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Cladophora rhodolithicola sp. nov. (Cladophorales, Chlorophyta), a diminutive species from European maerl beds

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Cladophora rhodolithicola sp. nov., a small green macroalgal epiphyte on rhodoliths, is described from the Atlantic coasts of the British Isles, France and Spain, based on morphological and molecular evidence. Molecular phylogenetic analyses reveal that the rhodolith epiphyte is closely related to *C. pygmaea*, a dwarf species that also grows on maerl and small stones and whose systematic position was previously uncertain. The fact that both species, along with two other distinct *Cladophora* species (*C. echinus* and *C. battersii*), are resolved among species traditionally assigned to the *Cladophora* section *Longi-articulatae*, has implications for our understanding of the evolution of the genus. The section *Longi-articulatae* is one of the most distinctive groups in *Cladophora*, being characterized by coarse thalli with conspicuous basal cells, strict acropetal growth and the lack of intercalary rhizoids. Here we show that deviant character states such as reduced and irregular growth, and intercalary rhizoids have evolved independently several times within this clade.

Key words: *Cladophora rhodolithicola*, Cladophorophyceae, green algae, maerl, marine, phylogeny, rhodolith beds, Siphonocladales, systematics, Ulvophyceae

Introduction

Rhodolith or maerl beds are composed of high concentrations of free-living, non-geniculate coralline red algae and are found worldwide in arctic, temperate and tropical marine waters (Foster, 2001; Wilson *et al.*, 2004). Maerl beds, which vary in size from tens to thousands of square metres, harbour high biodiversity and have significantly higher habitat heterogeneity than common adjacent substrata including gravel, sand, and impacted dead maerl beds (Bordehore *et al.*, 2003; BIOMAERL Team, 2003; Kamenos *et al.*, 2003; Steller *et al.*, 2003; Hall-Spencer *et al.*, 2006). This high diversity is partly a result of the branched twig-like thalli of the coralline red algae that lock together into a network, providing ecological niches for a diverse range of associated macroalgae and invertebrates, some of which may be confined to the maerl habitat (Hily *et al.*, 1992; Birkett *et al.*, 1998; Grall *et al.* 2006). The diversity of associated seaweeds in maerl beds is dominated by red algae, but also numerous green algae, including species of *Bryopsis*, *Cladophora*, *Codium*, *Derbesia* and *Ulva* grow as epiphytes on rhodoliths (Birkett *et al.*, 1998; BIOMAERL Team, 1999; Peña & Bárbara, 2008).

Cladophora is a large and common green macroalgal genus with a worldwide distribution in marine as well as freshwater habitats. Plants have simple thallus architecture, composed of branched, uniseriate filaments of multinucleate cells. The taxonomy of the genus is problematic. Molecular phylogenetic studies have shown that the circumscription of *Cladophora*, as well as the included morphological sections and species are in need of revision. The genus is polyphyletic with representatives in three different clades (Cladophorales-, Siphonocladales- and *Aegagropila*-clade), but nomenclatural changes have not yet been proposed (Bakker *et al.*, 1994; Hanyuda *et al.*, 2002; Leliaert *et al.*, 2003). At the species level, taxonomic problems arise from intraspecific morphological variability and cryptic genetic diversity.

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Examples include cryptic species in *C. vagabunda* (Linnaeus) van den Hoek (Bakker *et al.*, 1995*a*) and *C. coelothrix* Kützing (Leliaert *et al.*, 2007*a*), and *C. albida* (Nees) Kützing and *C. sericea* (Hudson) Kützing forming a morphologically diverse species complex (Bakker *et al.*, 1995*b*). Although the exact number of species along the Atlantic coasts of Europe is uncertain, 18 morphospecies are currently recognized based on differences in habit, mode of attachment, organization of branch systems (acropetal with cell divisions largely confined to the apical cells, or irregular with many intercalary cell division), number of branches per node, insertion of the branches (apical with a slightly inclined cross wall or lateral with a steeply inclined cross wall), cell shape and dimensions, and thickness of the cell walls (van den Hoek, 1963; Leliaert & Boedeker, 2007). Along the Atlantic European coast, several *Cladophora* species have been reported on maerl, including *C. albida*, *C. battersii* van den Hoek, *C. hutchinsiae* (Dillwyn) Kützing, *C. laetevirens* (Dillwyn) Kützing, *C. lehmanniana* (Lindenberg) Kützing, *C. pellucida* (Hudson) Kützing, *C. pygmaea* Reinke, *C. retroflexa* (Bonnemaison ex P.L. Crouan & H.M. Crouan) van den Hoek, *C. rupestris* (Linnaeus) Kützing and *C. sericea* (Donze, 1968; Cabioch, 1969; Blunden *et al.*, 1977; Birkett *et al.*, 1998; BIOMAERL Team, 1999; Mannino *et al.*, 2002; Bárbara *et al.*, 2004; Peña & Bárbara, 2008).

A small, distinct *Cladophora* has been commonly found in maerl beds of the British Isles (Maggs, 1983; Birkett *et al.*, 1998; Bunker & Camplin, 2006), and the Atlantic coast of Spain (Peña & Bárbara, 2006). These subtidal plants have been referred to as small growth forms of *Cladophora rupestris* (Linnaeus) Kützing, which normally forms large, rope-like tufts on intertidal rocks. The conspecificity with *C. rupestris* is questionable from a morphological as well as ecological perspective. In this paper we aim to determine the identity of the enigmatic rhodolith epiphyte and examine its systematic position using morphological and molecular data.

Material and methods

Specimens of *Cladophora* were collected from maerl beds in Wales, France and Spain, and voucher specimens from Ireland (Maggs, 1983) were received on loan from GALW (Tables 1, 2). Specimens were preserved in 5% formalin in seawater or kept alive in seawater for culturing purpose (Table 1). Unialgal cultures could not be established because clean, cut off apical cells failed to grow and died after 1 or 2 weeks. Instead, the *Cladophora* plants grew successfully (but slowly) on the rhodoliths in the presence of other epiphytic or associated algae. Algal cultures were grown in 1/8x modified Provasoli enriched seawater (West & McBride, 1999) at 17°C, under a 12-h:12-h light–dark cycle with a photon flux rate of c. $10\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$. Stronger enrichment solutions, higher temperatures and levels of irradiation resulted in the thalli being overgrown by epiphytic algae. Specimens were examined with an Olympus BX51 bright field light microscope and an Olympus SZX10 stereo microscope (Olympus Co., Tokyo, Japan). Photographs were taken with an Olympus E410 digital camera mounted on the light or stereo microscope. Additional specimens that were examined morphologically include cultures of *C. pygmaea* and *C. echinus* (Biasoletto) Kützing, and other specimens of the *Cladophora* section *Longi-articulatae* (Table 1).

Specimens for the molecular analyses were collected from two maerl beds, one in the British Isles (Milford Haven, UK), the other in north-western Spain (Ría de Vigo, Galicia). Additional taxa from which sequences were generated, or obtained from GenBank, are listed in Tables 1 and S1 (see supplementary material, available at: http://www.informaworld.com/mpp/uploads/ leliaert_et_al_supplementary-material.pdf). Molecular phylogenetic analyses were based on small subunit (SSU), partial large subunit (LSU) rDNA and partial rDNA ITS1-5.8S-ITS2 sequences. Clean portions of the living or silica-dried plants were isolated for DNA extraction. The minute plants of *C. pygmaea* were carefully isolated from small stones under a stereo microscope with fine tweezers. DNA of *C. dotyana*, *C. echinus*, *C. herpestica*, *C. pellucidoidea* and *C. sakaii* was extracted from unialgal cultures. DNA extraction, PCR amplification and sequencing were performed as described in Leliaert *et al.* (2007*a*, *b*).

The LSU sequences were included in a preliminary neighbor-joining analysis in PAUP* 4.0b10 (Swofford, 2002) with more than 200 cladophoralean sequences (Boedeker, unpublished data). Based on this result, closely related taxa of the maerl epiphytes along with representatives of the main cladophoralean clades were selected for subsequent phylogenetic analyses. Three data sets were considered. The first one was assembled to assess the phylogenetic relationship of the rhodolith epiphytes within the Cladophorophyceae (Cladophorales/Siphonocladales). This data set consisted of an alignment of 46 partial LSU rDNA sequences with *Cladophora horii* van den Hoek & M. Chihara selected as the outgroup taxon based on Leliaert *et al.* (2003). This dataset will further be referred to as the LSU alignment. In order to determine the phylogenetic position of *Cladophora battersii*, for which we were able to sequence only the first 999 base pairs of the SSU rDNA, a second dataset of 34 SSU sequences was assembled (Table S1, available at: http://www.informaworld.com/mpp/uploads/ leliaert_et_al_supplementary-material.pdf). Based on the results of the phylogenetic analysis inferred from the LSU alignment, a third dataset was assembled and analysed to examine the phylogenetic position of the putative new species on maerl among related species (from hereon referred to as the *Longi-articulatae* (LA) clade) with more confidence. This dataset consisted of

Table 1. Specimens for which new sequences were generated with collection data (location, collector, date of collection and voucher information) and EMBL accession numbers.

Species	Collection and voucher information	SSU rDNA	partial LSU rDNA	rDNA ITS
Chaetomorpha aerea (Dillwyn) Kützing	France: Normandy: Cherbourg, intertidal rockpool (W. Prud'homme van Reine, summer 2005, B19, L 0793563)		FM205025	
Chaetomorpha aerea	UK: Wales: Pembrokeshire: Milford Haven, subtidal (−4m), on maerl (F. Bunker, 6 Nov 2007, Bunker5/F720, GENT)		FM205026	
Cladophora battersii van den Hoek	Ireland: Co. Donegal: SW Mulroy Bay, unattached tufts in a very sheltered, enclosed sea lough with freshwater input (C. Maggs, J80, L 0793567)	FM205047	FM205027	
Cladophora dotyana Gilbert	Fiji: Great Astrolabe Reef (M & D. Littler, 15 Feb 1996, DML40094/F648, US)	FM205048	FM205028	
Cladophora dotyana	Australia: Queensland: Townsville: Arthur Bay (M. van Oppen, 1991, CrugABA/Crugu1l/F221, as 'Cladophora rugulosa')a			
Cladophora echinus (Biasoletto) Kützing	Algeria: Algiers, (R. Delepine, summer 1960, no. 60/55, UTEX LB 148/F533)a,b	FM205049	FM205029	
Cladophora feredayi Harvey	New Zealand: Kaikoura: Oaru stream, subtidal (−0.5m, on rock with coralline crusts (C. Boedeker, Nov 2002, C48, L 0793564)		FM205030	
Cladophora herpestica (Montagne) Kützing	China: Hainan (I. Bartsch, 1990, ClozC007/Cherp1/F211)a		FM205031	
Cladophora laetevirens (Dillwyn) Kützing	Netherlands: Zeeland: Westkapelle, intertidal, on rock (C. Boedeker, Aug 2004, B21, L 0793552)		FM205032	
Cladophora laetevirens	UK: Wales: Pembrokeshire: Milford Haven, subtidal (−4m), on maerl (F. Bunker, 6 Nov 2007, Bunker4/F719, GENT)	FM205050	FM205033	
Cladophora mirabilis (C. Agardh) Rabenhorst	South Africa: Cape Town: Hout Bay, drift (C. Boedeker, Sep 2005, E09, L 0793561)		FM205034	
Cladophora olkuboana Holmes	Oman: Dhofar: Mirbat: Hoon's Bay (T. Schils *et al.*, 25 Sep 2003, DHO151/F172, GENT)		FM205035	
Cladophora pellucida (Hudson) Kützing	Madeira: Porto da Cruz (E. Coppejans & O. De Clerck, 16 May 2006, HEC 15794/F529, GENT)		FM205036	
Cladophora pellucida	France: Brittany: La Conquet, intertidal rockpool (C. Boedeker, Jun 2004, A25, L 0793562)		FM205037	
Cladophora pellucidoidea van den Hoek	Canary Islands: Tenerife (collector unknown, 1988, CpoT/Cpeloid3/F218)a		FM205038	
Cladophora pygmaea Reinke	UK: Wales: Pembrokeshire: Milford Haven, subtidal (−4m), on pebbles (F. Bunker, 6 Nov 2007, Bunker3/F718, GENT)a		FM205039	FM205054
Cladophora pygmaea	UK: Wales: Pembrokeshire: Milford Haven, subtidal, on pebbles (C. Maggs, summer 2006, J94, L 0793569)	FM205051	FM205040	
Cladophora pygmaea	Denmark: Sønder Stenron (Baltic Sea), subtidal (−12m), on pebbles (R. Nielsen *et al.*, Sep 1993, J24/RN93105, L 0793547)		FM205041	
Cladophora radiosa (Suhr) Kützing	South Africa: Western Cape: Saldanha Bay, epilithic in intertidal rock pool (C. Boedeker, Sep 2005, E14, L 0793566)	FM205052	FM205042	
Cladophora rhodolithicola Leliaert	UK: Wales: Pembrokeshire: Milford Haven, on maerl (F. Bunker, Jun 2006, FL1036/F530, GENT)a		FM205043	
Cladophora rhodolithicola	UK: Wales: Pembrokeshire: Milford Haven, subtidal (−4m), on maerl (F. Bunker, 6 Nov 2007, Bunker1/F716, GENT, Holotype)a	FM205053	FM205044	FM205055
Cladophora rhodolithicola	Spain: Pontevedra: Ria de Vigo: Vigo: Baliza de Tofiño, subtidal (−11m), on maerl (V. Peña & I. Bárbara, 12 Sep 2007, FL1193/F715, GENT and SANT-Algae 19589)a		FM205045	FM205056
Cladophora sakaii I.A. Abbott	Japan: Choshi (C. van den Hoek, 1990, CsakCJ/Csak1/F223)a		FM205046	

aCulture maintained in Ghent. bIsolate examined by van den Hoek (1963: 50, table 5).

Table 2. Additional specimens of *Cladophora rhodolithicola* that were examined morphologically.

Locality	Habitat	Collector(s), collection date and voucher information
France: Bretagne: Finistère: Molène: Banc des Pourceaux	On maerl, 4–11 m deep	J. Grall, 1 Mar 2005, SANT-Algae 19590
Ireland: Galway Bay: Carraroe	On maerl, c. 3 m deep	C. Maggs, 27 Jun 1980, GALW
Ireland: Galway Bay: Carraroe	On maerl, c. 3 m deep	C. Maggs, 27 Aug 1980, GALW
Ireland: Galway Bay: Finavarra (south shore of bay)	On maerl, c. 10 m deep	C. Maggs, 1 Jul 1980, GALW
Spain: A Coruña: Ría de Arousa: A Pobra do Caramiñal: Isla Ratas	On maerl, 8 m deep	V. Peña & I. Bárbara, 28 Apr 2004, SANT-Algae 19571
Spain: A Coruña: Ría de Arousa: Riveira: Sur de Aguiño	On maerl, 1 m deep	V. Peña & I. Bárbara, 22 Nov 2005, SANT-Algae 19580
Spain: A Coruña: Ría de Muros y Noia: Ensenada Bornalle	On maerl, 9 m deep	V. Peña & I. Bárbara, 1 Jun 2006, SANT-Algae 19584
Spain: Asturias: Serantes: Playa del Sarello	On sand covered *Lithophyllum*, low intertidal	P. Díaz & I. Bárbara, 2 Jun 2006, SANT-Algae 17817
Spain: Pontevedra: Ría de Pontevedra: Bueu: Playa de la Tulla	On maerl, 10 m deep	V. Peña & I. Bárbara, 18 Feb 2005, SANT-Algae 19582
Spain: Pontevedra: Ría de Pontevedra: Marín: NW Isla de Tambo	On maerl, 6 m deep	V. Peña & I. Bárbara, 10 May 2005, SANT-Algae 19583
Spain: Pontevedra: Ría de Vigo: Cangas: Ensenada Limens	On maerl, 15 m deep	V. Peña & I. Bárbara, 7 Jun 2006, SANT-Algae 19587
Spain: Pontevedra: Ría de Vigo: Cangas: Oeste Con de Pego	On maerl, 4 m deep	V. Peña & I. Bárbara, 10 Dec 2005, SANT-Algae 17049
Spain: Pontevedra: Ría de Vigo: Cangas: Oeste Con de Pego	On maerl, 4 m deep	V. Peña & I. Bárbara, 14 Mar 2006, SANT-Algae 19586
UK: Wales: Pembrokeshire: Milford Haven	On maerl and pebbles, subtidal	C. Maggs, summer 2006, L 0793569

a concatenated alignment of SSU and partial LSU rDNA sequences from 21 representatives of the LA clade, including the putative new *Cladophora* species, *C. pygmaea* and *C. echinus*. This dataset will further be referred to as the SSU-LSU alignment. An incongruence length difference test (Farris *et al.*, 1995), implemented in PAUP, indicated that the SSU and LSU data were not significantly heterogeneous ($p = 0.99$), justifying a combined data approach. Sequences were aligned using ClustalW (Thompson *et al.*, 1994) with default settings implemented in BioEdit (Hall, 1999), and inspected visually.

The three datasets were analysed with maximum likelihood (ML) and Bayesian inference (BI). ML analyses were performed with PhyML v2.4.4 (Guindon & Gascuel, 2003). BI was performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). The LSU dataset was analysed under a Jukes–Cantor model, following Leliaert *et al.* (2003). The SSU and SSU-LSU alignment were analysed under a general time-reversible model with a proportion of invariable sites and gamma distribution split into four categories (GTR+I+G), as determined by the Akaike Information Criterion in PAUP/Modeltest 3.6 (Swofford, 2002; Posada & Crandall, 1998). BI analyses consisted of two parallel runs of each four incrementally heated chains, and 3 million generations with sampling every 1000 generations. A burn-in sample of 1000 trees was removed before constructing the majority rule consensus tree. For the ML trees, the reliability of each internal branch was evaluated based on 1000 bootstrap replicates.

Phylogenetic analyses were conducted on datasets with or without outgroup taxa. The root position of the LA clade was determined by using one of four different outgroups (*C. aokii*, *C. coelothrix*, *C. rupestris* or *C. vagabunda*) or the four combined. Additionally, the root placement was determined under a relaxed molecular clock (where the root of the tree is placed along its oldest branch, at exactly the same distance from each terminal taxon) with an uncorrelated log-normal model in BEAST v1.4.6 (Drummond & Rambaut, 2007).

The ITS sequences of *C. pygmaea* and the putative new species were used to check for conspecifity and assess sequence divergence. Uncorrected pairwise distances (p-distance) were calculated in PAUP.

Character evolution was traced along the LA-ML tree using parsimony reconstruction in Mesquite v1.11 (Maddison & Maddison, 2006).

Results

Morphology

Plants form medium to dark green, stiff tufts up to 1 (rarely 2) cm high (Figs 1, 2, 10, 15, and Fig. S1, available online at: http://www.informaworld.com/mpp/uploads/leliaert_et_al_supplementary-material.pdf) and are composed of irregularly branched, uniseriate filaments. Thalli are attached to rhodoliths at a single point by branched rhizoids developing from a basal cell (Fig. 6), or at multiple points by tightly clumped rhizoids produced by stolonoid cells (Figs 3, 4). In addition, the plants can attach by rhizoids developing from the proximal pole of cells in any part of the thallus, including apical cells (Figs 5, 9, 11). The plants thus consist of both erect and creeping parts. Growth of the thallus is accomplished by apical and intercalary cell
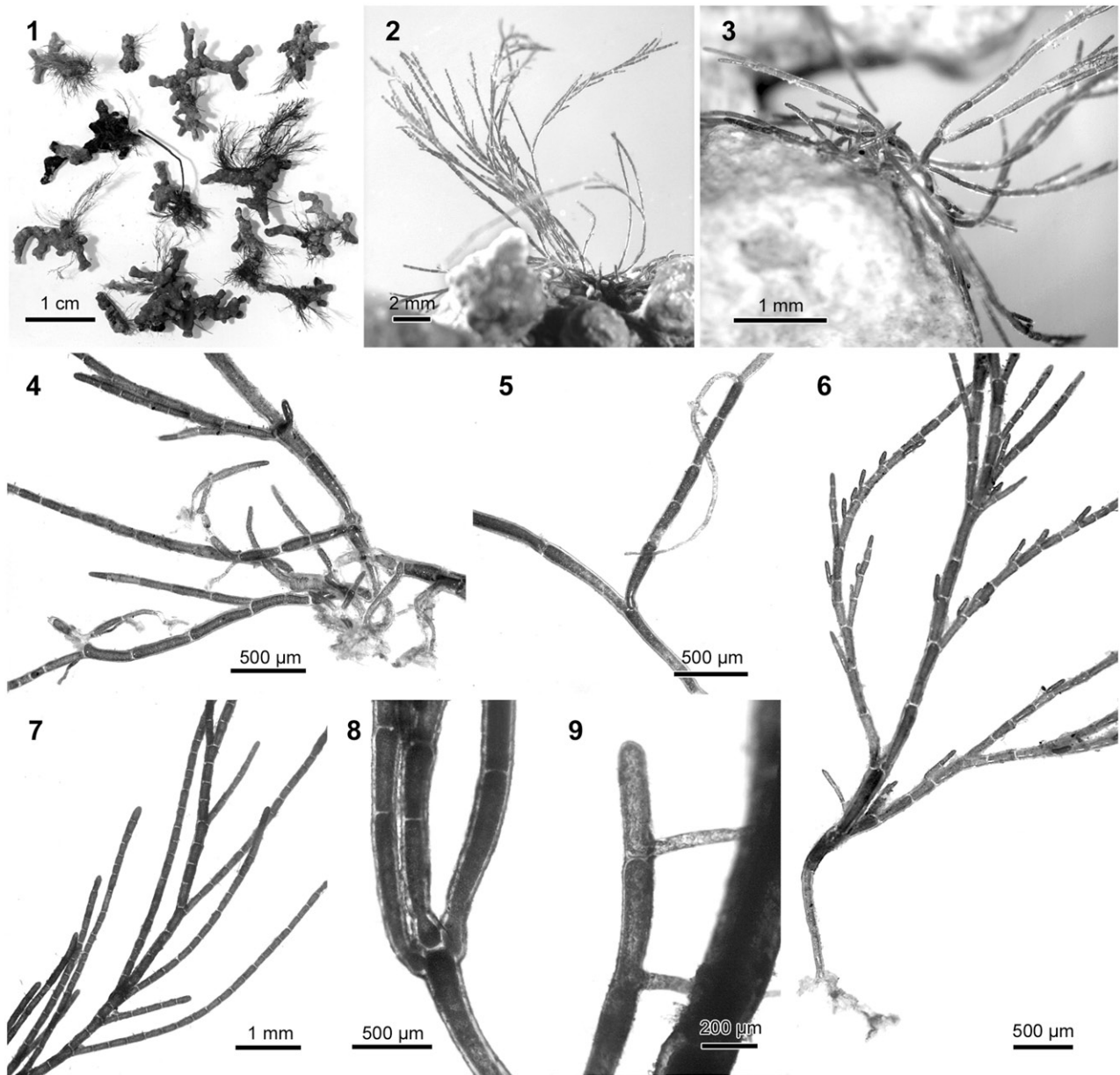
Figs 1–9. Holotype of *Cladophora rhodolithicola* from a subtidal maerl bed in Milford Haven, Wales (F. Bunker, 6 Nov 2007, GENT). Fig. 1. Habit of thalli growing on rhodoliths. Figs 2–3. Creeping stolonoid filaments giving rise to erect branches. Fig. 4. Detail of stolonoid filaments with rhizoids produced from the base of the cells. Fig. 5. Unbranched, aseptate rhizoid produced from the proximal pole of an intercalary cell. Fig. 6. Thallus composed of irregularly organized branch systems; rhizoids produced at the basal pole of the basal cell. Fig. 7. Irregularly organized branch systems. Fig. 8. Cells near the base with distinct bulges at the proximal poles. Fig. 9. Unbranched, aseptate rhizoids produced from the proximal poles of an intercalary and apical cell.

divisions, followed by cell elongation. New cells produce lateral branches at their apical poles, resulting in rows of branches, younger ones inter-calated between older ones (Figs 6, 7, 15, 16). Older cells may produce a second, third and sometimes fourth or fifth branch (Figs 6, 18). Some filaments undergo successive intercalary cell division with delayed branch formation, resulting in unbranched portions of filaments of up to 20 cells (Fig. 7). Young branches are inserted at the apical cell pole by an oblique wall cutting it off from the parent cell; the position of the wall becoming nearly horizontal in older branches, resulting in pseudodi-, tri- or poly-chotomously branching main axes (Figs 6, 12, 17, 18). Angle of ramification is very variable, generally 5–40°, sometimes 90° or more in basal branches. Apical cells are cylindrical to slightly tapering with rounded tips (Fig. 19), 37–65 µm in diameter (average 45 µm), up to 175 (–200) µm long, 1–4 times as long as broad. Cells of the term-inal branch systems are generally cylindrical, some-times slightly clavate or barrel-shaped, 50–95 µm in diameter (average 75 µm), up to 350 µm long, length/width ratio highly variable, 2–5 in regions with few cell divisions, 0.5–1.5 in filaments with numerous intercalary divisions. Basal cells are
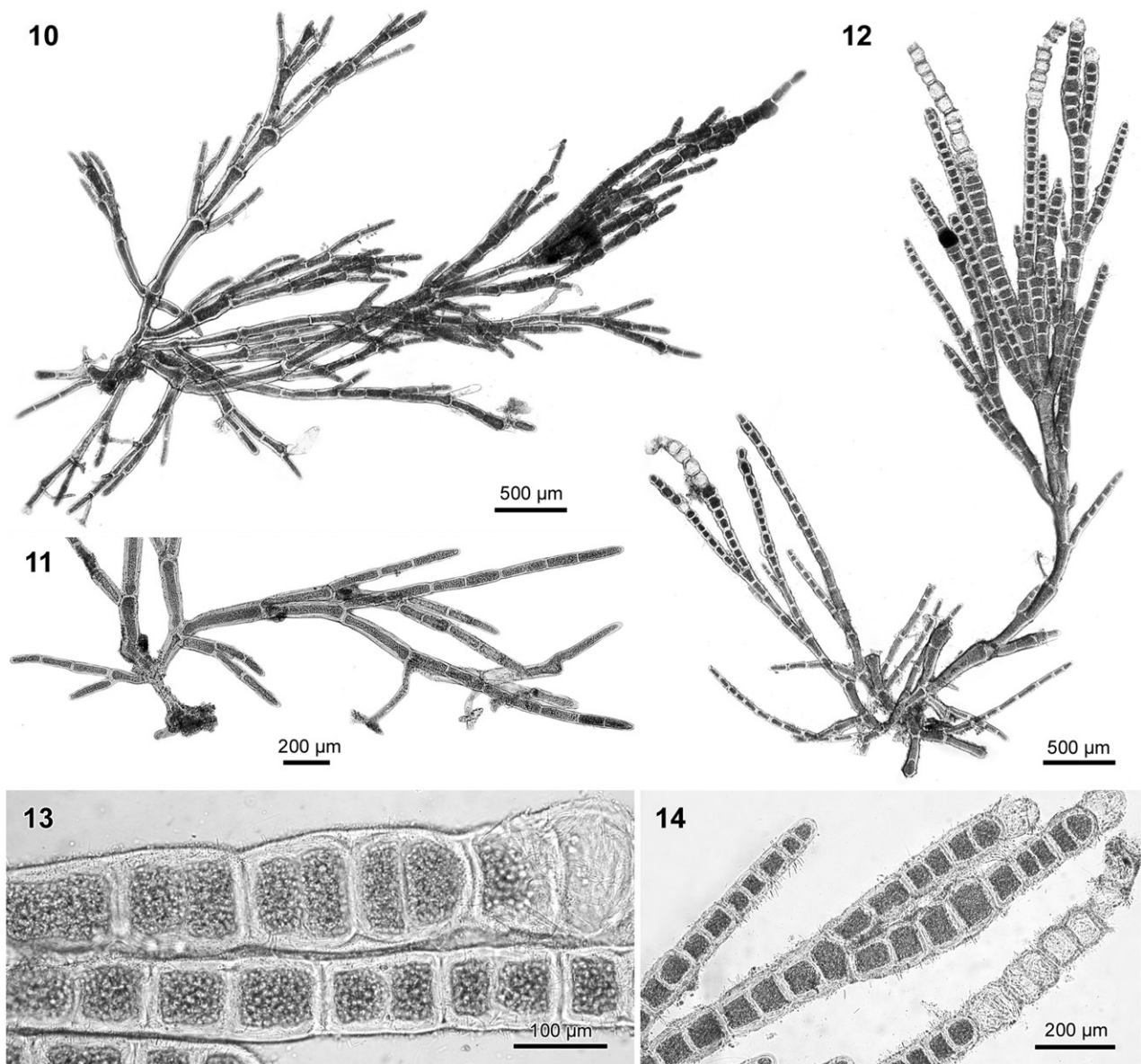
Figs 10–14. *Cladophora rhodolithicola* from subtidal maerl beds of Galway Bay, Ireland (Figs 10–11: Finavarra, C. Maggs, 1 Jul 1980, GALW; Figs 12–14: Carraroe, C. Maggs, 27 Aug 1980, GALW) C. Maggs, Jun–Aug 1980, GALW). Fig. 10. Creeping filaments giving rise to erect branches. Fig. 11. Creeping filaments with intercalary rhizoids. Fig. 12. Reproductive plant with terminal chains of swollen cells, which become zoidangia. Fig. 13. Intercalary cell divisions prior to zoidangia formation. Fig. 14. Detail of zoidangia.

cylindrical to club-shaped, often with a distinct basal bulge (Figs 4, 8), sometimes curved, 110–160 (–210) µm in diameter, up to 1100 µm long, 3.5–8 times as long as broad. Cell walls are relatively thick and striate, 4–8 µm thick in the apical cells, 20–40 µm thick in the basal cells. Chloroplasts are polygonal or round, 8–12 µm in diameter, with a relatively large pyrenoid, c. 6 µm in diameter, forming a more or less closed parietal layer (Fig. 20). Cells lack crystalline cell inclusions. In reproductive plants, the terminal filaments undergo numerous intercalary cell divisions (Fig. 13) resulting in long chains of swollen, barrel-shaped cells, which become zoidangia (90–140 µm in diameter, 1–1.2 times as long as broad),

each releasing its zoids through a single lateral pore halfway along the cell (Figs 12, 14).

Plants were cultured for 5 months. Within this period only limited growth was observed: filaments increased c. 3–7 mm in length. Cell divisions were mainly intercalary, resulting in long, unbranched filaments of 25 cells or more. In some plants the cells of these filaments became distinctly barrel-shaped but did not form zoidangia.

Habitat, seasonality and geographical distribution

Plants have been found as epiphytes on rhodoliths from maerl beds in the British Isles (Wales and Ireland), France (Brittany) and Spain (Galicia).
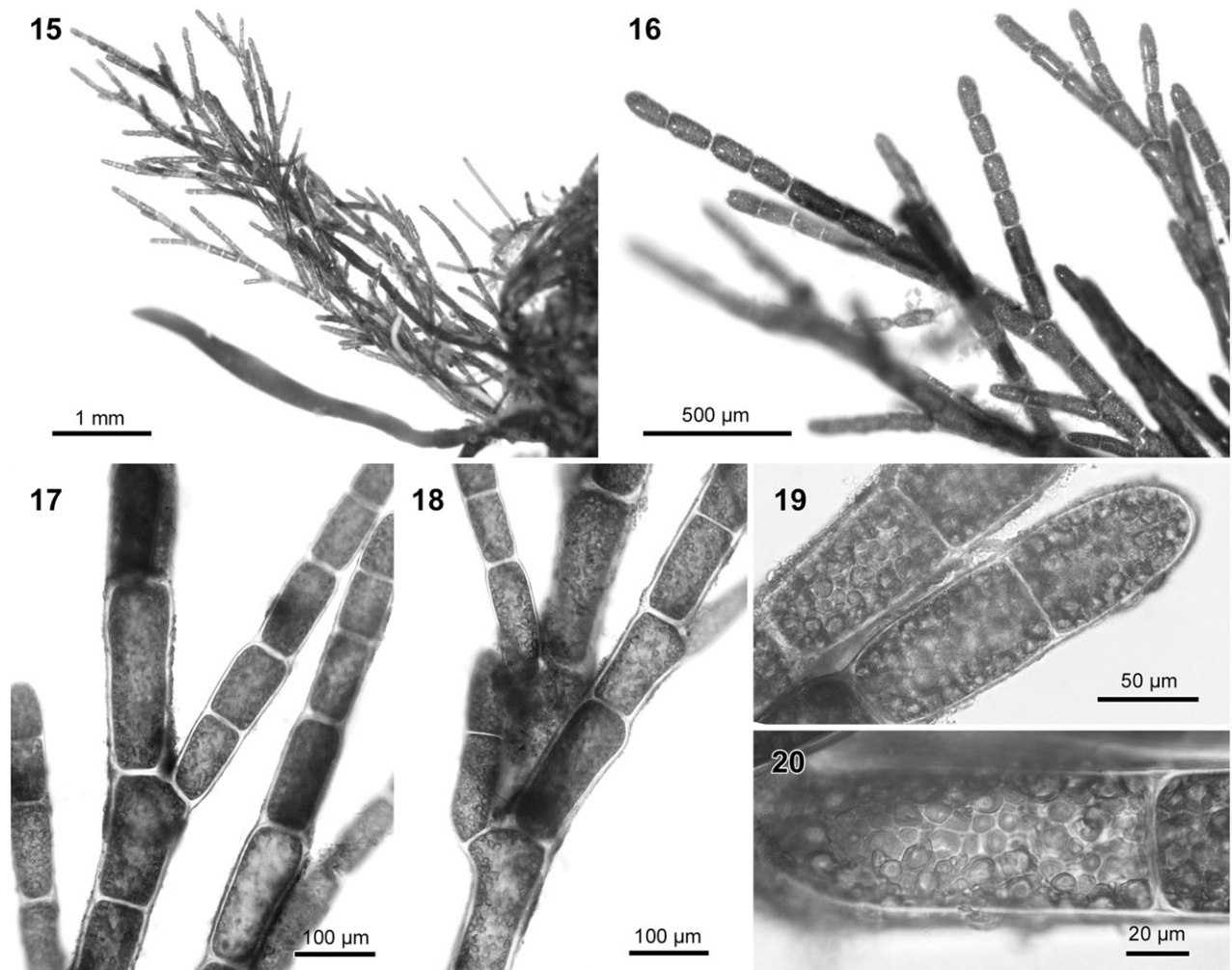
Figs 15–20. *Cladophora rhodolithicola* from a subtidal maerl bed of Ría de Vigo, Spain (V. Peña & I. Bárbara, 12 Sep 2007, GENT). Fig 15. Thallus composed of irregularly organized branch systems. Fig. 16. Terminal filaments composed of sub-cylindrical cells. Figs 17, 18. Details of branches; young branches inserted at the apical cell pole by an oblique wall cutting it off from the parent cell, the position of the wall becoming nearly horizontal in older branches, resulting in pseudodichotomies or polychotomies. Fig. 19. Cylindrical apical cell with rounded tip. Fig. 20. Polygonal chloroplasts forming a closed parietal layer.

The geographical distribution is possibly much wider given that the species has likely been confused with *C. rupestris*, which it vaguely resembles. The Galician specimens were recorded throughout the year in different subtidal maerl beds at 1–15 m depth. In Asturias (Spain) a specimen was found in the low intertidal on a single occasion, growing on sand-covered crustose coralline red algae. Specimens from Brittany were collected from a maerl bed at 4–15 m depth in March 2005. The plants from Wales were growing in shallow subtidal maerl beds (c. 4 m depth) on *Phymatolithon calcareum* (Pallas) W.H. Adey & D.L. McKibbin and *Lithothamnion corallioides* (P.L. Crouan & H.M. Crouan, 1867) P.L. Crouan & H.M. Crouan. During a study of an impacted maerl bed in Milford Haven it has been observed that *C. rhodolithicola* grew on both live and dead rhodoliths and occasionally on adjacent shell fragments (Bunker, unpublished data). A single specimen was also found, together with *C. pygmaea*, on a small pebble that was lying amongst the rhodoliths. Observations made at two maerl bed sites in Galway Bay (Ireland) in 1981–1982 (Finavarra, c. 10 m depth and Carraroe, c. 3 m depth) showed that this species (reported as 'Cladophora rupestris') grew throughout the year and only reproduced in September (Maggs, 1983).

Molecular phylogeny and ancestral state reconstruction

The LSU alignment of 46 sequences was 626 sites in total, including 259 phylogenetic informative characters. The phylogenetic tree obtained from the ML analysis is shown in Fig. 21. Two main clades (Cladophorales and Siphonocladales clade) were recovered with high support. The new species, *Cladophora rhodolithicola*, is most closely related to *C. pygmaea*, a minute species collected in the

subtidal on small stones. Both species fall within a clade of *Cladophora* species that are characterized by coarse thalli with strict acropetal growth, traditionally placed in the *Cladophora* section *Longi-articulatae*. Two other anomalous taxa within this LA clade are the cushion-forming species *C. herpestica* (Montagne) Kützing (with reduced branching, formerly placed in *Cladophoropsis*) and *C. echinus* (with irregular growth, previously allied with the section or genus *Aegagropila*). The identity of two other cladophoralean plants that were collected on rhodoliths in Wales, *C. laetevirens* and *Chaetomorpha aerea* (Dillwyn) Kützing, was confirmed by our molecular data. A second dataset of 32 SSU rDNA sequences was analysed to determine the phylogenetic position of *C. battersii*. This alignment was 1681 sites in total, 252 of which were phylogenetic informative. The ML tree is shown in Fig. S2, available online at: http://www.informaworld.com/mpp/uploads/leliaert_et_al_supplementary-material.pdf).

Cladophora battersii was recovered in the LA clade, but the exact position within this clade could not be determined with confidence.

The SSU–LSU alignment of 21 representatives of the LA clade was 2251 sites in total (SSU: 1661, LSU: 590 positions), including 206 phylogenetic informative characters (SSU: 87 and LSU: 119). Phylogenetic trees constructed with ML and BI methods gave nearly identical topologies. Inclusion of the outgroup sequences in the phylogenetic analyses did not affect in-group topology but in-group only analyses were better resolved. Three of the four outgroups (*C. vagabunda*, *C. aokii* and *C. coelothrix*) and the four outgroups combined rooted the tree on the branch separating *C. rhodolithicola*, *C. pygmaea*, *C. mirabilis* and *C. dotyana* from the rest of the clade. This root placement was also obtained with molecular clock rooting. On the other hand, outgroup rooting with *C. rupestris* placed the root on the branch towards *C. pygmaea*, resulting in
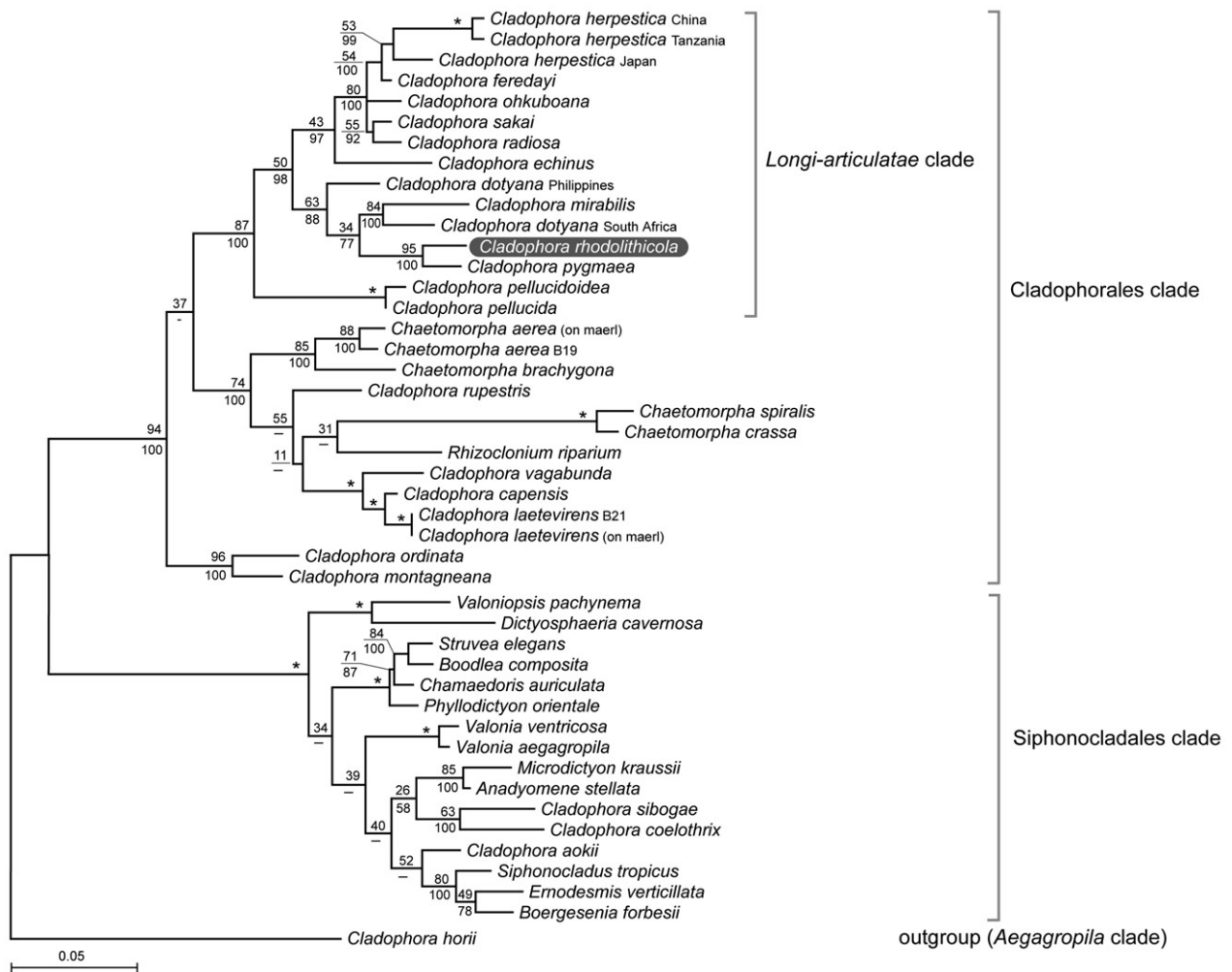

Fig. 21. Maximum likelihood (ML) tree of the Cladophorales/Siphonocladales inferred from large subunit rDNA sequences, showing the phylogenetic position of *Cladophora rhodolithicola*. ML bootstrap values and Bayesian inference (BI) posterior probabilities are indicated above and below branches, respectively. The nodes that received full support in both BI and ML analyses are denoted by asterisks.

a highly asymmetrical tree with low overall branch support. The phylogram obtained from the ML analysis of ingroup sequences, rooted manually along the branch as determined by the first set of outgroups and molecular clock rooting is shown in Fig. 22. The sequences of *C. rhodolithicola*, which were nearly identical, grouped in a single clade, preceded by a relatively long, well supported branch. The close relationship of *C. rhodolithicola* and *C. pygmaea* is strongly supported. Uncorrected pairwise distances between the two

species ranged between 0.02 and 0.03, corresponding with 17 fixed base pair differences in the LSU and four differences in the SSU. *Cladophora echinus* is placed on a relatively long branch, sister to a clade containing *C. pellucida* and *C. pellucidoidea*. The specimens of *C. herpestica* fall within a clade of closely related taxa (*C. feredayi* Harvey, *C. ohkuboana* Holmes, *C. radiosa* (Suhr) Kützing and *C. sakaii* Abbott).

The rDNA ITS1-5.8S-ITS2 sequences of *C. rhodolithicola* were 782 bases long (ITS1: 410 bases, 5.8S: 157 bases, ITS2: 215 bases). The sequences from Wales and Spain differed from each other in three positions, two of which were situated in the ITS1 region and one in the 5.8S rDNA. Close examination of the electropherograms at these variable positions showed distinct underlying peaks matching the corresponding base of the other sequence, suggesting that the observed differences from direct cycle sequencing might be attributable to intra-genomic variation. The rDNA ITS1-5.8S-ITS2 sequence of *C. pygmaea* was 725 bases long (ITS1: 350 bases, 5.8S bases: 157, ITS2: 218 bases) and differed from the sequences of *C. rhodolithicola* in 206 positions, corresponding with an uncorrected pairwise distance of 0.29. Of the base-pair differences, 121 were situated in the ITS1 region (p-distance of 0.35), 83 in the ITS2 region (p-distance 0.40), and two in the 5.8S.

The evolution of three morphological characters was traced along the LA phylogeny via parsimony reconstruction (Fig. 23). Two types of thallus organisation were distinguished in the LA clade. Most taxa showed a strict acropetal organisation, where growth of the thallus is by division of the apical cells and subsequent elongation and enlargement of the cells. These cells produce branches, which gradually increase in age and length from the apex towards the base. Irregular thallus organization, caused by
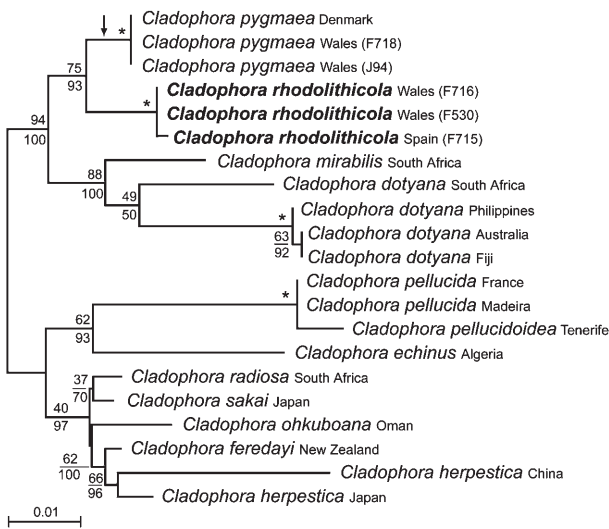

Fig 22. Maximum likelihood (ML) tree of the *Longi-articulatae* clade inferred from a concatenated dataset of small subunit and large subunit rDNA ingroup sequences, rooted along the branch as determined by outgroup rooting (see text) and molecular clock rooting. Arrow indicates alternative root placement when *Cladophora rupestris* is used as outgroup. ML bootstrap values and Bayesian inference (BI) posterior probabilities are indicated above and below branches, respectively. The nodes that received full support in both BI and ML analyses are denoted by asterisks.
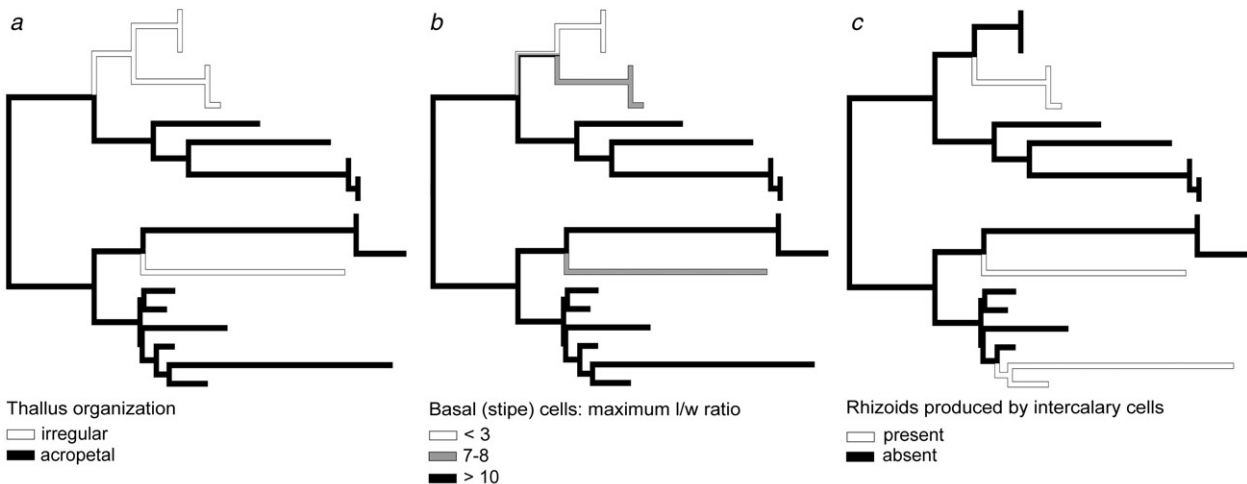

Fig. 23. Ancestral state reconstruction along the *Longi-articulatae* phylogram (see Fig. 17).

intercalary cell divisions, was only found in *C. echinus*, *C. pygmaea*, *C. rhodolithicola*. Ancestral state reconstruction showed that acropetal growth is plesiomorphic in the LA clade and that irregular growth evolved independently on two occasions (Fig. 23*a*). Many species of the LA-clade are characterized by long and conspicuous basal cells (that are distinctly larger than the apical cells), a character state that emerges as ancestral in the LA clade, and which has been gradually lost twice, once in the clade of *C. rhodolithicola* and *C. pygmaea* and once in *C. echinus* (Fig. 23*b*). Intercalary and/or apical rhizoids appear to have evolved independently three times, in *C. rhodolithicola*, *C. echinus* and *C. herpestica* (Fig. 23*c*).

Discussion

The taxonomy of the genus *Cladophora* worldwide is problematic, but European representatives have been relatively well documented (Söderström, 1963; van den Hoek, 1963; Noailles, 1995; Leliaert & Boedeker, 2007). Currently, about 18 marine morphospecies are recognised along the Atlantic coasts of Europe. Morphologically, our rhodolith epiphyte does not correspond with any previously described species of the European flora. Furthermore, a literature survey of *Cladophora* species worldwide yielded no matches. The inability to match our plants with a described taxon is confirmed by molecular data. Our phylogenetic analyses show that the maerl epiphytes from Wales and Spain group in a single clade, clearly separated from its sister species, *C. pygmaea*, by a long well supported branch, suggestive of a single phylogenetic species. The conspecificity of the plants from Wales and Spain is implied based on near identical ITS sequences. We therefore propose formal recognition of a new species of *Cladophora*.

Cladophora rhodolithicola Leliaert sp. nov

DIAGNOSIS: *Thallus atroviridis, usque ad 2 cm altus, erectus vel repens, filamentibus irregulariter ramificatis consistens, rhizoidibus ex cellulis basalibus orientibus, vel rhizoidibus ex basibus cellularum in partibus omnibus thalli (cellulis apicalibus quoque) orientibus ad substratum affixus. Thalli augmentum apicalium atque intercalarium cellularum divisione. Cellulae unum, duos vel usque ad quinque ramos generantes; filamenti nonnulli (usque ad 25 cellulas longi) non ramificati. Cellulae apicales cylindricae vel paulo conicae, nonnumquam doliiformes, 40–65 (–80) µm latae, usque ad 175 (–200) µm longae. Cellulae basales cylindricae vel paulo clavatae, saepe protuberationem basalem ostendentes, nonnunquam arcuatae, 110–160 (–210) µm latae,*

usque ad 1100 µm longae. Cellularum parietes crassae et striatae, 5 µm circiter crassae in cellulis apicalibus, 25–40 µm crassae in basalibus cellulis. Chloroplasti stratum parietalem paene continuum formantes. Thalli epiphytici super rhodolitos in zona sublitorale. GenBank numeri holotypi: FM205053 (*SSU rDNA*), FM205044 (*LSU rDNA*), FM205055 (*rDNA ITS1-5.8S-ITS2*).

Plants dark green, up to 2 cm high, erect or creeping, composed of irregularly branched filaments, attached to the substratum by rhizoids that develop from basal cells, or by rhizoids arising from the basal parts of cells in any part of the thallus (including the apical cells). Growth of the thallus by apical and intercalary cell divisions. Cells producing one, two, or up to five branches; some unbranched filaments (up to 25 cells long). Apical cells cylindrical to slightly tapering with obtuse tips, sometimes barrel shaped, 40–65 (–80) µm wide, up to 175 (–200) µm long. Basal cells cylindrical to slightly clavate, often with a distinct basal bulge, sometimes curved, 110–160 (–210) µm wide, up to 1100 µm long. Zoids produced in long terminal chains of barrel-shaped zoidangia. Cell walls thick and striate, c. 5 µm thick in the apical cells, 25–40 µm thick in the basal cells. Chloroplasts forming an almost uninterrupted parietal layer. Thalli epiphytic on rhodoliths in the subtidal region. Genbank numbers of holotype: FM205053 (SSU rDNA), FM205044 (LSU rDNA), FM205055 (rDNA ITS1-5.8S-ITS2).

HOLOTYPE: Collected as an epiphyte on rhodoliths from Milford Haven, Pembrokeshire, Wales, British Isles, by Francis Bunker, 6 November 2007, subtidal (−4 m). Formalin preserved specimen deposited in the Herbarium of Ghent University, Belgium (GENT). Culture maintained in the Phycology Research Group, Ghent University, Belgium.

ISOTYPE: Natural History Museum, London, UK (BM).

ETYMOLOGY: The specific epithet refers to the biotic substratum (rhodoliths) on which the species typically grows.

Despite the simple thallus architecture of *Cladophora* representatives, 12 architectural types have been distinguished, representing the sections of *Cladophora* as conceived by van den Hoek (1963, 1982). Molecular phylogenetic studies have shown that most of these morphological sections are untenable (Bakker *et al.*, 1994; Hanyuda *et al.*, 2002; Leliaert *et al.*, 2003, 2007*a*). One notable exception is the *Cladophora* section *Longiarticulatae* (*sensu* van den Hoek & Chihara, 2000), which forms a morphologically well-defined clade, except that *C. herpestica*, a species with reduced branch formation that was previously placed in

Cladophoropsis, also belongs to this clade (Hanyuda et al., 2002; Leliaert et al., 2003; Leliaert & Coppejans, 2006). Species in this section are characterized by large, coarse plants (some, like *C. mirabilis*, grow up to 1 m long) composed of acropetally organized branch systems. Growth is almost exclusively by division of conspicuous apical cells, followed by considerable cell elongation and enlargement, resulting in long and conspicuous basal cells; plants are attached by branched rhizoids developing from a basal stipe cell. The members of this clade not only differ morphologically from the rest of the Cladophorales but there is also a pronounced tendency to occupy subtidal habitats, where they experience less fluctuation in environmental conditions, possibly resulting in low intraspecific morphological variability as compared to some intertidal *Cladophora* species.

The position of *C. rhodolithicola* in the LA clade is unexpected from a morphological point of view. Unlike other members of the clade, *C. rhodolithicola* forms small thalli (about 1 cm high) that mainly grow by intercalary cell divisions, resulting in irregular branch systems. Attachment in the new species is by branched rhizoids sprouting from basal, often stolonoid cells and by unbranched, aseptate rhizoids developing from the proximal pole of intercalary and apical cells, a character shared with *C. herpestica* and *C. echinus*, two other anomalous species of the LA clade. Species of the section LA generally attach at a single point by rhizoids developing from the basal pole of a large stipe cell. It should be noted that some species, including *C. blomquistii* van den Hoek (1982), *C. sakaii* and *C. minisakaii* van den Hoek & Chihara (2000), and *C. feredayoides* Kraft & A. Millar (Kraft, 2000) also occasionally form rhizoids at the basal pole of cells, but these are always situated in the basal region of the thallus. It should be noted that many other taxa, from different clades of the Cladophorales/Siphonocladales, produce similar intercalary rhizoids (Leliaert et al., 2003, 2007a). Despite these differences, *C. rhodolithicola* shares a number of morphological features with other LA representatives: thick cell walls, resulting in stiff thalli; chloroplasts forming a more or less closed parietal layer, giving the plants a dark green colour; coarse and club-shaped basal cells. The distinct basal bulges of the basal cells are shared by *C. dotyana* (Leliaert & Coppejans, 2003).

Cladophora pygmaea is here revealed as the sister species of *C. rhodolithicola*. The former species is only known from scattered localities in north-western Europe and north America, and forms minute thalli, barely visible to the naked eye (Figs 24–26) (Waern, 1940; Söderström, 1963; van den Hoek, 1963, 1982; Wilce, 1970; Irvine et al., 1975; Maggs & Guiry, 1981; Maggs, 1983; Leliaert & Boedeker, 2007). Like *Cladophora rhodolithicola*, *C. pygmaea* grows in maerl beds at depths of 2–30 m or more, but it is also commonly found attached to small stones, pieces of shell and in crevices in the sublittoral. Based on morphological characters the systematic position of *C. pygmaea* was uncertain. Its special nature was recognised by Reinke (1889) and van den Hoek (1963) who placed it in a separate subgenus or section *Chamaethamnion*, based on the diminutive size of the thallus, the special mode of attachment by a discoid holdfast (Fig. 26), and the typical reproductive cells (Fig. 27). *Cladophora rhodolithicola* and *C. pygmaea* share a number of morphological features, including intercalary growth, apically inserted branches and the formation of terminal chains of barrel-shaped zooidangia, each opening by a pore halfway along the cell (Jónsson & Chesnoy, 1991; van den Hoek, 1963; Maggs & Guiry, 1981).

Cladophora echinus is here revealed as another unusual member of the LA clade. This southern European species has been reported from calcified habitats in the Mediterranean Sea, growing on the
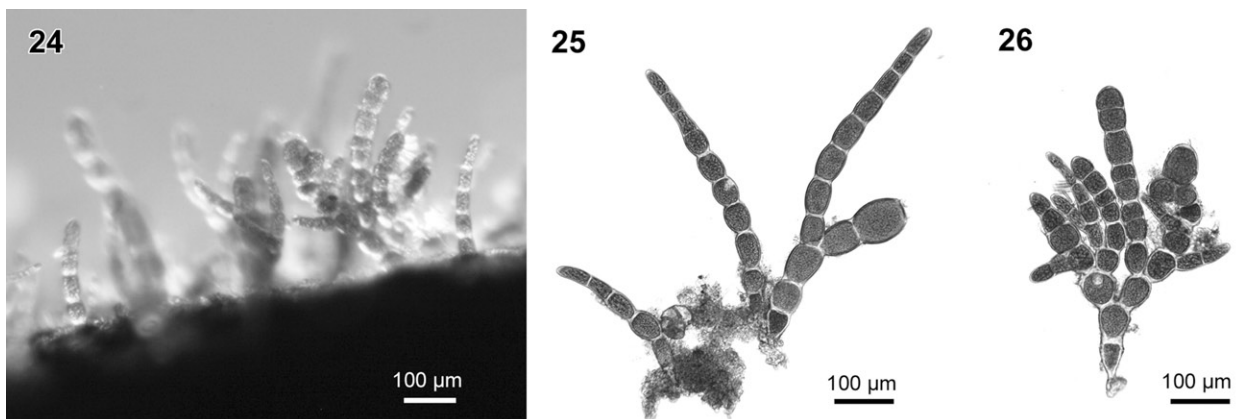
Figs 24–26. *Cladophora pygmaea* from Milford Haven, Wales (F. Bunker, 6 Nov 2007, F718, GENT). Fig. 14. Habit of thalli growing on a small stone. Figs 25, 26. Thalli with irregular branch-systems and a basal, discoid holdfast.

coralline alga *Lithothamnion* (Zederbauer, 1902) or on *Serpula*-tubes (Friedmann, 1955). The thalli are cushion-like when attached, but can also form unattached spherical balls ('aegagropilas'). Branch systems are irregularly organized with frequent intercalary cell divisions (Fig. 28). As in *C. rhodolithicola*, rhizoids are frequently produced

from the proximal pole of intercalary cells, but in *C. echinus* rhizoids also develop from the distal pole of apical cells (Figs 29, 30). *Cladophora echinus* is morphologically very similar to the freshwater, ball-forming *Aegagropila linnaei* Kützing, commonly known as Marimo. Both species, together with the marine species *C. aegagropiloidea* van den Hoek & Womersley, *C. catenata* (Linnaeus) Kützing and *C. corallicola* Børgesen were placed in the *Cladophora* section *Aegagropila* by van den Hoek (1963) and were believed to be related to *Basicladia* and *Wittrockiella* (van den Hoek, 1984). Molecular phylogenetic studies have confirmed the close relationship of *A. linnaei* to *Wittrockiella* and *Basicladia*, which form a distinct lineage, sister to the Cladophorales and Siphonocladales clades (Hanyuda *et al.*, 2002). On the other hand, *C. catenata* was found to be closely related to *Anadyomene* and *Microdictyon* in the Siphonocladales clade (Leliaert *et al.*, 2007a). No molecular data are yet available for *C. corallicola*, a rare Caribbean species growing in deep waters on dead corals and shells (Børgesen, 1913; van den Hoek, 1982), and *C. aegagropiloidea*, which is only known from its type from Kangaroo Island, South Australia.

The position of *C. battersii* in the LA clade is also surprising from a morphological perspective.
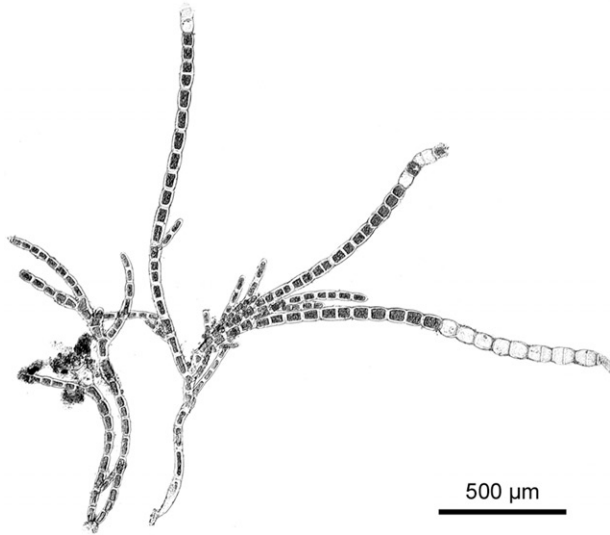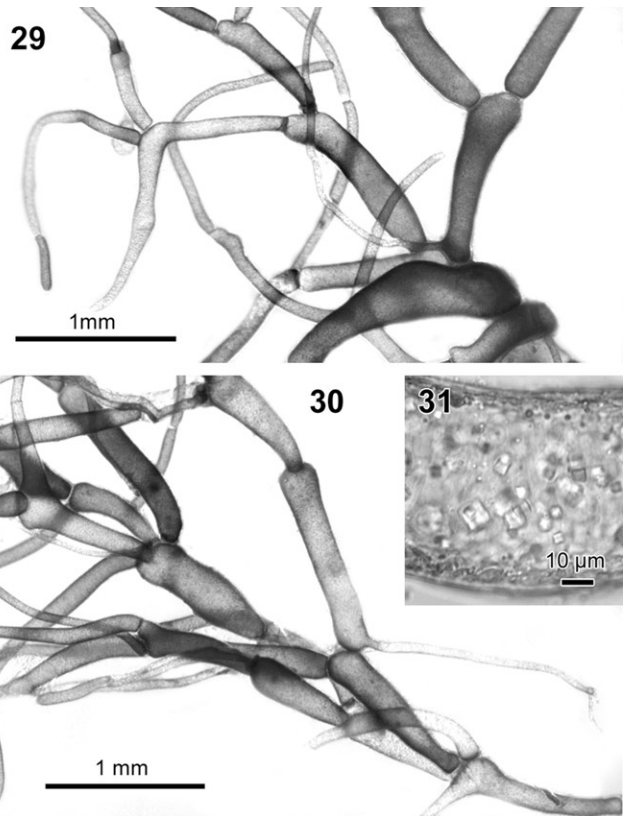

Fig. 27. *Cladophora pygmaea* from Carraroe, Galway Bay, Ireland (C. Maggs, 26 Feb 1980, GALW). Fertile thallus with terminal chains of barrel-shaped zoidangia.

Figs 28–31. Culture of *Cladophora echinus* from Algiers, Algeria (R. Delepine, summer 1960, no. 60/55, UTEX LB 1480). Figs 28–30. Thalli composed of entangling, irregularly branched filaments. Unbranched, aseptate rhizoids produced at the proximal pole of intercalary cells and at the apex of terminal cells. Fig. 31. Cubical crystalline cell inclusions.

This rare European species grows unattached in quiet bays and lagoons, or hooked around *Zostera* rhizomes, where it forms aegagropiloid aggregates composed of irregularly organized branch systems (van den Hoek, 1963; Leliaert & Boedeker, 2007). The systematic position of the species has been uncertain. Hamel (1929) allied it with *C. liebetruthii* Grunow (*Cladophora* section *Boodleoides*) because of its spongy, ball-forming morphology, while Crouan & Crouan (1867) and van den Hoek (1963) considered it to be related to *C. albida* (section *Glomeratae*) based on its irregular growth and lack of intercalary rhizoids.

The fact that five 'morphological strangers' (*C. battersii*, *C. echinus*, *C. herpestica*, *C. pygmaea* and, *C. rhodolithicola*) are found in the *Cladophora* section *Longi-articulatae* means that this, formerly well defined taxonomic entity, cannot be retained. This reinforces the notion that a revised classification of the Cladophorales/Siphonocladales, based primarily on molecular data is inevitable, because several morphological characters traditionally employed to circumscribe the orders, families, genera and sections have evolved independently on many occasions (Bakker *et al.*, 1994; Hanyuda *et al.*, 2002; Leliaert *et al.*, 2003, 2007a). Parallel or convergent evolution of various morphological characters is also seen within the section *Longi-articulatae*. Ancestral character state reconstruction revealed that the ancestor of the LA clade probably had a strict acropetal thallus organization with conspicuous basal cells, and lacked intercalary rhizoids. The aberrant morphologies of *C. rhodolithicola*, *C. pygmaea*, *C. echinus* and *C. herpestica* appear to have been derived independently from this ancestral morphology.

Rhodolith beds provide habitat for a high diversity and abundance of marine species (Foster, 2001). Surveys in the British Isles have found over 400 species of associated animals (Scott & Moore, 1996) and around 180 species of macroalgae (Farnham & Jephson, 1977; Blunden *et al.*, 1981; Maggs, 1983; Birkett *et al.*, 1998; BIOMAERL Team, 1999; De Grave & Whittaker, 1999; De Grave *et al.*, 2000). Similar levels of seaweed diversity have been found in Brittany (Cabioch, 1969; Blunden *et al.*, 1977, 1981; Hily *et al.*, 1992; BIOMAERL Team, 1999) and in the Atlantic Spanish maerl beds where more than 230 species have been recorded (Miranda, 1934; Donze, 1968; Seoane-Camba & Campo-Sancho, 1968; Bárbara *et al.* 2004; Peña & Bárbara, 2006, 2008). Most of these seaweed species are not confined to rhodolith beds as their only habitat. Some of these species, like *Halymenia latifolia* P.L. Crouan & H.M. Crouan ex Kützing and *Scinaia interrupta* (A.P. de Candolle) M.J. Wynne, might prefer calcareous habitats, which provide a suitable substratum in which their shell-boring microthalli can grow (Maggs & Guiry, 1982). Other rhodolith epiphytes, such as *Halarachnion ligulatum* (Woodward) Kützing and *Atractophora hypnoides* P.L. Crouan & H.M. Crouan, may have a preference for mobile substrata because of the poor competitive ability of the crustose tetrasporophytic phase of their life cycle (Birkett *et al.*, 1998). The heteromorphic life history of some other species may also be a distinct advantage on mobile substrata because the boring or crustose phase can survive periods of physical or biological stress (Lubchenco & Cubit, 1980; Maggs & Guiry, 1987; Bárbara *et al.*, 2004). Only a few seaweeds, such as *Gelidiella calcicola* Maggs & Guiry, *Gelidium maggsiae* Rico & Guiry and *Cruoria cruoriaeformis* (P.L. Crouan & H.M. Crouan) Denizot have been found to be entirely restricted to maerl habitats (Maggs & Guiry, 1987, 1989; Maggs, 1990; Rico & Guiry, 1997). *Cladophora rhodolithicola* represents another species that has a high preference for maerl biotopes. The life cycle of sexually reproducing cladophoralean representatives is diplohaplontic and isomorphic (no micro-thalli occur), therefore eliminating the possibility that calcareous substrata are a prerequisite for completion of the life cycle in this species. It should be noted that some other cladophoralean species are restricted to calcareous substrata. These include the marine species *Cladophora conchopheria* Sakai, which is only found on the shells of the gastropod *Lunella coronata* (van den Hoek & Chihara, 2000), the freshwater species *Arnoldiella conchophila* V. Miller, which grows on shells of the bivalves *Anodonta* and *Unio* and on other calcareous substrata, such as dolomite and limestone (Downing, 1970), and the terrestrial species *Cladophorella calcicola* Fritsch, which is only found on limestone (Fritsch, 1944). Based on our culture observations, *C. rhodolithicola* is probably slow-growing in nature. The species may therefore prefer mobile substrata which allow it to escape intense competition for space and light that characterizes adjacent stable rocky substrata. Like many other rhodolith epiphytes (e.g. *Gelidiella calcicola* and *Gelidium maggsiae*), *C. rhodolithicola* forms creeping, stolonoid axes, attached at intervals by rhizoidal holdfasts. In *C. rhodolithicola*, cells of the erect filaments also frequently form rhizoids which can re-attach. This sprawling nature enables the thalli to grow around the rhodoliths, giving them a survival advantage on such mobile substrata. Jacquotte (1962) and Cabioch (1969) discussed the importance of various creeping species in stabilizing maerl beds by the formation of stolons and secondary attachments. Although not observed in nature, a stabilizing effect was also demonstrated in our

cultures of *C. rhodolithicola* where plants attached to adjacent rhodoliths by rhizoids produced from the apical parts of the filaments, thereby forming a network of *Cladophora* filaments and rhodoliths.

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Supplementary material

Cladophora rhodolithicola sp. nov. (Cladophorales, Chlorophyta), a diminutive species from European maerl beds

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Table S1. Additional sequences used in the phylogenetic analyses

| Species	Location	SSU rDNA	LSU rDNA
Anadyomene stellata (Wulfen) C. Agardh	Panama (Caribbean Sea)	AF510147	
Anadyomene stellata	Philippines		AJ544746
Boergesenia forbesii (Harvey) J. Feldmann	Japan	AF510164	
Boergesenia forbesii	Tanzania		AJ544742
Boodlea composita (Harvey) Brand	Tanzania	AF510157	AJ544731
Chaetomorpha antennina (Bory de Saint-Vincent) Kützing	Japan	AB062700	
Chaetomorpha brachygona Harvey	Tanzania		AJ544759
Chaetomorpha crassa (C. Agardh) Kützing	Tanzania		AJ544767
Chaetomorpha linum (O.F. Müller) Kützing	Japan	AB062702	
Chaetomorpha moniligera Kjellman	Japan	AB062703	
Chaetomorpha spiralis Okamura	Sri Lanka		AJ544766
Chamaedoris auriculata Børgesen	Socotra		AJ544739
Cladophora albida (Nees) Kützing	France (Atlantic Ocean)	Z35317	
Cladophora aokii Yamada	Japan	AM498747	AM503434
Cladophora capensis (C. Agardh) De Toni	South Africa (Atlantic Ocean)		AJ544763
Cladophora coelothrix Kützing	Kenya		AJ544754
Cladophora dotyana Gilbert	Philippines		AJ544755
Cladophora dotyana	South Africa (Indian Ocean)		AJ544756
Cladophora glomerata (Linnaeus) Kützing	Japan	AB062706	
Cladophora herpestica (Montagne) Kützing	Japan	Z35419	AM503460
Cladophora herpestica	Tanzania		AJ544751
Cladophora horii van den Hoek & Chihara	Japan	AB078731	
Cladophora horii	South Africa (Indian Ocean)		AJ544728
Cladophora japonica Yamada	Japan	AB062707	
Cladophora montagneana Kützing	Tanzania		AJ544762
Cladophora ohkuboana Holmes	Japan	AB062708	
Cladophora ordinata (Børgesen) van den Hoek	South Africa (Indian Ocean)		AJ544757
Cladophora pellucida (*Hudson*) *Kützing*	France (Atlantic Ocean)	Z35314	
Cladophora rupestris (Linnaeus) Kützing	France (Atlantic Ocean)	Z35319	AJ544764
Cladophora sericea (Hudson) Kützing	France (Atlantic Ocean)	Z35320	
Cladophora sibogae Reinbold	Tanzania		AJ544752
Cladophora vagabunda (Linnaeus) van den Hoek	Japan	AB062710	
Cladophora vagabunda	Tanzania		AJ544760
Dictyosphaeria cavernosa (Forsskål) Børgesen	Seychelles	AM498756	
Dictyosphaeria cavernosa	Tanzania		AJ544745
Ernodesmis verticillata (Kützing) Børgesen	Canary Islands	AM498758	
Ernodesmis verticillata	Costa Rica		AJ544743
Microdictyon japonicum Setchell	Japan	AM498760	
Microdictyon kraussii J. Gray	South Africa (Indian Ocean)		AJ544747
Phyllodictyon orientale (A. Gepp & E. Gepp) Kraft & Wynne	Maldives		AJ544738
Rhizoclonium riparium (Roth) Harvey	Japan	AB202076	
Rhizoclonium riparium var. *implexum* (Dillwyn) Rosenvinge	France (Atlantic Ocean)		AJ544765
Siphonocladus tropicus (P. Crouan & H. Crouan) J. Agardh	Canary Islands	AM498761	
Siphonocladus tropicus	Dominican Republic		AJ544744
Struvea elegans Børgesen	Bahamas	AF510149	
Struvea elegans	Papua New Guinea		AJ544737
Valonia aegagropila C. Agardh	Japan	AM498762	
Valonia aegagropila	Tanzania		AJ544748
Valoniopsis pachynema (G. Martens) Børgesen	Australia (Pacific Ocean)	AM498765	
Valoniopsis pachynema	Tanzania		AJ544741
Ventricaria ventricosa (J. Agardh) Olsen & J. West	Tanzania		AJ544750
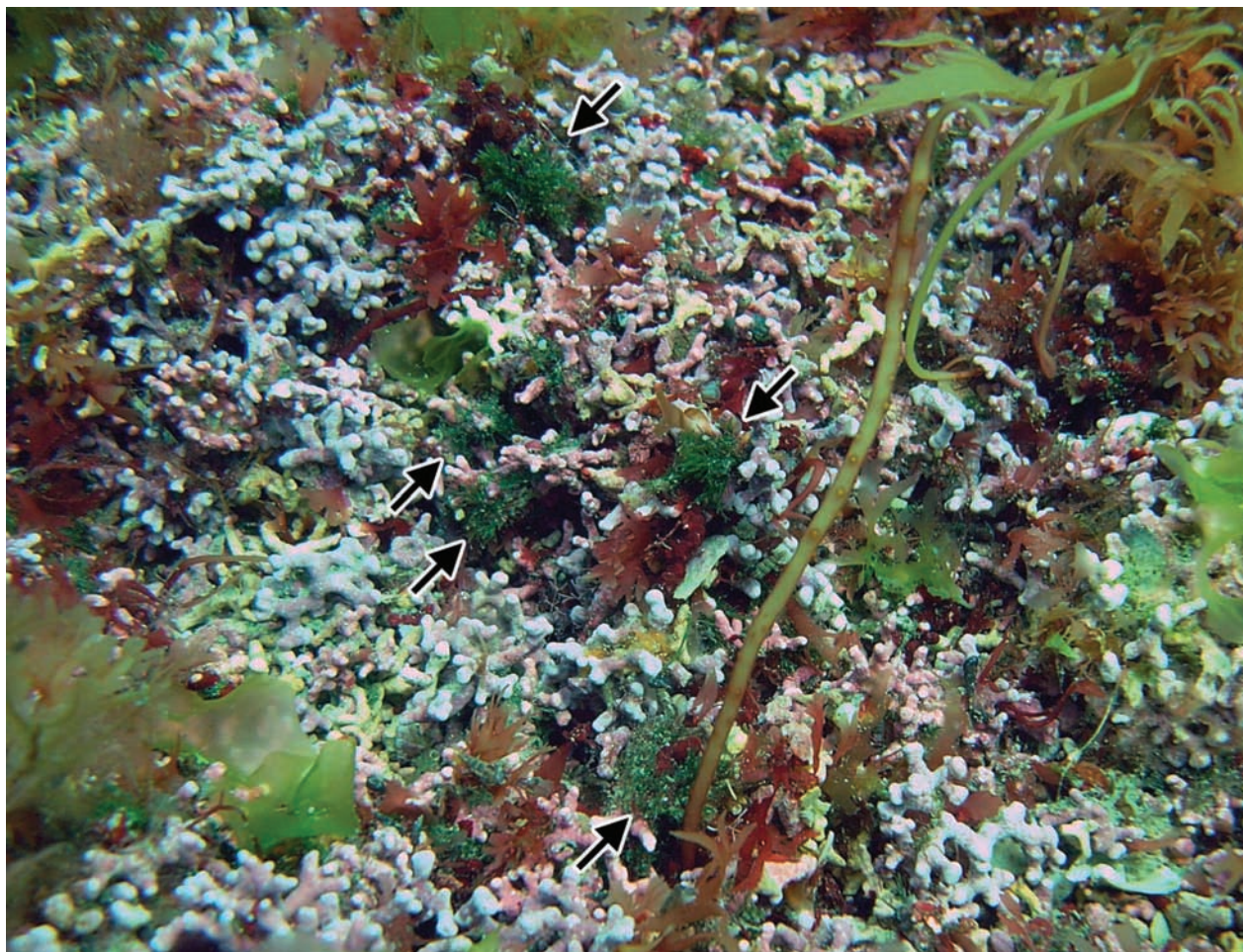
Fig. S1. *Cladophora rhodolithicola*. Habit of *in situ* plants on rhodoliths in a shallow subtidal maerl bed (−4 m) from Oeste Con de Pego, Ría de Vigo, Spain (Photo by V. Peña).
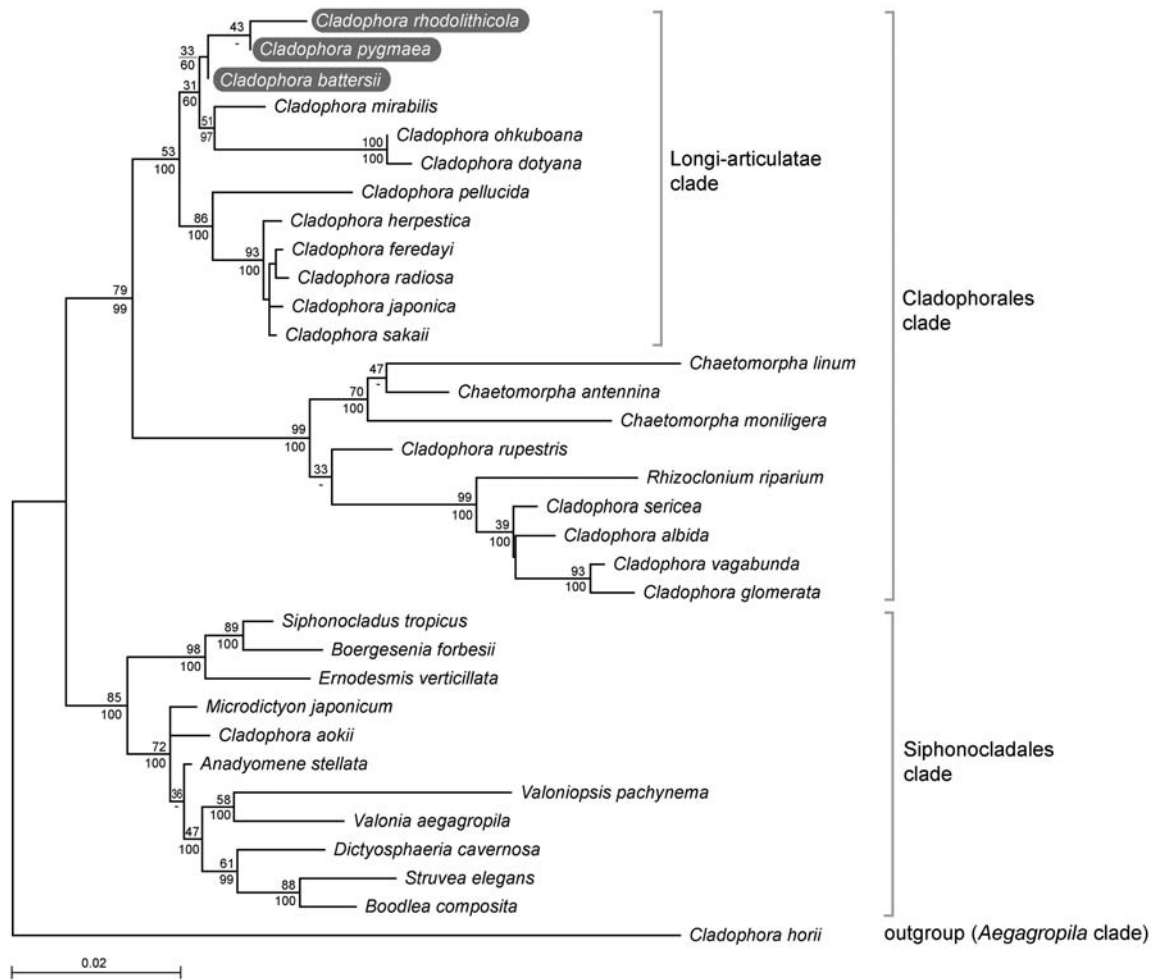
Fig. S2. Maximum likelihood (ML) tree of the Cladophorales/Siphonocladales inferred from SSU rDNA sequences, showing the phylogenetic position of *Cladophora battersii*, *C. pygmaea* and *C. rhodolithicola*. ML bootstrap values are indicated above branches; Bayesian inference posterior probabilities are indicated below branches.