pseudodiscs, usually from the central part (Fig. 31); and (3) erect filaments regenerating from squashed portions of the original blade used in culture (Figs 32–34). Erect filaments continued to increase in size through a series of transverse and longitudinal divisions to result in a multiaxial organization. Continuous cell divisions produced an aggregation of a large number of erect filaments similar in structure to that found in gametophytes collected from nature; however, these filaments are not united in a gelatinous matrix.

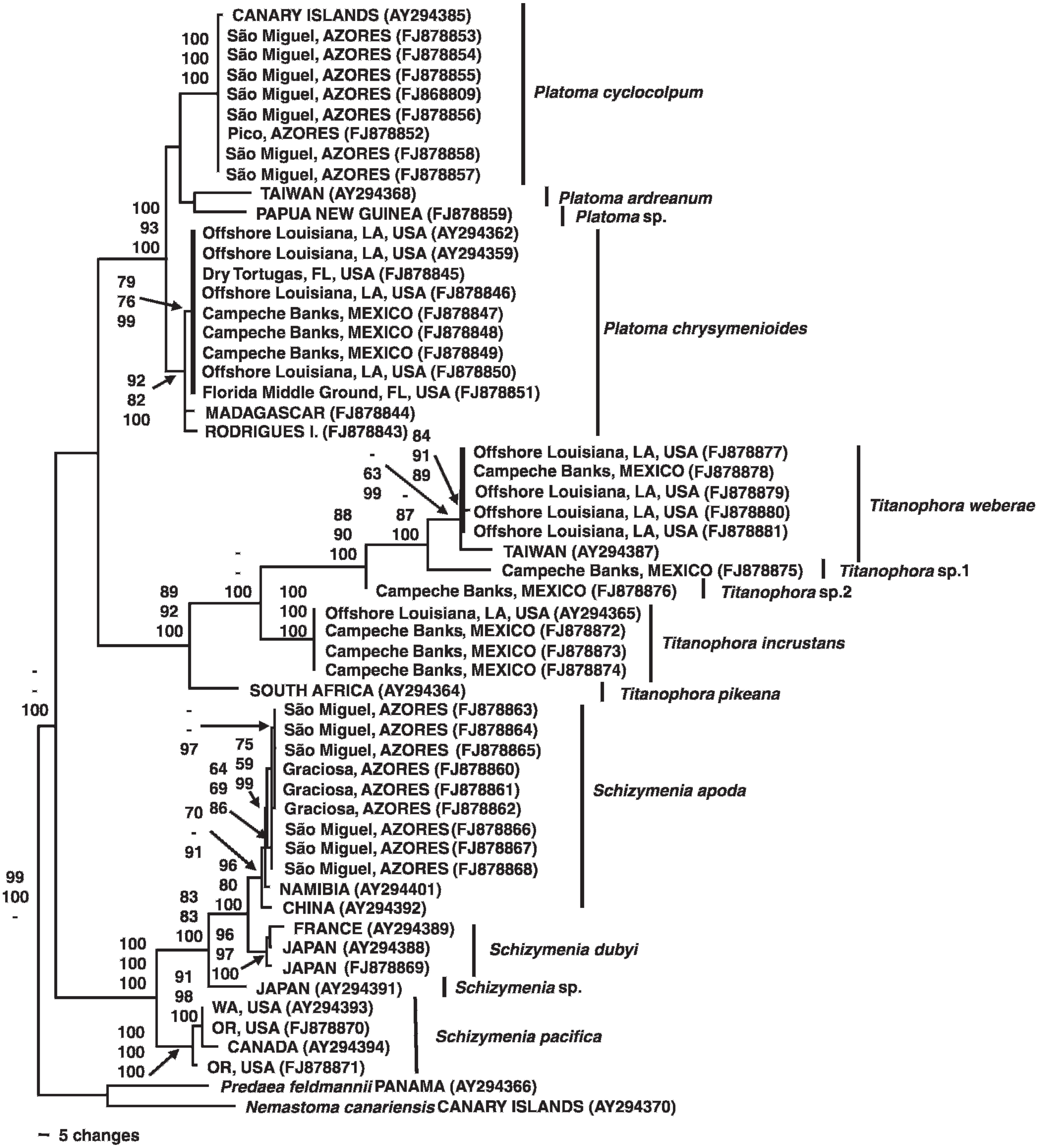


Fig. 35. Life history diagram of *Platoma cyclocolpum* from São Miguel. (1) Direct development of an apparently permanent crustose thallus; (2) immediate production of an erect thallus; (3) successive growth of erect thalli from initially crustose phases.

The present observations indicate that Azorean *P. cyclocolpum* has a complex life history (Fig. 35), in which the carpospores show three different developments.

Molecular analysis

With two members of the family Nemastomataceae as the outgroup, *rbc*L-based sequence analyses of members of the Schizymeniaceae resulted in a phylogenetic tree (Fig. 36) encompassing three main clades that comprise the monophyletic genera *Platoma*, *Titanophora* and *Schizymenia*. The MP tree demonstrates four distinct clades within the genus *Platoma*, each of which in the past would most likely have been identified as *P. cyclocolpum* but which now represent *P. chrysymenioides* (Figs 37–40), *Platoma ardreanum* from

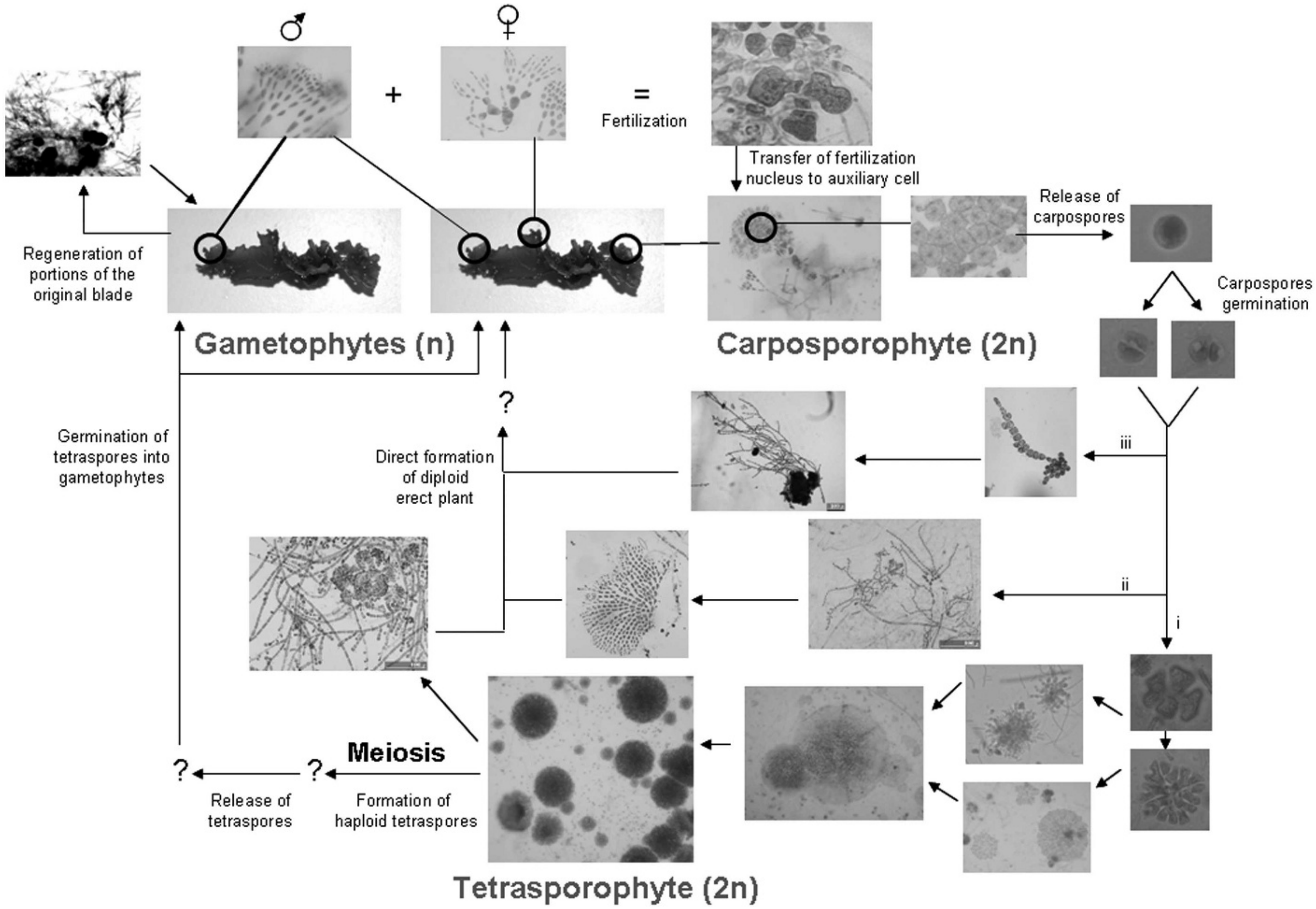


Fig. 36. One of 176 equally most parsimonious trees for chloroplast-encoded *rbc*L sequences showing the position of *Platoma cyclocolpum* in the genus *Platoma* and the Schizymeniaceae. Branch lengths are proportional to sequence change as indicated by the five-nucleotide-changes scale. Series of three numbers at nodes represent (respectively, from top) maximum parsimony bootstrap, maximum likelihood bootstrap and Mr. Bayes posterior probabilities values.

Taiwan and an undescribed species from Papua New Guinea in addition to genuine *P. cyclocolpum*. All four clades are well supported as distinct species by MP bootstrap, ML bootstrap, and Bayesian posterior probabilities (Fig. 36).

DISCUSSION

The type species of *Platoma*, *P. cyclocolpum*, is shown to be present throughout the Azores and the Canary Islands. The Azorean vouchers of *P. cyclocolpum* are in agreement with the description and illustrations provided by Masuda & Guiry (1994) working with plants from the Canary Islands. A comparison of Azorean material with specimens from other localities reveals differences with respect to variation in thallus shape, colour and texture as well as cell size.

All examined specimens previously identified as *P. cyclocolpum* from Madagascar, Rodrigues Island and from throughout the Gulf of Mexico are shown in this report to belong to *P. chrysymenioides* (see Table 1 for a comparison between both taxa). When Huisman (1999) synonymised the Western Australian-endemic *Nemastoma damaecorne* with *P. cyclocolpum*, he considered the larger size of the plants and the smaller vegetative cells of the former as a reflection of the disjunct distribution of a single species. Data from the present work indicate that *P. cyclocolpum* has a great morphological plasticity covering apparently distinct morphotypes even when environmental conditions did not vary between specimens. The ability of branches from different parts of the blade to coalesce, coupled with the ability of marginal parts of the blade to transform into holdfasts when in contact with the substratum, may be causes of much of the great morphological plasticity this species exhibits both in culture and in the field.

Anatomically, variation in the number of carpogonial branch cells was observed in *P. cyclocolpum* from the Azores, most being three-celled but with small numbers also four-celled. In some cases the four-celled carpogonial branches were clearly nonfunctional as the trichogynes continued to septate; whereas, in others this configuration may be a false impression resulting from the intercalation of a dislodged subsidiary cell. In any case, no instance was seen in which a clearly four-celled carpogonial branch was involved in the diploidization of generative auxiliary cells. The well-documented alternation of generation between the gametophytic blades of *Schizymenia* and the tetrasporangial crusts of *Haematocelis* (Ardré 1977, 1980; DeCew *et al.*

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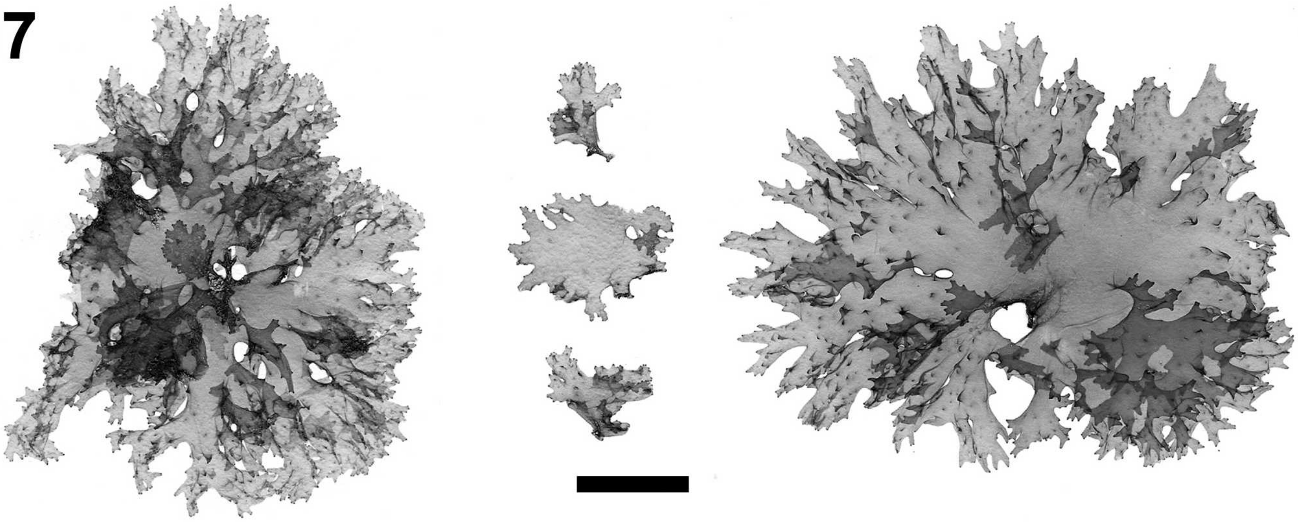
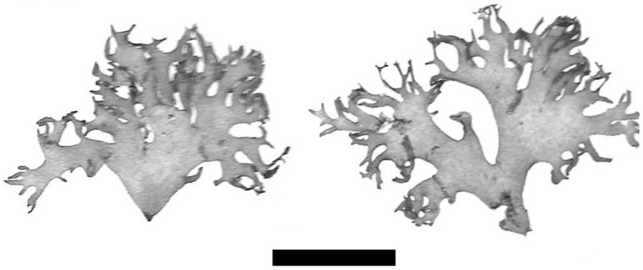
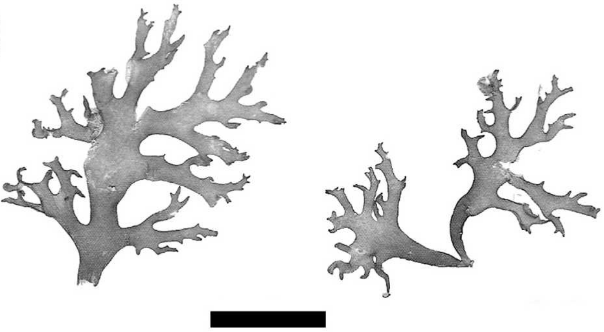


38



39



40

Figs 37–40. Habits of *Platoma chrysymenioides*.

Fig. 37. Herbarium-pressed specimens from Madagascar, HEC10222. Scale bar = 2 cm.

Fig. 38. Herbarium-pressed specimens from the Campeche Banks, Mexico, SW Gulf of Mexico. NSF-II-3-7. Scale bar = 2 cm.

Fig. 39. Same as in Fig. 38. NSF-II-100-4. Scale bar = 1 cm.

Fig. 40. Herbarium-pressed specimens from the Florida Middle Ground, NE Gulf of Mexico, NSF-III-7-09-06-5-1. Scale bar = 2 cm.

1992) suggests that this life history might be general throughout the Schizymeniaceae. However, no other study has succeeded in completing the life history of other species from this family, and only the development of vegetative crusts from carpospores has been conclusively demonstrated in culture for any species of *Platoma* (Masuda & Guiry 1994, Huisman 1999). The present work confirms the occurrence of a crustose stage in the life history of *P. cyclocolpum* but has failed to discover tetrasporophytes.

The present study has shown three different pathways following carpospore germination in culture: (1) direct development of an apparently permanent crustose thallus; (2) immediate production of an erect thallus; and (3) the successive growth of erect thalli from initially crustose phases. In each case, either the crust or the erect thallus has a different morphology. Our results are very similar to those of Masuda & Guiry (1994), both in regard to germination patterns of carpospores and the subsequent formation of crusts, such that the development of some discs, after detaching from the substratum, into knot-ball structures is a new observation for this species, and it is likely to be a culture artifact. A similar phenomenon has been reported for other groups, including the brown alga *Endarachne* (Parente *et al.* 2003).

The absence of tetrasporangial development in these and several previous life-history studies of *P. cyclocolpum* suggests the possibility of either apomixis or of somatic meiosis in some yet-undetermined site. Either that, or natural conditions conducive to the initiation of tetrasporogenesis could not have been mimicked in the culture experiment. Arguing against the latter possibility is the fact that no unidentified or unattributed tetrasporic crusts have yet been found in nature that could represent the *P. cyclocolpum* tetrasporophyte. Substratum specificity might be an important factor that has not been tested in the culture experiments.

Dawson (1961) suggested conspecificity of *Platoma tenue* M.A. Howe & W.R. Taylor and *Predaea masonii*, a suggestion subsequently endorsed by Bula-Meyer (1992), who provided supporting diagnostic details of the auxiliary-cell complex. However, Bula-Meyer's new combination *Predaea tenuis* (M.A. Howe & W.R. Taylor) G. Bula-Meyer (1992: 64) needs validation because certain strictures of the International Code of Botanical Nomenclature were not correctly followed. The following validation of Bula-Meyer's combination is thus provided:

***Predaea tenuis* (M.A. Howe & W.R. Taylor) Bula-Meyer in Gabriel *et al.* 2008**

BASIONYM: *Platoma tenue* M.A. Howe & W.R. Taylor (1931, p. 32).

SYNONYM: *Predaea tenuis* (M.A. Howe & W.R. Taylor) G. Bula-Meyer (1992, p. 64), nom. inval.

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