

Research Article

Cladophora ruchingeri (C. Agardh) Kützing, 1845 (Cladophorales, Chlorophyta): a new biofouling pest of green-lipped mussel *Perna canaliculus* (Gmelin, 1791) farms in New Zealand

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Abstract

Cladophora is a genus of branched filamentous green algae (Ulvophyceae). It contains many species that are challenging to differentiate based on morphology because of the scarcity of diagnostic characters and extensive phenotypic plasticity. Within the past five years, *Cladophora* blooms have been observed on the ropes of green-lipped mussel farms in the Marlborough Sounds, New Zealand. When *Cladophora* reaches high biomass, it can clog mussel-harvesting equipment; thus, it is considered a nuisance organism in the region. This study used morphological and molecular techniques to identify the species responsible for the blooms, and to investigate whether this might be a recent incursion. *Cladophora* samples (n = 21) were collected from nine mussel farms, one salmon farm, and a marina. Morphological and phylogenetic analyses (partial large subunit and internally transcribed spacer regions 1 and 2 of the nuclear ribosomal cistron), revealed the identity of the bloom forming species as *Cladophora ruchingeri* (C. Agardh) Kützing, 1845. This represents the first report of this species in the Southern Hemisphere and Pacific region. Given the distinct morphology of *C. ruchingeri* (when mature), its absence from previous surveys of macro-algae from this region, and increasing reports of blooms, our findings suggest that this species has only recently been introduced to New Zealand. This study provides a robust taxonomic identification and initial baseline data. Further directed studies on *Cladophora* are required to advance knowledge on its ecology and distribution in New Zealand, and assist in the development of mitigation strategies.

Key words: aquaculture, green tide, marine pest, nuisance algae, ribosomal internal transcribed spacer

Introduction

Biofouling represents an important biosecurity risk for the aquaculture industry. Negative effects include direct impacts on cultured species (e.g. smothering, competition for space and food), deterioration of farm infrastructure (immersed structures such as cages, netting and pontoons), and effects on natural ecosystem functioning of adjacent areas (Fitridge et al. 2012; Fletcher et al. 2013). Biofouling assemblages often include a large proportion of non-indigenous species (Carlton 1989; Ruiz et al. 2009), which represent an important threat to coastal ecosystems

globally (Molnar et al. 2008; Ruiz et al. 1999). Non-indigenous species can cause economic and ecological harm (Falk-Petersen et al. 2006), and there are multiple examples of high-profile species (e.g., *Ciona intestinalis* (Linnaeus, 1767)) that have severely affected aquaculture regions globally (Ramsay et al. 2008).

In New Zealand, the green-lipped mussel (*Perna canaliculus* Gmelin, 1791) industry is the largest aquaculture sector, producing US\$ 218 million in annual exports (AQNZ 2012). The Pelorus Sounds, located in the Marlborough region at the top of New Zealand's South Island (Figure 1), supports 69% of the annual production of

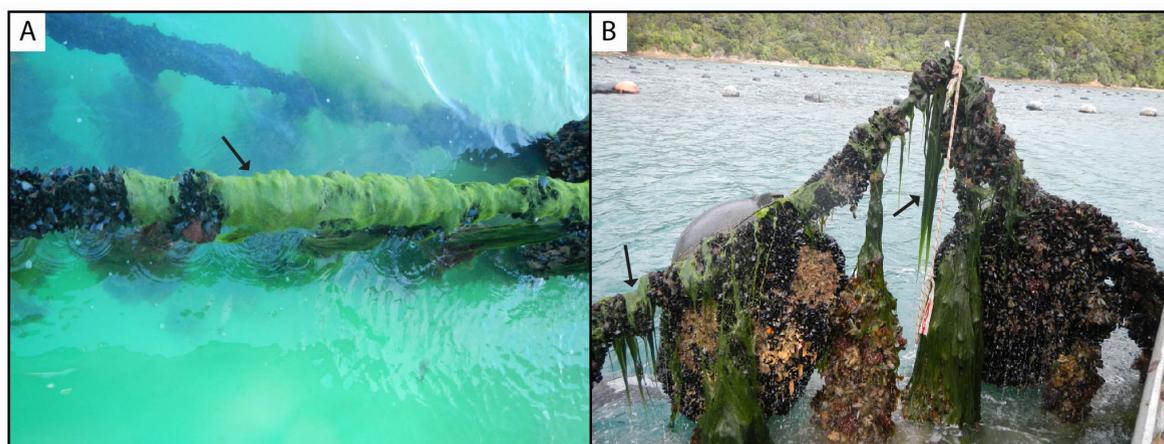


Figure 1. Photos of *Cladophora ruchingeri* mats (see black arrows) overgrowing (A) green-lipped mussel farm structures and (B) crop lines in the Marlborough Sounds, New Zealand. Photos AshleighWatts.

green-lipped mussel (AQNZ 2012). Green-lipped mussels are cultured on long-lines of a rough fibrous and porous texture, which is favourable for larval settlement of fouling organisms. These lines are suspended from backbone ropes above the seabed and supported by a series of buoys (Anderson and Underwood 1994). A typical three hectare mussel farm has nine backbone ropes, measuring 110 m each, and each rope supports a crop long-line of 3,750 metres (Marine Farming Association Inc., Blenheim, New Zealand, pers. comm.). Mussel production occurs over 12 to 24 month cycles from initial spat settlement to the harvesting of adult mussels (Woods et al. 2012). During intermediate and final seed crop stages, culture ropes are stripped and reseeded to reduce the density of the mussels for grow-out to market size and to reduce mussel biofouling (Woods et al. 2012).

Previous studies have recorded > 70 biofouling taxa growing on culture ropes, representing a large proportion of the total biomass (Woods et al. 2012). Since 2009, mussel culture structures have been fouled by a mat-forming green filamentous macroalga, which was tentatively identified as *Cladophora* sp. (Figure 1). When it reaches high biomass, it can clog the mussel harvesting equipment, resulting in costly delays. It has also been observed growing on other artificial structures (e.g. floating pontoons, floats and wharf piles) and on natural substrates (e.g. shallow rocky reefs) in the region.

Cladophora is a large genus of branched, uniseriate, filamentous green algae (Ulvophyceae) characterized by multinucleate cells. It is found

worldwide in marine, brackish, and freshwater environments and is known to form conspicuous algal mats, sometimes referred to as green tides or macroalgal blooms, where nutrients accumulate and competition is reduced (Flindt et al. 1997; Curiel et al. 2004; Gubelit and Kovalchuk 2010; Zulkifly et al. 2013). Molecular studies show the genus is polyphyletic (Bakker et al. 1994; Hanyuda et al. 2002; Leliaert et al. 2003), leading to the recent description of new genera (Boedeker et al. 2012). Due to the limited number of morphological features and the pronounced phenotypic plasticity (van den Hoek 1963; Leliaert and Boedeker 2007), species identification is difficult. In addition, cryptic diversity has been revealed in several morphospecies (Bakker et al. 1995; Bakker et al. 1995a, b; Leliaert et al. 2007). Thus, molecular identification and phylogenetics are important tools for assessing evolutionary relationships and diversity within *Cladophora* (e.g., Leliaert et al. 2009; Hayakawa et al. 2012).

The diversity of *Cladophora* in New Zealand has not yet been fully documented. Chapman (1956) lists 25 species, including a number of dubious taxa. Currently, 15 accepted names of *Cladophora* are listed in AlgaeBase for New Zealand (Guiry and Guiry 2014). Of these, five have been documented as marine species occurring around the North and South Islands of New Zealand (Adams 1994; Nelson 2013). However, the true diversity and the correct taxonomic identities of *Cladophora* in New Zealand are unknown (see also Hurd et al. 2004; Nelson 2013), and a comprehensive study including large-scale DNA sequencing is clearly required.

In the interim, the aim of this study was to identify the *Cladophora* species that forms blooms in Pelorus Sound, using both morphological examinations and molecular techniques. It was also of interest to evaluate whether the *Cladophora* species responsible for these algal blooms might represent a new incursion to New Zealand.

Methods

Sample collection

Between March and June 2013, 21 specimens of *Cladophora* spp. were collected by hand from 9 mussel farms in the Pelorus Sound, the Havelock marina, and a salmon farm in the Queen Charlotte Sound, Marlborough, New Zealand (Figure 2). In Pelorus Sound, samples were collected from the top 3 m of mussel long-lines and from wharf piles in Havelock marina. In Queen Charlotte Sound, samples were collected from the top 3 m of a salmon cage net. Samples were cleaned of epiphytes using filtered sea water, and fragments from each specimen were preserved in 70% ethanol for future morphological analyses and stored at -20°C for later DNA extraction.

Morphological analyses

The ethanol-preserved samples were identified as morphospecies using several taxonomic treatments of the genus *Cladophora* (e.g., Söderström 1963; van den Hoek 1963, 1982; van den Hoek and Womersley 1984; van den Hoek and Chihara 2000; Kraft 2007; Leliaert and Boedeker 2007).

All identifications were performed using a light microscope (Olympus BH2, Olympus Optical Co. GmbH, Germany) and a stereo microscope (Leica MZ9.5, Leica Microsystems, Germany), and images were taken with a digital camera (ColorView Illu, Olympus Soft Imaging Systems, Germany). Voucher specimens were deposited in the Te Papa Tongarewa National Museum of New Zealand herbarium (WELT, see Table 1).

DNA extractions, PCR, and sequencing

The DNA from the frozen *Cladophora* specimens was extracted using the PureLink™ Genomic DNA Kit (Life Technologies, USA) according to the protocol supplied by the manufacturer. All PCR reactions were undertaken on a DNA Engine thermal cycler (Bio-Rad, USA) or an Eppendorf Mastercycler (Eppendorf, Germany). Two regions of the nuclear ribosomal cistron were investigated:

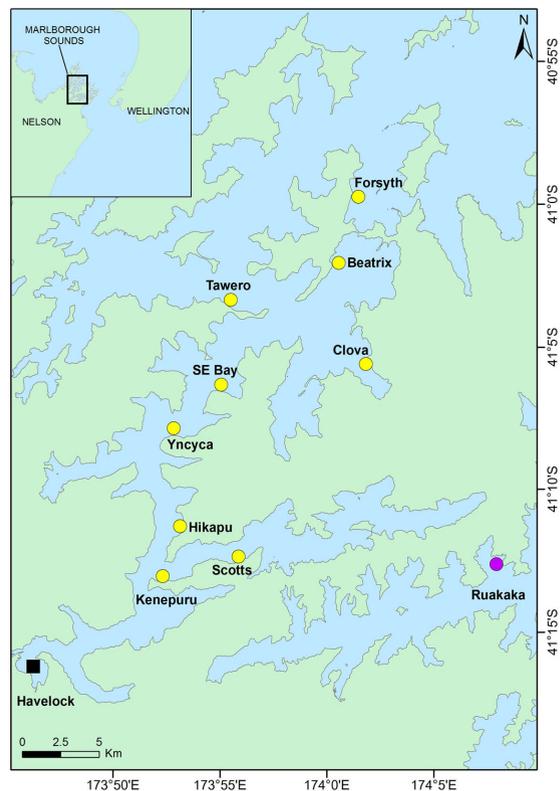


Figure 2. Map of Marlborough Sounds in relation to New Zealand. Yellow circles denote mussel farm sites, the purple circle a salmon farm, and the black square the Havelock marina.

partial large subunit (LSU rDNA) and internally transcribed spacer regions 1 and 2 (ITS rDNA). PCRs for the LSU gene were performed in a total volume of 25 μl using the high-fidelity Platinum® PCR Supermix (Life Technologies), 0.4 μM of each primers (D1R-F 5'-ACCCGCTGAATTT AAGCATA-3' [Scholin et al. 1994] and D3B-R 5'-TCGGAGGGAACCAG CTAATA-3' [Nunn et al. 1996]), and template DNA (30–50 ng). PCR cycling conditions were: 94°C for 2 min, followed by 38 cycles of 94°C for 45 s, 54°C for 45 s, 72°C for 60 s, and a final extension of 72°C for 8 min. PCRs for the ITS amplicons were performed using the forward primer ITS1 (5'-GGTGAACCTGAGGA AGGAT-3' [modified from Gottschling et al. 2005]) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3' [White et al. 1990]). Thermocycling conditions were identical to those described for LSU except for the annealing temperature which was lowered to 52°C . PCR products were visualized with 1.5% agarose gel electrophoresis with ethidium bromide staining and UV illumination. Positive PCR products were purified using the AxyPrep PCR

Table 1. List of 28 Cladophorales species sequenced in this study.

Species	Strain ID.	Origin	Location/Region	Voucher	GenBank (LSU)	GenBank (ITS)
<i>Cladophora albida</i> *	CAW #21	New Zealand	South Bay	A033064 ^a	n.i.	LN679098
<i>Cladophora albida</i> *	CAW #23	New Zealand	Ruakaka (salmon farm)	A033065 ^a	n.i.	LN679097
<i>Cladophora glomerata</i>	K89	New Zealand	Wanganui River	A033066 ^a	LN679067	LN679077
<i>Cladophora hutchinsioides</i> *	CAW #36	New Zealand	Hikapu Reach	n.i.	n.i.	LN679078
<i>Cladophora laetevirens</i>	A72; Lt88.E	Spain	Estartit	n.i.	n.i.	LN679099
<i>Cladophora ruchingeri</i>	C62	Sweden	Gulmarnsfjord	A033067 ^a	LN679069	n.i.
<i>Cladophora ruchingeri</i>	H95; Ru84.60	Norway	Bergen	Cruch3 ^b	LN679070	n.i.
<i>Cladophora ruchingeri</i> *	CAW #01	New Zealand	Yncyc Bay	n.i.	LN679071	LN679079
<i>Cladophora ruchingeri</i> *	CAW #02	New Zealand	South East Bay	n.i.	LN679072	LN679080
<i>Cladophora ruchingeri</i> *	CAW #03	New Zealand	Kenepuru	n.i.	LN679073	LN679081
<i>Cladophora ruchingeri</i>	CAW #04	New Zealand	Havelock marina	n.i.	LN679074	LN679082
<i>Cladophora ruchingeri</i> *	CAW #05	New Zealand	Yncyc Bay	n.i.	n.i.	LN679083
<i>Cladophora ruchingeri</i> *	CAW #06	New Zealand	Clova Bay	n.i.	n.i.	LN679084
<i>Cladophora ruchingeri</i> *	CAW #07	New Zealand	Tawero Point	n.i.	n.i.	LN679085
<i>Cladophora ruchingeri</i> *	CAW #10	New Zealand	Forsyth Bay	n.i.	n.i.	LN679086
<i>Cladophora ruchingeri</i> *	CAW #12	New Zealand	Beatrix Bay	A033068 ^a	n.i.	LN679087
<i>Cladophora ruchingeri</i> *	CAW #14	New Zealand	Yncyc Bay	A033069 ^a	n.i.	LN679088
<i>Cladophora ruchingeri</i> *	CAW #18	New Zealand	South East Bay	A033070 ^a	n.i.	LN679089
<i>Cladophora ruchingeri</i> *	CAW #31	New Zealand	Scotts Bay	A033071 ^a	n.i.	LN679090
<i>Cladophora ruchingeri</i> *	CAW #37	New Zealand	Hikapu Reach	A033072 ^a	n.i.	LN679091
<i>Cladophora ruchingeri</i> *	CAW #38	New Zealand	Hikapu Reach	A033073 ^a	n.i.	LN679092
<i>Cladophora ruchingeri</i> *	CAW #39	New Zealand	Hikapu Reach	n.i.	n.i.	LN679093
<i>Cladophora ruchingeri</i> *	CAW #40	New Zealand	Yncyc Bay	n.i.	n.i.	LN679094
<i>Cladophora ruchingeri</i> *	CAW #41	New Zealand	South East Bay	n.i.	n.i.	LN679095
<i>Cladophora ruchingeri</i> *	CAW #42	New Zealand	Tawero Point	n.i.	n.i.	LN679096
<i>Cladophora vagabunda</i>	E62	New Zealand	Hawke's Bay	A033074 ^a	LN679068	n.i.
<i>Rhizoclonium</i> sp.	C16	New Zealand	Havelock	A033075 ^a	n.i.	LN679075
<i>Rhizoclonium</i> sp.	D88	South Africa	Knysna Lagoon	A033076 ^a	n.i.	LN679076

n.i.: no information; LSU: large subunit; ITS: internal transcribed spacer

^aWELT: Te Papa Tongarewa National Museum of New Zealand herbarium collection number

^bGENT: Ghent University (Belgium) herbarium collection number

* Species found on aquaculture facilities

Clean-up Kit (Axygen Biosciences, CA, USA) and sequenced bi-directionally using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

Sequences generated during this work were deposited in the GenBank-EMBL database and additional sequences of Cladophorales from GenBank were included in the analyses (see Table S1 and Table S2). Furthermore, we included a number of ITS sequences from Bakker (1995) and seven additional *Cladophora* samples from the Boedeker collection (unpublished) were selected (Table 1). DNA sequences were inspected and assembled using Geneious v6.1.4 (Biomatters Ltd, New Zealand) and manually aligned with

BioEdit v5.0.9 sequence alignment software (Hall 1999). Uncorrected pairwise distances were calculated using PAUP (Swofford 2002). For the LSU alignment, five species (*C. coelothrix* Kützing, *C. socialis* Kützing, *C. sibogae* Reinbold, *C. prolifera* (Roth) Kützing, and *Siphonocladus tropicus* (P.L. Crouon and H.M. Crouon, C. Agardh) were used as outgroup, while *Rhizoclonium* sp. served as the outgroup for the ITS analysis.

Each DNA alignment (LSU and ITS2 rDNA) was analyzed independently with both Maximum Likelihood (ML) and Bayesian Inference (BI). Best-fit models of evolution were estimated for each alignment using TREEFINDER v12.2.0 (Jobb et al. 2004). Maximum Likelihood analyses

were carried out using PhyML v3.0 (Guindon et al. 2009), and the reliability of internal branches was assessed using 1000 bootstraps with subtree pruning-regrafting branch swapping. Bayesian tree reconstructions with posterior probabilities were inferred using MrBayes v3.2.2 (Ronquist et al. 2012), using the same model of DNA evolution as for the ML analyses. Four simultaneous Markov chains were run for 1,000,000 generations with trees sampled every 10 generations, with 25% of initial trees discarded as ‘burn-in’.

Results

Morphological analysis

Of the 21 *Cladophora* samples analysed morphologically and genetically, three distinct morphospecies were identified (Table 1). The majority (n = 18) corresponded to *C. ruchingeri* (C.Agardh) Kützing, 1845. Key characteristics for this species include: a sparsely branched thallus growing mainly by intercalary cell divisions, with few long branches originating from pseudodichotomies that have the same thickness as the main axis (van den Hoek 1963, 1982 and Figure 3); and terminal branches are short and relatively stiff with cell walls greater than 5 μm , never more than one lateral per cell. Macroscopically, these samples can be described as filamentous green algae displaying very long, sparsely branched thalli that become intertwined and form rope-like strands (see van den Hoek 1963, 1982).

Two samples (CAW#21, CAW#23; Table 1) were characterised by much smaller cell diameter in the terminal branches (10–50 μm), which were more densely branched than *C. ruchingeri*, and were identified as *C. albida* (Nees) Kützing. The remaining sample (CAW#36; Table 1) was not analyzed morphologically but identified genetically (see below).

Phylogenetic analysis

The LSU sequence alignment (n=49 sequences) was 553 bp long (including gaps). The model of evolution corresponded to the General Time Reversible (GTR; Lanave et al. 1984) + Gamma model (Gamma value = 0.22). Four *Cladophora* sp. samples collected in four distinct locations of the Marlborough Sounds (CAW #1 to #4; Table 1; Figure 1) yielded identical LSU sequences to previously published sequences of *Cladophora ruchingeri* (Table S1), and clearly grouped within the well-supported clade of marine *Cladophora* species. Other closely related species include:

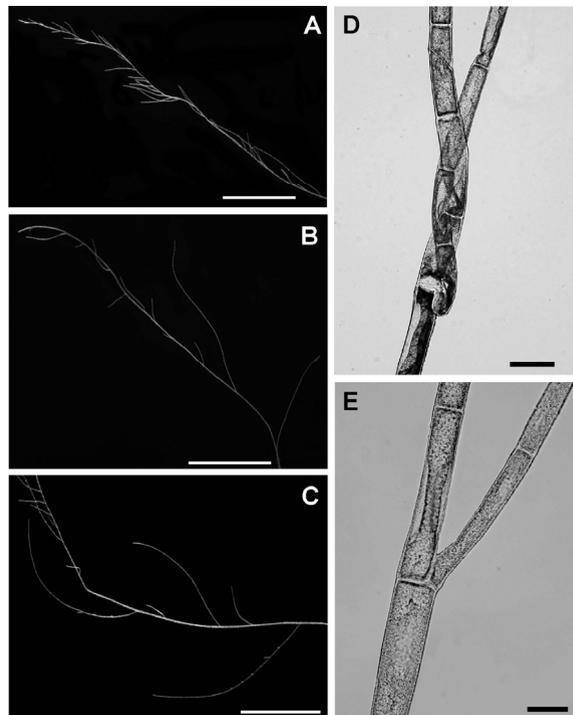


Figure 3. Morphology of *Cladophora ruchingeri*. (A) Upper part of sparsely branched thallus with simultaneous ramifications in the apical region, younger branches intercalated between older branches. (B) Upper part of thallus showing sparsely branched habit, older sidebranches long and unbranched. (C) Lower part of thallus showing sparsely branched habit with long sidebranches. Secondary laterals are rare. (D) Sidebranch twisted around the main axis, a typical feature of *C. ruchingeri* that might contribute to the rope-like strands often formed by this species. (E) Pseudodichotomous branching, the lateral branch being shifted to the apical pole of the cell. Scale bars: (A)–(C) 500 μm , (D) 200 μm , (E) 100 μm .

C. albida; *C. laetevirens* (Dillwyn) Kützing; *C. sericea*; and *C. capensis* (C. Agardh) De Toni. This clade of marine *Cladophora* species is sister to a clade containing the brackish species *C. vagabunda* (L.) C. Hoek and the freshwater species *C. glomerata* (L.) Kützing.

The *Cladophorales* samples (n=21) collected from sites in the Marlborough Sounds (including the four samples analysed using LSU, see Table 1; Figure 2) were investigated phylogenetically using the 5.8S/ITS-2 gene region to increase phylogenetic resolution within the true *Cladophora* clade (see discussion for terminology). The sequence alignment (n = 49 sequences) was 847 bp (including gaps). The model of evolution corresponded to the GTR + Gamma model (Gamma value = 0.56). Eighteen samples clearly corresponded to *C. ruchingeri* in a highly supported clade (Figure 5), with negligible pairwise

distances of maximum 0.6%. Among remaining sequences, we found two specimens of *C. albida* (CAW#21, CAW#23), and one specimen which was a close match (98% sequence similarity; BLASTn e-value of 0) to the ITS sequence JQ308254 of *C. hutchinsioides* Hoek and Womersley (CAW#36, no voucher/morphological identification available), differing by only two bp.

Discussion

Taxonomical and biogeographical considerations

All *Cladophora* samples from the Marlborough Sounds grouped with marine *Cladophora* species, which are sister to a clade containing the brackish species *C. vagabunda* and the freshwater species *C. glomerata*. Together, they represent the true *Cladophora* clade of this polyphyletic genus. Morphological and phylogenetic analyses revealed the identity of the dominant *Cladophora* species from the mussel farms in the Marlborough Sounds to be *C. ruchingeri*.

The LSU and ITS2 sequences of *C. ruchingeri* were virtually identical to sequences previously identified as *C. flexuosa* (O. F. Müller) Kützinger (Cflex84.82, Rua84.60, and Rua84.24 [Bakker 1995] and UTEX LB2875 [Ichihara et al. 2013], Tables S1 and S2), with a maximum difference of only 0.4% in ITS2. According to van den Hoek (1963), *C. flexuosa* should be regarded as a synonym of *C. sericea*. However, based on the genetic data presented here (Figures 4 and 5), it is clear that the '*C. flexuosa*' samples do not represent *C. sericea*, but *C. ruchingeri* instead. Since young individuals of *Cladophora ruchingeri* are morphologically similar to *C. sericea*, we assume that the '*C. flexuosa*' samples corresponded to juvenile individuals that had not been correctly identified and instead represent *C. ruchingeri*.

Cladophora ruchingeri has previously been recorded from the European Atlantic and the Mediterranean (van den Hoek 1963) as well as the Atlantic coasts of the USA (van den Hoek 1982). In addition, this species has been confirmed in Ghana (van den Hoek 1982) and has been reported in Venezuela (Ganesan 1990), resulting in a tropical to temperate ampho-Atlantic distribution. The finding of *C. ruchingeri* in New Zealand represents the first record of this species for the Southern Hemisphere and for the Pacific region.

Sample CAW#36 differs by only two bp from the published ITS sequence of *C. hutchinsioides* (GenBank no. JQ308254) from China, and must thus be regarded as conspecific. *Cladophora*

hutchinsioides has been described from southern Australia (van den Hoek and Womersley 1984), and has also been reported from East Asia (van den Hoek and Chihara 2000; Zeng 2009). This species has been reported to grow on a commercial fishing boat in South Australia (van den Hoek and Womersley 1984), and on fishing nets and ropes as well as oyster cages in Japan (van den Hoek and Chihara 2000). Thus, there is a possibility that this species is spreading globally via fishing vessels and equipment. This is the first record of this species for New Zealand; however, the lack of distribution data means we cannot determine whether it is a recent arrival.

Cladophora albida has not been recorded previously in New Zealand, but this species has a cosmopolitan distribution and can be assumed to be a natural part of New Zealand's algal flora (Nelson 2013). *Cladophora albida* has also been collected in other parts of New Zealand (e.g. Auckland, Taranaki, Wellington, Kaikoura; C. Boedeker, unpublished data).

Cladophora ruchingeri, a new marine pest of mussel farms in New Zealand

Cladophora ruchingeri is a poorly known species. In its young stages, it is difficult to differentiate from several other marine *Cladophora* species (e.g. van den Hoek 1963). However, the long, sparsely branched thalli of mature individuals that intertwine into rope-like threads are very characteristic (e.g. van den Hoek 1963, 1984). In an earlier, comprehensive, survey of marine algae along the coasts of northern South Island of New Zealand (Nelson et al. 1992), the only dominant species reported from the Marlborough Sounds was *C. subsimplex*, a species that is morphological distinct from *C. ruchingeri*. If present in its mature form, it is unlikely that *C. ruchingeri* would have been overlooked or misidentified in previous surveys of the Marlborough Sounds (Hurd et al. 2004; Nelson 2013).

Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum, 1792)) and green-lipped mussel farms have been present in the Marlborough Sounds region for ca. 30 years, and culture intensity has not increased markedly in the last 10 years (see further discussion below). This in concert with *C. ruchingeri*'s distinctive macroscopic appearance (when mature), its absence from previous Marlborough Sounds surveys, and the fact that it has not previously been reported from the Southern Hemisphere or Pacific region suggest that this species has only recently arrived in New

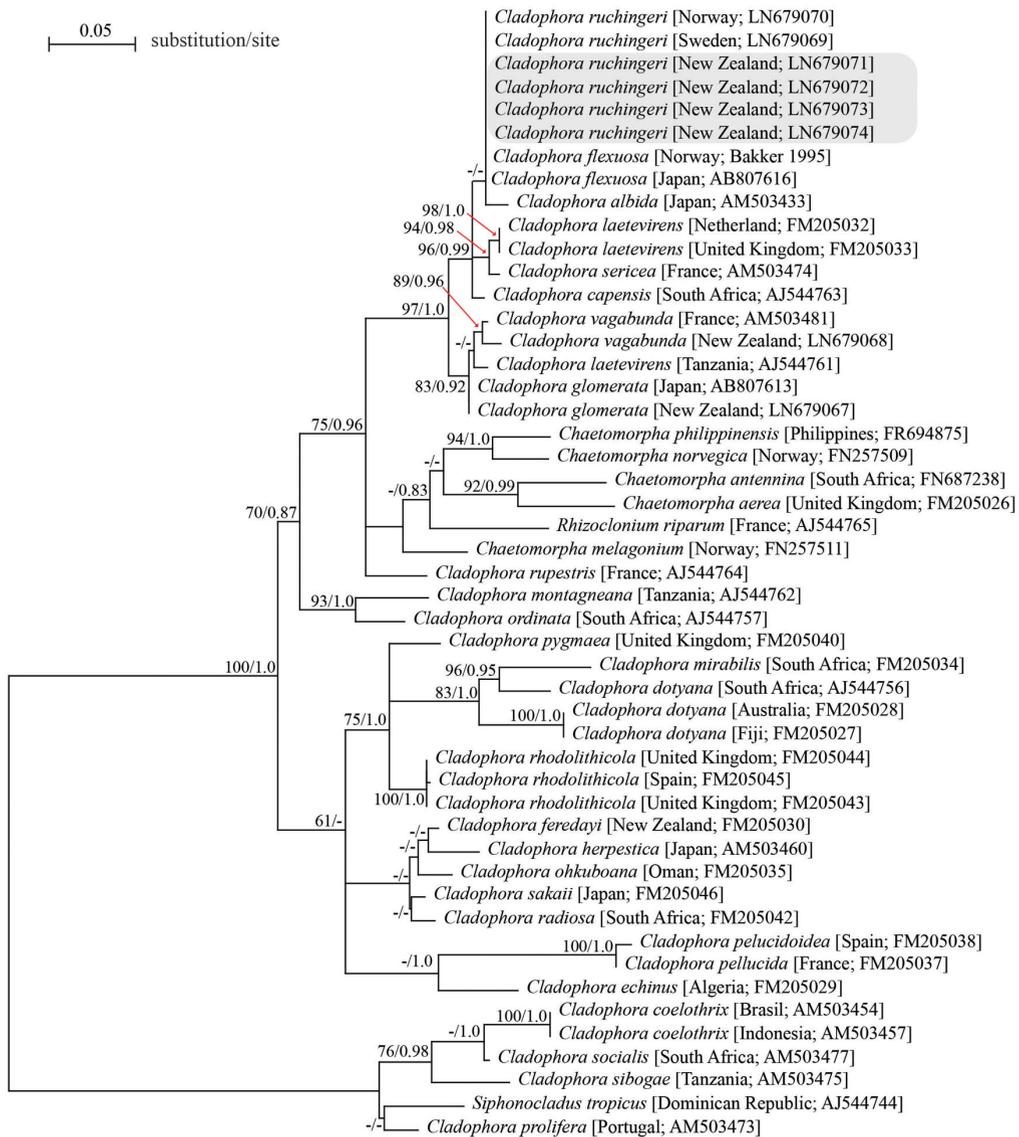


Figure 4. Phylogenetic reconstruction of Cladophorales species inferred from partial large subunit (LSU) rDNA based on Maximum Likelihood (ML). The tree includes 49 LSU sequences. For each sequence, the species name is followed by the geographical origin of the specimen and sequence accession number. The numbers at nodes correspond to bootstrap values in ML and posterior probabilities (PP) in MrBayes analyses. Values <70 ML and <0.8 PP are replaced by a dash (-); Branches at nodes displaying both values of <50 ML and <0.5 PP were manually collapsed. Grey area indicates *Cladophora ruchingeri* specimens collected in the Marlborough Sounds.

Zealand. This is further substantiated by the identification of this species primarily on artificial substrates used in aquaculture, a typical first point of establishment for non-indigenous organisms (Minchin 2007). An assessment of the diversity of New Zealand marine Cladophorales using a polyphasic approach is required to confirm this hypothesis.

Human activities in the Marlborough Sounds area, particularly aquaculture and vessel movements, are a significant mechanism for the introduction, establishment, and spread of marine pests (Acosta and Forrest 2009; Woods et al. 2012). The large surface area provided by farming floating structures (e.g. ropes, floats, pontoons) provide ideal habitat for biofouling organisms, such as *C. ruchingeri*,

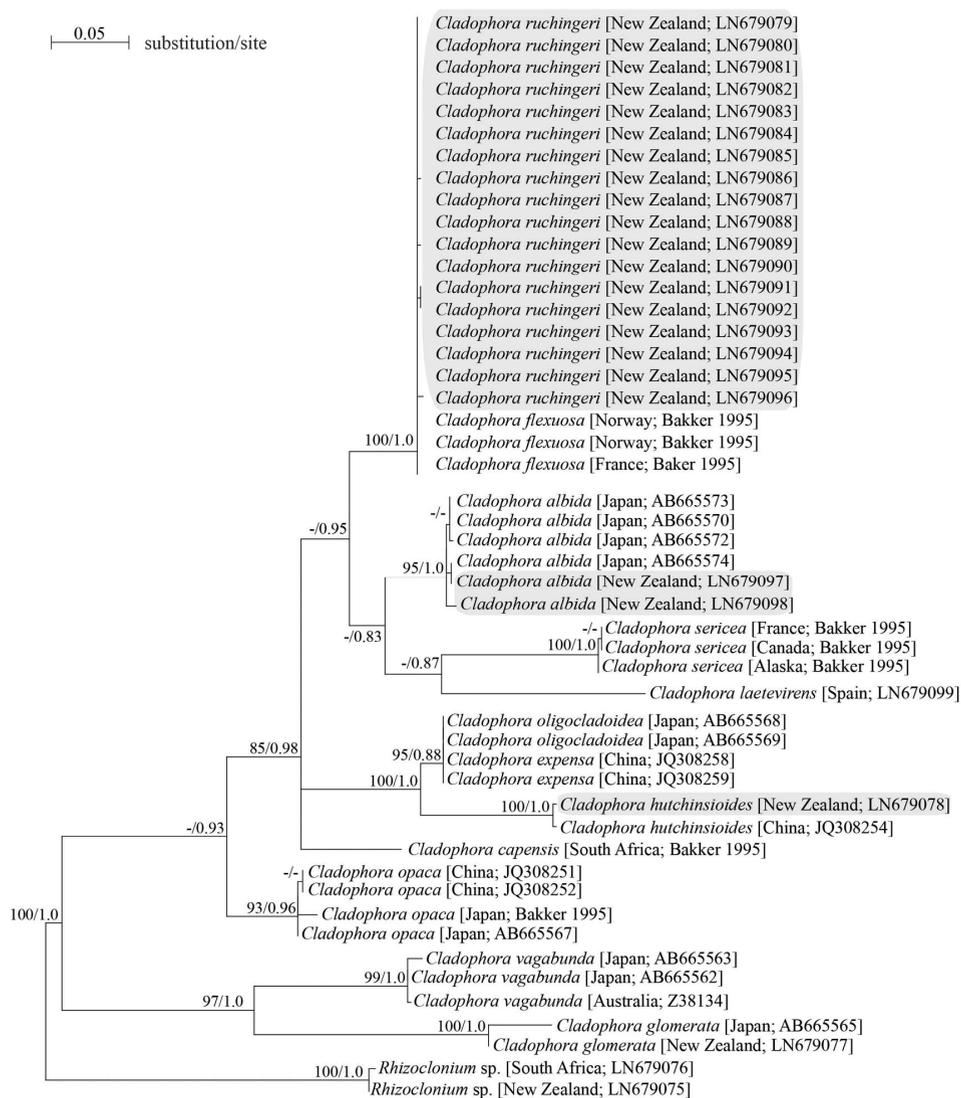


Figure 5. Phylogenetic reconstruction of Cladophorales species inferred from partial 5.8S and internal transcribed spacer 2 (ITS2) rDNA gene based on Maximum Likelihood (ML). The tree includes 49 sequences. For each sequence, the species name is followed by the geographical origin of the specimen and sequence accession number. The numbers at nodes correspond to bootstrap values in ML and posterior probabilities (PP) in MrBayes analyses. Values <70 ML and <0.8 PP are replaced by a dash (-); Branches at nodes displaying both values of <50 ML and <0.5 PP were manually collapsed. Grey areas indicate *Cladophora* spp. specimens collected in the Marlborough Sounds.

to proliferate (Forrest and Blakemore 2006; Minchin 2007). Once floating artificial structures become infected, they can act as a reservoir for the spread of the pest into adjacent areas (Bulleri and Airolidi 2005; Carver et al. 2003). For example, high densities of *C. ruchingeri* on floating structures in the Marlborough Sounds may provide

a propagule supply to natural habitats, where it has also been recorded in large abundance, especially during summer months (J. Atalah; pers. observation).

Cladophora ruchingeri has the potential to negatively impact the mussel industry by overgrowing and smothering mussel lines. This

can increase their weight and lead to crop losses, affect seed-stock survival, and result in additional production and processing expenses (Fitridge et al. 2012; Fletcher et al. 2013). In natural habitats, *Cladophora* species are regarded as ecological engineers, where they have the ability to alter the flow of organic matter and light and provide a structurally complex habitat for benthic microfauna (Zulkifly et al. 2013). Several *Cladophora* species form conspicuous algal mats in coastal and freshwater systems worldwide (Dodds 1991; Zulkifly et al. 2013). These mass developments frequently occur in brackish estuaries and lagoons, where nutrients accumulate and competition is reduced (Flindt et al. 1997; Curiel et al. 2004; Gubelit and Kovalchuk 2010).

Many *Cladophora* species, including *C. ruchingeri*, have high growth rates, short generation times, broad ecological tolerance, and produce significant quantities of offspring via the release of large amounts of asexual spores, resulting in large seasonal populations (Dodds and Gudder 1992; Zulkifly et al. 2013). Several species of *Cladophora* can form akinetes, thick-walled resting stages that can persist during unfavourable conditions (e.g. van den Hoek 1963; Whitton 1970; Dodds and Gudder 1992). These features provide some species of *Cladophora* with a competitive advantage over other macroalgae and this may explain why *C. ruchingeri* has become problematic in the Marlborough Sounds.

An alternative hypothesis for the sudden appearance of *C. ruchingeri* in recent years is a change in environmental conditions (e.g., nutrient enrichment) that has facilitated the rapid increase in biomass. Most *Cladophora* species have high nutrient requirements, and phosphorus seems to be the limiting factor for growth (e.g. Whitton 1970; Dodds and Gudder 1992). However, the Marlborough Sounds are considered to be low-mesotrophic, with moderate nutrient concentrations and primary productivity (Gibbs et al. 1992; Zeldis et al. 2008). There has been no evidence of an increase of nutrient concentration in the Marlborough Sounds at a regional scale in recent years (Gibbs et al. 1992; Zeldis et al. 2008). Nevertheless, mussels' biodepositions (i.e. faeces and pseudofaeces) and ammonium excretion result in localised nutrient enrichment (Kaspar et al. 1985), which may stimulate the mass proliferation of *C. ruchingeri* attached to farm structures. Further studies of *C. ruchingeri* blooms and nutrients availability around aquaculture farms are required to test this hypothesis.

Conclusions

Within the past five years, large *Cladophora* blooms have been observed on the ropes of green-lipped mussel farms in the Marlborough Sounds. These blooms represent a nuisance for the mussel farming industry and may alter ecosystem functioning. We identified this green filamentous alga as *C. ruchingeri* and believe this species has only recently arrived in New Zealand. This study is a crucial first step for understanding *C. ruchingeri* ecology and distribution, and in the development of management strategies to minimise risks for the aquaculture industry and protect natural values.

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References

- Acosta H, Forrest BM (2009) The spread of marine non-indigenous species via recreational boating: A conceptual model for risk assessment based on fault tree analysis. *Ecological Modelling* 220: 1586–1598, <http://dx.doi.org/10.1016/j.ecolmodel.2009.03.026>
- Adams NM (1994) Seaweeds of New Zealand: an illustrated guide. Christchurch, Canterbury University Press, 360 pp
- AQNZ (2012) Aquaculture New Zealand Ltd. New Zealand aquaculture: A sector overview with key facts, statistics and trends, 20 pp
- Anderson MJ, Underwood AJ (1994) Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage. *Journal of Experimental Marine Biology and Ecology* 184: 217–236, [http://dx.doi.org/10.1016/0022-0981\(94\)90006-X](http://dx.doi.org/10.1016/0022-0981(94)90006-X)
- Bakker FT, Olsen JL, Stam WT, van den Hoek C (1994) The *Cladophora* complex (Chlorophyta): New views based on 18S rRNA gene sequences. *Molecular Phylogenetics and Evolution* 3: 365–382, <http://dx.doi.org/10.1006/mpev.1994.1043>
- Bakker FT (1995) Time spans and spacers: molecular phylogenetic explorations in the *Cladophora* complex (Chlorophyta) from the perspective of rDNA gene and spacer sequences. PhD thesis, University of Groningen, Netherlands
- Bakker FT, Olsen JL, Stam WT (1995a) Global phylogeography in the cosmopolitan species *Cladophora vagabunda* (Chlorophyta) based on nuclear rDNA internal transcribed spacer sequences. *European Journal of Phycology* 30: 197–208, <http://dx.doi.org/10.1080/09670269500650981>
- Bakker FT, Olsen, JL, Stam WT (1995b) Evolution of nuclear rDNA ITS sequences in the *Cladophora albida/sericea* clade (Chlorophyta). *Molecular Biology and Evolution* 40: 640–651, <http://dx.doi.org/10.1007/BF00160512>
- Boedeker C, O'Kelly CJ, Star W, Leliaert F (2012) Molecular phylogeny and taxonomy of the Aegagropila clade (Cladophorales, Chlorophyta), including the description of *Aegagropilopsis* gen. nov. and *Pseudocladophora* gen.

- nov. *Journal of Phycology* 48: 808–25, <http://dx.doi.org/10.1111/j.1529-8817.2012.01145.x>
- Bulleri F, Airoldi L (2005) Artificial marine structures facilitate the spread of a non-indigenous green alga, *Codium fragile* ssp. *tomentosoides*, in the north Adriatic Sea. *Journal of Applied Ecology* 42:1063–1072, <http://dx.doi.org/10.1111/j.1365-2664.2005.01096.x>
- Carlton JT (1989) Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. *Conservation Biology* 3: 265–273, <http://dx.doi.org/10.1111/j.1523-1739.1989.tb00086.x>
- Carver CE, Chisholm A, Mallet AL (2003) Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. *Journal of Shellfish Research* 22: 621–631
- Chapman VJ (1956) The marine algae of New Zealand. Part I. Myxophyceae and Chlorophyceae. *Botanical Journal of the Linnean Society* 55: 333–501, <http://dx.doi.org/10.1111/j.1095-8339.1956.tb00019.x>
- Curiel D, Rismondo A, Bellemo G, Marzochi M (2004) Macroalgal biomass and species variations in the Lagoon of Venice (Northern Adriatic Sea, Italy): 1981–1998. *Scientia Marina* 68: 57–67
- Dodds WK (1991) Factors associated with dominance of the filamentous green alga *Cladophora glomerata*. *Water Research* 25: 1325–1332, [http://dx.doi.org/10.1016/0043-1354\(91\)90110-C](http://dx.doi.org/10.1016/0043-1354(91)90110-C)
- Dodds WK, Gudder DA (1992) The ecology of *Cladophora*. *Journal of Phycology* 28: 415–427, <http://dx.doi.org/10.1111/j.0022-3646.1992.00415.x>
- Falk-Petersen J, Bohn T, Sandlund OT (2006) On the numerous concepts in invasion biology. *Biological Invasions* 8: 1409–1424, <http://dx.doi.org/10.1007/s10530-005-0710-6>
- Fitridge I, Dempster T, Guenther J, et al. (2012) The impact and control of biofouling in marine aquaculture: a review. *Biofouling* 28: 649–669, <http://dx.doi.org/10.1080/08927014.2012.700478>
- Fletcher LM, Forrest BM, Bell JJ (2013) Impacts of the invasive ascidian *Didemnum vexillum* on green-lipped mussel (*Perna canaliculus*) growth on a New Zealand aquaculture farm. *Aquaculture Environment Interactions* 4: 17–30, <http://dx.doi.org/10.3354/aei00069>
- Flindt MR, Kamp-Nielsen L, Marques JC, Pardal MA, Bocci M, Bendoricchio G, Salomonsen J, Nielsen SN, Jørgensen SE (1997) Description of three shallow estuaries: Mondego River (Portugal), Roskilde Fjord (Denmark) and the Lagoon of Venice (Italy). *Ecological Modelling* 102: 17–31, [http://dx.doi.org/10.1016/S0304-3800\(97\)00092-6](http://dx.doi.org/10.1016/S0304-3800(97)00092-6)
- Forrest BM, Blakemore KA (2006) Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* 257: 333–345, <http://dx.doi.org/10.1016/j.aquaculture.2006.03.021>
- Ganesan EK (1990) A catalog of benthic marine algae and seagrasses of Venezuela. Fondo Editorial Conicit, Caracas, 237 pp, 15 maps
- Gibbs M, Pickmere S, Woods P, et al. (1992) Nutrient and chlorophyll a variability at six stations associated with mussel farming in Pelorus Sound, 1984–85. *New Zealand Journal of Marine and Freshwater Research* 26: 197–211, <http://dx.doi.org/10.1080/00288330.1992.9516515>
- Gottschling M, Keupp H, Plotner J, Knop R, Willems H, Kirsch M (2005) Phylogeny of calcareous dinoflagellates as inferred from ITS and ribosomal sequence data. *Molecular Phylogenetics and Evolution* 36: 444–455, <http://dx.doi.org/10.1016/j.ympev.2005.03.036>
- Gubelit YI, Kovalchuk NA (2010) Macroalgal blooms and species diversity in the transition zone of the eastern Gulf of Finland. *Hydrobiologia* 656: 83–86, <http://dx.doi.org/10.1007/s10750-010-0425-2>
- Guindon S, Delsuc F, Dufayard JF, Gascuel O (2009) Estimating maximum likelihood phylogenies with PhyML. *Methods in Molecular Biology* 537: 113–137, http://dx.doi.org/10.1007/978-1-59745-251-9_6
- Guiry MD, Guiry GM (2014) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org> (Accessed 14 May 2014)
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95–98
- Hanyuda T, Wakana I, Arai S, Miyaji K, Watano Y, Ueda K (2002) Phylogenetic relationships within Cladophorales (Ulvophyceae, Chlorophyta) inferred from 18S rRNA gene sequences, with special reference to *Aegagropila linnaei*. *Journal of Phycology* 38: 564–71, <http://dx.doi.org/10.1046/j.1529-8817.2002.01151.x>
- Hayakawa Y-I, Ogawa T, Yoshikawa S, Ohki K, Kamiya M (2012) Genetic and ecophysiological diversity of *Cladophora* (Cladophorales, Ulvophyceae) in various salinity regimes. *Phycological Research* 60: 86–97, <http://dx.doi.org/10.1111/j.1440-1835.2012.00641.x>
- Hurd CL, Nelson WA, Falshaw R, Neill KF (2004) History, current status and future of marine macroalgal research in New Zealand: Taxonomy, ecology, physiology and human uses. *Phycological Research* 52: 80–106, <http://dx.doi.org/10.1111/j.1440-1835.2004.tb00318.x>
- Ichihara K, Shimada S, Miyaji K (2013) Systematics of *Rhizoclonium*-like algae (Cladophorales, Chlorophyta) from Japanese waters, based on molecular phylogenetic and morphological analyses. *Phycologia* 52: 398–410, <http://dx.doi.org/10.2216/12-102.1>
- Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* 4: 18, <http://dx.doi.org/10.1186/1471-2148-4-18>
- Kaspar HF, Gillespie PA, Boyer IC, MacKenzie AL (1985) Effects of mussel aquaculture on the nitrogen cycle and benthic communities in Kenepuru Sound, Marlborough Sounds, New Zealand. *Marine Biology* 85: 127–136, <http://dx.doi.org/10.1007/BF00397431>
- Kraft GT (2007) Algae of Australia. Marine benthic algae of Lord Howe Island and the southern Great Barrier Reef, 1. Green algae. Australian Biological Resources Study and CSIRO Publishing, Canberra and Melbourne, Australia
- Lanave C, Preparata G, Saccone C, Serio G (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* 20: 86–93, <http://dx.doi.org/10.1007/BF02101990>
- Leliaert F, Boedeker C (2007) Cladophorales. In: Brodie J, Maggs CA, John D (eds), Green Seaweeds of Britain and Ireland. Natural History Museum Publications, London, UK, pp 131–183
- Leliaert F, Rousseau F, de Reviers B, Coppejans E (2003) Phylogeny of the Cladophorophyceae (Chlorophyta) inferred from partial LSU rRNA gene sequences: is the recognition of a separate order Siphonocladales justified? *European Journal of Phycology* 38: 233–246, <http://dx.doi.org/10.1080/1364253031000136376>
- Leliaert F, De Clerck O, Verbruggen H, Boedeker C, Coppejans E (2007) Molecular phylogeny of the Siphonocladales (Chlorophyta: Cladophorophyceae). *Molecular Phylogenetics and Evolution* 44: 1237–1256, <http://dx.doi.org/10.1016/j.ympev.2007.04.016>
- Leliaert F, Boedeker C, Pena V, Bunker F, Verbruggen H, De Clerck O (2009) *Cladophora rhodoliticola* sp. nov. (Cladophorales, Chlorophyta), a diminutive species from European Maerl beds. *European Journal of Phycology* 44: 155–69, <http://dx.doi.org/10.1080/09670260802573113>
- Minchin D (2007) Aquaculture and transport in a changing environment: Overlap and links in the spread of alien biota. *Marine Pollution Bulletin* 55: 302–313, <http://dx.doi.org/10.1016/j.marpolbul.2006.11.017>

- Molnar JL, Gamboa RL, Revenga C, et al. (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* 6: 485–492, <http://dx.doi.org/10.1890/070064>
- Nelson WA (2013) *New Zealand Seaweeds: an illustrated guide*. Te Papa Press, Wellington, New Zealand, 328 pp
- Nelson WA, Adams NM, Fox JM (1992) Marine algae of the Northern South Island: a list of species. National Museum of New Zealand. Miscellaneous Series No 26, 79 pp
- Nunn GB, Theisen BF, et al. (1996) Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order Isopoda. *Journal of Molecular Evolution* 42: 211–223, <http://dx.doi.org/10.1007/BF02198847>
- Ramsay A, Davidson J, Landry T, Arsenault G (2008) Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. *Biological Invasions* 10: 1311–1316, <http://dx.doi.org/10.1007/s10530-007-9205-y>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542, <http://dx.doi.org/10.1093/sysbio/sys029>
- Ruiz GM, Fofonoff P, Hines AH, et al. (1999) Non-indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. *Limnology and Oceanography* 44: 950–972, http://dx.doi.org/10.4319/lo.1999.44.3_part_2.0950
- Ruiz GM, Freestone AL, Fofonoff PW, et al. (2009) Habitat distribution and heterogeneity in marine invasion dynamics: the importance of hard substrate and artificial structure. In: Rilov G and Crooks JA (eds) *Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives*. Springer-Verlag, Berlin Heidelberg, pp 321–332
- Scholin CA, Anderson DM (1994) Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU ribosomal RNA genes. *Journal of Phycology* 30: 744–754, <http://dx.doi.org/10.1111/j.0022-3646.1994.00744.x>
- Söderström J (1963) Studies in *Cladophora*. *Botanica gothoburgensia* 1: 1–147
- Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, Massachusetts, USA
- van den Hoek C (1963) Revision of the European species of *Cladophora*. Brill E.J., Leiden, Netherlands
- van den Hoek C (1982) A taxonomic revision of the American species of *Cladophora* (Chlorophyceae) in the North Atlantic Ocean and their geographic distribution. North Holland Publishing Company, Amsterdam, Netherlands
- van den Hoek C, Chihara M (2000) A taxonomic revision of the marine species of *Cladophora* (Chlorophyta) along the coasts of Japan and the Russian Far-east. National Science Museum (Tokyo) Monographs 19. National Science Museum, Tokyo, Japan
- van den Hoek C, Womersley HBS (1984) Genus *Cladophora*. In: Womersley HBS (ed) *The marine benthic flora of southern Australia. Part I*. Government Printer, Adelaide, pp 185–213
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, et al. (eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc., New York, pp 315–322, <http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Whitton BA (1970) Biology of *Cladophora* in freshwater. *Water Research* 4: 457–476, [http://dx.doi.org/10.1016/0043-1354\(70\)90061-8](http://dx.doi.org/10.1016/0043-1354(70)90061-8)
- Woods CMC, Floerl O, Hayden BJ (2012) Biofouling on Greenshell (TM) mussel (*Perna canaliculus*) farms: a preliminary assessment and potential implications for sustainable aquaculture practices. *Aquaculture International* 20: 537–557, <http://dx.doi.org/10.1007/s10499-011-9484-2>
- Zeldis J, Howard-Williams C, Carter C, et al. (2008) ENSO and riverine control of nutrient loading, phytoplankton biomass and mussel aquaculture yield in Pelorus Sound, New Zealand. *Marine Ecology Progress Series* 371: 131–142, <http://dx.doi.org/10.3354/meps07668>
- Zeng C (Tseng C) (2009) *Seaweeds in Yellow Sea and Bohai Sea of China*. Science Press, Beijing, China
- Zulkifly SB, Graham JM, Young EB, et al. (2013) The Genus *Cladophora* Kützinger (Ulvophyceae) as a Globally Distributed Ecological Engineer. *Journal of Phycology* 49: 1–17, <http://dx.doi.org/10.1111/jpy.12025>

The following supplementary material is available for this article:

Table S1. List of 49 *Cladophorales* species included in the phylogenetic analysis of LSU rDNA gene (Figure 4).

Table S2. List of 49 *Cladophorales* species included in the phylogenetic analysis of ITS rDNA gene (Figure 5).

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